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3 **Vertebrate seed predation can limit recruitment of later-successional species in tropical**
4 **forest restoration**

5

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16 **Abstract**

17 The effects of vertebrate seed predation on the regeneration of restored forests are not well
18 understood because most past studies have focused on seed predation within the first few years
19 after restoration and have measured seed removal without quantifying subsequent seedling
20 establishment of seeds that avoid predation. Quantifying establishment of seeds that escape
21 predation in restored forests at later stages of regrowth is crucial for anticipating longer-term
22 recovery trajectories. Here, we evaluated the potential role of vertebrate seed predators in
23 limiting recruitment of later-successional tree species in nine forests actively restored ≥ 15 years
24 prior and in four paired remnant forest fragments embedded in an agricultural landscape in
25 southern Costa Rica. We conducted seed addition experiments with four tree species inside and
26 outside vertebrate exclosures and used camera trapping to detect seed predators. To determine
27 the fate of seeds that avoided predation, we also measured seedling establishment after one year,
28 given that other mortality factors may compensate in the absence of vertebrate seed predation.
29 We detected two species of birds and five species of granivorous mammals removing seeds.
30 Seed tagging indicated that most removal resulted in predation. For three of the four tree species
31 tested, vertebrate seed predation reduced seedling establishment. The magnitude of this effect
32 depended on species' susceptibility to other causes of mortality during the seed-to-seedling
33 transition. Our study demonstrates that vertebrate seed predators can substantially reduce later-
34 successional seedling recruitment in restored forests and should be considered alongside
35 dispersal limitation and microsite conditions as factors slowing forest recovery.

36 **Keywords:** Costa Rica, ecological restoration, exclosure experiment, seed addition, seedling
37 establishment, succession, tree plantation

38 1. INTRODUCTION

39 Vertebrate seed predation can play important roles in tropical forest dynamics by shaping spatial
40 and temporal patterns of plant recruitment and the relative abundances of different species (Clark
41 et al., 2012; Janzen, 1971; Paine et al., 2016; Rosin & Poulsen, 2016; Silman et al., 2003;
42 Wright, 2003). Although post-dispersal seed predation by vertebrates is a natural ecosystem
43 process long recognized to influence seedling community structure (Asquith et al., 1997; Orrock
44 et al., 2006), it has the potential to slow forest recovery and is typically considered a barrier
45 rather than a restoration target (Doust, 2011; Pearson et al., 2022).

46 Vertebrate seed predation could affect tree seedling recruitment differently in restoration
47 plantings ('active restoration') compared to intact or remnant forests for multiple reasons. First,
48 restoration sites (both passive and active) often lack reproductive individuals of many tree
49 species during the initial years of succession (Caughlin et al., 2019; Van Breugel et al., 2007), so
50 seed rain for many species is comprised primarily of immigrant seeds. Even in restored forests
51 planted with native species, the trees require time to reproductively mature, and the diversity of
52 planted species is typically much lower than that in remnant forests (de Almeida et al., 2024).
53 This means that for later-successional tree species, especially larger-seed species which are more
54 strongly dispersal-limited, most recruitment is from low-density immigrant seed rain, in the tails
55 of seed shadows (Peña-Domene et al., 2013). In this scenario negative density-dependence
56 resulting from natural enemies is expected to be weaker (Comita et al., 2014), so the relative
57 importance of seed predation by generalist granivores may increase. Second, the faunal
58 assemblages using restoration plantings are often distinct from those using remnant forests
59 (Zagal-García et al., 2022), which may result from altered habitat structure and resources, small
60 area, and isolation from source populations (Galetti et al., 2017; Ribeiro da Silva et al., 2015).

61 Despite these differences and their potential implications for tropical forest recovery, a recent
62 meta-analysis found that seed predation was the least common plant-animal interaction examined
63 in studies comparing restored and reference systems (Genes & Dirzo, 2022). Furthermore, many
64 seed predation studies related to tropical forest recovery historically focused on seed predation as
65 a barrier to initial recruitment in abandoned pasture (Holl & Lulow, 1997; Jones et al., 2003;
66 Myster, 2003). But the role of vertebrate seed predation may change as succession proceeds
67 (Yang et al., 2018). Better understanding the outcomes of seed predation interactions may be key
68 to predicting and managing successional pathways at existing and future restoration sites to
69 improve outcomes for biodiversity and carbon storage (Culot et al., 2017).

70 Identifying which vertebrate species are responsible for post-dispersal seed removal is
71 necessary to understand if and how altered faunal assemblages at restoration sites influence the
72 seed-to-seedling transition for immigrant seeds. For example, the absence of large-bodied
73 animals at restoration sites could release large-seeded species from seed predation and increase
74 seedling establishment, as has sometimes occurred in response to loss of large fauna (Dirzo et al.,
75 2007; Mendoza & Dirzo, 2007; Silman et al., 2003). Alternatively, if extant smaller vertebrates
76 or seed-eating insects are able to compensate for the absence of larger seed predators, different
77 faunal communities could yield similar seed predation outcomes (Brewer et al., 1997; Rosin &
78 Poulsen, 2018). It is also especially important to identify the vertebrates removing seeds because
79 some species act as both seed predators and secondary dispersers (Gómez et al., 2019; Mittelman
80 et al., 2021; Vander Wall et al., 2005).

81 Evaluating whether effects of vertebrate seed predation carry through to seedling
82 establishment is key to understanding its role in recruitment limitation. Many seed predation
83 studies only examine seed removal over the short term (days to weeks) (Dylewski et al., 2020;

84 Moles & Westoby, 2003), yet the remaining seeds are not guaranteed to complete the seed-to-
85 seedling transition. So the importance of vertebrate seed predation for recruitment outcomes can
86 be overestimated when other causes of mortality fully or partially compensate for the loss of
87 vertebrate seed predators (Terborgh, 2013; Williams et al., 2021). Therefore, monitoring seed
88 fates through the full seed-to-seedling transition provides a more realistic picture of vertebrate
89 seed predation effects.

90 Here, we conducted a seed addition and vertebrate exclosure experiment in two habitat types
91 (restoration plantings and remnant forest) using four large-seeded (>10-mm), animal-dispersed,
92 later-successional tree species, within a long-term, well replicated restoration experiment in
93 southern Costa Rica. We paired the seed addition experiments with camera trapping to assess
94 overall vertebrate use of habitats and link faunal communities to seed predation outcomes. We
95 asked: (a) Which species are seed predators on larger seeds in this fragmented agricultural
96 landscape? (b) To what degree does seed predation by granivorous mammals and birds reduce
97 seedling recruitment? (c) Does seed predation, and its net effects on seedling establishment, vary
98 between remnant forests and restoration plantings in their second decade of succession? We
99 expected that experimentally added seeds would have higher survival when vertebrates were
100 excluded and that rodents would be the main seed predators (Cole, 2009; Kuprewicz & García-
101 Robledo, 2019). How closely seedling establishment success would match the proportion of
102 seeds that avoided predation by vertebrates was an open question. Furthermore, we expected that
103 the greater resource levels, habitat area, and structural complexity of remnant forests might result
104 in greater activity of seed predators, but that high functional redundancy within faunal
105 communities could result in similar seed predation rates between the two habitat types.

106

107 2. METHODS

108 2.1 Study area and long-term restoration experiment design

109 The study area is an agricultural landscape in southern Costa Rica (Coto Brus, Puntarenas
110 Province). The region has a 3000-yr record of human habitation, forest clearing, and agriculture
111 (Clement & Horn, 2001), but the majority of land is thought to have been forested until
112 settlement by non-indigenous people in the mid-20th century, at which point land was rapidly
113 deforested for conversion to agriculture (Zahawi et al., 2015). Remnant and secondary forest
114 fragments are interspersed among pastures and row crops. The native ecosystem is transitional
115 between premontane wet and rain forest (Holdridge et al., 1971). The study area receives ~3500-
116 4000 mm annually, with a dry season from December to March, and has a mean annual
117 temperature of ~21 °C. Elevation of study sites ranges from ~1100-1200 m. The region hosts a
118 high diversity of mammals (>100 spp.) (Pacheco et al., 2006) and birds (>400 spp.), although six
119 species of large-bodied vertebrates have been locally extirpated (Daily et al., 2003). These
120 include *Tapirus bairdii* (Baird’s tapir) and *Tayassu pecari* (white-lipped peccary), a prominent
121 seed predator in the Neotropics (Beck, 2005).

122 We conducted this study at nine sites (Figure 1, Table S1), a subset of the sites established in
123 2004-2006 as a long-term restoration experiment (Zahawi et al., 2013). Each site contains a
124 50×50 m plot (hereafter “restored forest”) planted with two native timber tree species,
125 *Terminalia amazonia* (Combretaceae) and *Vochysia guatemalensis* (Vochysiaceae), and two N-
126 fixing species, *Erythrina poeppigiana* and *Inga edulis* (Fabaceae), with a consistent spacing of
127 2.8 m along the diagonal (Cole et al., 2010). Over the course of this study (January 2021-October
128 2023), restored forests ranged from 14-19 years old. Four of the nine sites had paired areas of
129 remnant forest used as a reference for later-successional conditions in the region. Although nine

130 sites were used overall, the number of restored forest sites used in seed addition experiments
131 varied from six to eight, depending on species and experimental stage (seed removal or seedling
132 establishment) because of the loss of one site to land use change (Table S1). The minimum
133 distance between replicate sites was 0.7 km (median = 4.0 km, max = 7.8).

134

135 **2.2 Baseline faunal surveys**

136 To quantify habitat use by ground-dwelling mammals and birds and to identify the pool of
137 potential seed predator species, we conducted camera trapping in restored and remnant forests. In
138 January-June 2021 we deployed cameras at all sites except for one, which we surveyed in May
139 2022,. Within each restored or remnant forest, we deployed two unbaited Bushnell Core DS
140 cameras (models 119975C and 119977C; Bushnell, Kansas, USA), set to high sensitivity and
141 programmed to capture five 8 MP images per trigger. To prevent disturbance to long-term
142 research plots, we did not clear vegetation in front of cameras, and instead secured cameras to
143 trees adjacent to suitable fields of view, but avoided aiming cameras at trails used by humans.
144 The minimum distance between cameras was ~25 m. Our 50 cm deployment height is standard
145 for monitoring mammals with camera traps (Kays et al., 2020), although consistently detecting
146 and identifying small nocturnal mammals using camera traps is a common challenge (Gracanin
147 et al., 2019). The duration of each camera deployment was ~55 days, but effective survey effort
148 was lower for some deployments due to camera failures. Total survey effort was 1311 trap-nights
149 (524 in remnant forests, 787 in restored forests).

150

151 **2.3 Focal tree species selection and seed collection**

152 We used four focal tree species for seed addition experiments (Table 1), which we selected on
153 the basis of (a) seed availability during the study period; (b) occurrence in late-successional
154 forest within the study region and some degree of natural recruitment within the long-term
155 restoration experiment (Werden et al., 2020); and (c) larger seed size (≥ 10 mm width, ≥ 1 g fresh
156 mass). This size class corresponds to the approximate maximum diameter of seeds that smaller
157 frugivorous birds in this system can swallow (Reid et al., 2021), and is similar to the 12-mm
158 threshold used to define large seeds in the Atlantic Forest biome (Bello et al., 2015). Seed
159 species included *Pseudolmedia mollis*, *Quercus benthamii*, and *Otoba novogranatensis*, and
160 *Ocotea puberula* (hereafter referred to by generic names) collected from the ground under at
161 least three mother trees per species. We manually removed pulp from *Pseudolmedia*, whereas
162 birds had already removed pulp from most *Ocotea* and *Otoba* seeds. We measured fresh mass
163 and width (minimum dimension) for a subsample of >50 seeds of each species. Before seed
164 additions, we thoroughly mixed seeds to avoid plot-level bias in seed source or quality. We also
165 briefly submerged all seeds in water to identify and remove insect-damaged or otherwise non-
166 viable seeds (indicated by floating).

167

168 **2.4 Seed addition experimental design**

169 In June 2021, we set up four stations within each 50×50 m restoration plot and each remnant
170 forest fragment. In each restored forest plot, one station was distributed in each quarter of the
171 plot (Figure 2). The minimum distance between stations was ~ 15 m. We installed one enclosure
172 (1×1 (wide) $\times 0.3$ (tall) m rebar frame secured to the ground and covered with 1.27 cm wire mesh)
173 in each station and demarcated two adjacent 1×1 m seed addition quadrats that were exposed to

174 vertebrate seed predators (Figure 2). Previous research in this region found that this mesh size
175 was sufficient to exclude small mice (Holl & Lulow, 1997). We marked the corners of exposed
176 quadrats with stake flags and secured a roll of fine mesh ~5 cm high on the downhill side to
177 catch any seeds washed downslope by runoff. We did not remove natural vegetation from seed
178 addition quadrats, since vegetation removal could have increased detectability by predators and
179 also affected subsequent seedling establishment.

180 We sequentially added seeds of the four focal tree species in July 2021, April 2022, and
181 August 2022 (Table 1). We placed seeds directly on top of the litter layer or soil surface to
182 simulate how seeds would naturally be deposited by primary dispersal, pressing down slightly to
183 prevent seeds from rolling away on steeper slopes. Seeds were distributed systematically
184 following a predetermined grid configuration with 10-20 cm minimum spacing.

185 We censused all quadrats at ~7-11 weeks post-addition to count the number of seeds
186 remaining (n = 3024 seeds added). We also quantified vertebrate-seed encounters for each
187 species in one exposed quadrat per plot by deploying a camera trap 1.5-2 m above the ground,
188 with the entire seed quadrat in the field of view. Camera traps were deployed for the initial ~7
189 weeks after seeds were set out, with individual camera deployment durations (range 7-51 days)
190 depending on seed species, camera functioning, and whether all seeds had already been removed.

191 We also censused seedling establishment (n = 2880 seeds) for each species after one year
192 (12-15 months). When monitoring quadrats, we scattered any leaf litter accumulated on the top
193 or sides of an enclosure inside that cage to reduce shading and litter depletion effects. Because
194 seed additions were staggered by species, the intervals between monitoring visits varied from 1-5
195 months. Litter depth was slightly reduced within enclosures compared to open seed addition

196 quadrats, from 2.7 ± 0.8 to 1.9 ± 1.1 cm (mean \pm SD, $p < 0.001$, Figure S2). There was no
197 evidence that this effect differed between forest types ($p = 0.20$), nor was there a difference in
198 overall litter depth between forest types ($p = 0.19$, Table S2).

199

200 **2.5 Seed tagging**

201 To assess the degree to which seed removal might represent seed dispersal by scatter-hoarding
202 rodents, rather than seed predation, in July-August 2022 we conducted a follow-up seed-tagging
203 experiment at two of the sites that had paired remnant forest and had previously shown high
204 levels of seed removal. We sequentially quantified secondary dispersal for two of the previously
205 added species. In each plot we set up five stations. First, we glued 50-cm lengths of pink nylon
206 string with labeled flagging to *Pseudolmedia* seeds and placed ten seeds (eight tagged, two non-
207 tagged) in each station, spaced 20 cm apart, with leaf litter covering the string and flagging so
208 that only the seeds themselves were exposed. We monitored two of the five stations in each plot
209 using camera traps positioned as previously described. We checked stations weekly for six
210 weeks, assessing in situ seed fate and searching the area surrounding each station for dispersed
211 seeds. We searched for missing seeds at each station for ~ 5 minutes, starting within 5 m of the
212 original location and expanding the search radius up to ~ 15 m as needed. We marked the
213 location of each scatter-hoarded seed with a bamboo skewer and re-checked their location and
214 predation status weekly until the seed was predated or the end of the tagging experiment,
215 whichever occurred first. Then, we repeated the process for *Quercus* with four seeds per station
216 and monitored the seeds for three weeks. Since we had not observed an effect of tagging on
217 *Pseudolmedia* removal rates, we tagged all four *Quercus* seeds.

218

219 **2.6 Data analysis**

220 We processed camera trap images in *camelot* software (Hendry & Mann, 2017) and calculated a
221 relative activity index (RAI, independent detections per 1000 trap nights) for each species in
222 both habitat types. We used 30 minutes as the threshold for independent detections (Burton et al.,
223 2015). Because detectability varies by species and habitat, this index is not a reliable proxy for
224 abundance (Sollmann et al., 2013), but at a minimum, this approach documents species presence
225 within the two habitat types and provides initial insights into coarse-level activity patterns. Small
226 nocturnal rodents (families Muridae, Heteromyidae, Echimyidae) were not identifiable in camera
227 trap images and thus binned into a single ‘unidentified mouse/rat’ category.

228 Camera trap monitoring of exposed seed addition quadrats sometimes provided clear
229 evidence of seed removal or consumption, but we were not consistently able to attribute the
230 removal of each individual seed to a particular interaction or even vertebrate species. Instead, we
231 quantified independent detections of granivorous species encountering seed addition quadrats.
232 We used the package *bipartite* to visualize bipartite networks of vertebrate-seed encounters
233 between experimental seeds and the granivorous vertebrates confirmed to consume at least one
234 focal species, aggregated by forest type (restored or remnant forest). These networks visualize
235 overall exposure of experimental seed quadrats to granivores but cannot be interpreted as a
236 robust quantification of interaction strength.

237 To test for the main effects of vertebrate exclosures and forest type, as well as their
238 interaction, on the proportion of seeds remaining and the proportion of seeds established as
239 seedlings after one year, we fit binomial generalized linear mixed models (GLMMs) using

240 package *lme4* v 1.1. For each response variable, we fit an overall model with species and station
241 within site as random predictors. We also explored species-specific effects by modeling response
242 variables for species individually, although this was not possible for all species \times response
243 variable combinations because some models failed to converge. For post-hoc comparisons we
244 obtained estimated marginal means using package *emmeans* v. 1.8.1. All analyses were
245 conducted in R v.4.2.1 (R Core Team 2022).

246

247 **2. RESULTS**

248 **3. 1 Faunal surveys**

249 Camera trap surveys in restored forest plots and remnant forests prior to seed addition
250 experiments detected 21 mammal taxa and 41 bird taxa (Table S3), 17 of which are granivorous.
251 The three most frequently-detected granivore species were *Dasyprocta punctata* (Central
252 American agouti, Figure S1a), *Leptotila cassinii* (gray-chested dove), and *Odontophorus*
253 *gujanensis* (marbled wood-quail, Figure S1b), which collectively accounted for 36% of
254 independent mammal and bird detections.

255 Camera trapping in seed quadrats detected two bird species and at least five mammal
256 species consuming or removing experimental seeds (Table 2, Figure 3, Table S3). Great
257 tinamous (*Timanus major*) have sufficiently wide bills to swallow large seeds. Marbled wood-
258 quail (*Odontophorus gujanensis*), in contrast, were observed pecking at seeds and eating the
259 fragments. Smaller granivorous birds such as doves (*Leptotila*, *Geotrygon*) and sparrows
260 (*Arremon* spp.) were never detected interacting with added seeds.

261 Some granivore species that consumed large seeds were more frequently detected in
262 remnant than restored forests in both rounds of camera trapping (Table 2, Figure 3). For
263 example, agouti (*Dasyprocta punctata*) detections were more than ten times as frequent in
264 remnant forests as in restored forests. Furthermore, two species, *Odontophorus gujanensis* and
265 *Dicotyles tajacu* (collared peccary, Figure S1c), were only detected at the two sites embedded
266 within the largest forest fragment in the landscape.

267 The relative frequency of mouse/rat encounters with seed addition quadrats was higher in
268 restored forests (Figure 3), although this was driven by high numbers of detections in particular
269 quadrats that presumably overlapped with the small home ranges of mice/rats that were detected
270 repeatedly. In most cases, mice/rats were not observed removing experimental seeds.

271

272 **3.3 Seed removal**

273 Overall, vertebrate seed predators strongly reduced the proportion of seeds remaining in exposed
274 quadrats ($z = -14.32$, $p < 0.001$, Figure 3a, Table S4); the percentage (mean \pm 1 SD) of seeds
275 remaining at 7-11 weeks in exposed quadrats was $50.8 \pm 5.2\%$, compared to $94.4 \pm 1.6\%$ in
276 exclosure quadrats. In some cases (especially for *Ocotea*), the percentage of seeds remaining in
277 caged quadrats was lower than 100%, but these seeds likely were not found due to burial under
278 shifting litter or because they died and rotted before they were censused. The proportion of seeds
279 remaining was slightly but significantly lower in restored than in remnant forests both inside and
280 outside exclosures ($z = -2.90$, $p = 0.003$, Table S4). Differences in census timing preclude direct
281 comparisons among species, but we observed highest removal for *Pseudolmedia*, intermediate
282 removal for *Quercus* and *Ocotea*, and lowest removal for *Otoba* (Figure 3a).

283

284 3.4 Seed tagging

285 Most (78%) of *Pseudolmedia* and *Quercus* seeds removed by vertebrates in the seed-tagging
286 study were consumed within a 2-m radius of their original position, as indicated by seed
287 fragments or strings with no seed attached. Weekly monitoring and camera trap images revealed
288 that 18 seeds (9% of *Pseudolmedia* seeds) were scatter-hoarded (secondarily dispersed) by
289 agoutis (*Dasyprocta punctata*) within 5 m of the station. These dispersal events were only
290 observed for *Pseudolmedia* and within remnant forest. Sometimes a seed was repeatedly moved
291 and recached, but the majority of scatter-hoarded seeds (10 of 18) were eventually consumed
292 within the six-week experiment.

293

294 3.5 Seedling establishment

295 Seedling establishment after one year was significantly greater inside exclosures compared to
296 exposed quadrats ($z = -15.76$, $p < 0.001$, Table S5); $46.3 \pm 4.3\%$ of added seeds established in
297 exclosures and $15.6 \pm 3.1\%$ of added seeds established in exposed quadrats (Figure 4b). The
298 probability of a caged seed establishing was not different between restored and remnant forests
299 ($z = -0.7$, $p = 0.48$), but exposed seeds were significantly less likely to establish in remnant forest
300 ($p = 0.038$, Table S5). This was driven by *Quercus*, which was less likely to establish as a
301 seedling in remnant forest ($p = 0.01$, Table S6). In contrast, only caged *Otoba* seeds were less
302 likely to establish as seedlings in remnant forest, whereas for *Ocotea* and *Pseudolmedia* there
303 was no significant interaction between exclosure treatment and forest type on establishment
304 success.

305

306 4. DISCUSSION

307 Our use of exclosures, camera trapping, and seed tagging provided direct evidence that
308 vertebrate seed predation reduced seed survival (Figure 4a), consistent with shorter-term studies
309 in restoration and secondary forest contexts (Holl & Lulow, 1997; Peña-Claros & de Boo, 2002).
310 By monitoring seed addition quadrats for a full year, we further showed that these initial effects
311 translated to differences in seedling establishment (Figure 4b), although exclosure effects on
312 establishment were smaller than their effects on removal and varied by species. Seedling
313 establishment both inside and outside of exclosures was much lower than the proportion of non-
314 removed seeds for all species except *Pseudolmedia*, indicating other mortality factors that varied
315 by species. Encouragingly, in our study seed predation did not consistently result in lower
316 seedling establishment at restoration sites compared to remnant forests, unlike the pattern of
317 herbivory effects on vegetation recovery documented in a recent global meta-analysis (Xu et al.
318 2023).

319 Although we did not quantify all mortality factors throughout the seed-to-seedling
320 transition, we offer some context for species variation in patterns of seed removal, seedling
321 establishment, and their correspondence. Variation in time to germination means that caution is
322 warranted when comparing among species (Chen et al., 2021; Martin & Hargreaves, 2023), but
323 we do not believe that the qualitative patterns observed were strongly confounded by exposure
324 time. We observed high *Otoba* and *Quercus* seed mortality from predation by beetles (consistent
325 with Santamaría-Aguilar & Lagomarsino, 2021) but were unable to quantify these effects
326 without destructive sampling of seeds. Although vertebrate seed predation reduced seedling
327 establishment for experimentally added *Quercus* seeds, it is worth noting that acorns of this
328 species would be unlikely to arrive at restoration sites in the absence of scatter-hoarding by

329 vertebrates, even if most interactions with mammals result in seed death. Many remaining
330 *Ocotea* seeds germinated but suffered moderate mortality as seedlings. In contrast, *Pseudolmedia*
331 showed very low mortality within exclosures and thus the strongest effects of vertebrate seed
332 predation. Moreover, it also had long time to germination (>6 months in some cases), suggesting
333 that levels of seed removal at 11 weeks underestimated total seed predation effects.

334 A varied assemblage of vertebrate seed predators encountered and consumed our focal
335 seeds, although composition varied by site and habitat type. Agoutis were important seed
336 predators but were only present at a subset of sites and showed greater activity in remnant forest.
337 In contrast to reports of increased seed predation by small rodents in defaunated and fragmented
338 landscapes elsewhere in the tropics (Galetti et al., 2015; Krishnan et al., 2022; Rosin & Poulsen,
339 2016), we rarely detected mice and rats removing seeds, despite their high levels of activity in
340 restored forests. Red-tailed squirrels (*Sciurus granatensis*) also occurred in restoration plantings
341 and were detected removing seeds more frequently than mice/rats. Marbled wood-quail
342 (*Odontophorus gujanensis*) were unexpectedly frequent seed predators within the largest forest
343 fragment. Although we are not aware of any studies documenting the seed predation interactions
344 of *Odontophorus gujanensis*, the similar congener *O. capoeira* has been documented to eat large
345 (>8-mm diameter) seeds in the Atlantic Forest (Galetti et al., 2015). As suggested by Pizo and
346 Vieira (2004), granivorous birds may be important post-dispersal seed predators in some
347 contexts (Christianini & Galetti, 2007; Palmer & Catterall, 2018) with effects comparable to
348 mammals. Post-dispersal seed removal is not always a reliable proxy for seed predation (Vander
349 Wall et al., 2005), and we detected two species known to effectively disperse seeds by
350 scatterhoarding (*Dasyprocta punctata* and *Sciurus novogranatensis*), but the results of our
351 tagging experiment suggest that most seed removal observed in our study resulted in predation.

352 Although the exclosure treatment affected several variables that could have influenced
353 seedling establishment, in addition to their intended effect of excluding vertebrates, these are
354 unlikely to change overall conclusions. First, exclosures reduced the amount of litter, although
355 on average there was still almost 2 cm of litter in exclosures. Since deeper litter can benefit
356 larger-seeded species (e.g., through reduced desiccation, Muscarella et al., 2013; Sayer, 2006), if
357 this difference biased seed and seedling survival at all, it likely would have caused us to
358 underestimate rather than overestimate the benefits of vertebrate exclusion. Likewise, additional
359 shading from litter on top of exclosure cages might have reduced the positive effects of exclusion
360 on seedling establishment, even though focal species are generally shade tolerant. It is possible
361 that shading could have increased mortality from phytopathogens favored by humid conditions
362 (Milici et al., 2020). Second, exclosures protected seedlings from physical damage, for example
363 from branch fall and trampling, that they would otherwise be subjected to (Clark & Clark, 1989)
364 which would overestimate the positive effects of exclosures on seedling establishment. Based on
365 an artificial seedling experiment at these sites (Quirós et al., in review), over a 1-year period
366 ~13% of seedlings in remnant forests and ~18% of seedlings in restored forests would be
367 expected to suffer physical damage though not necessarily fatal. These probabilities are unlikely
368 to explain the magnitude of observed differences between caged and exposed establishment.
369 Finally, the vertebrate exclosures could have also excluded some insect herbivores (e.g.,
370 lepidopterans laying eggs, large orthopterans), although they remained accessible to smaller
371 insect herbivores (e.g., we observed leaf-cutting ants and herbivory inside exclosures). As such,
372 some portion of the positive effects of exclosures may have been due to protection from insect
373 herbivory, which reduces seedling survival (see Kulikowski et al. 2022).

374 Together, our results suggest that at sites with relatively low seed rain, recruitment of
375 larger-seeded later-successional species could be further limited by high levels of seed predation,
376 although other abiotic or biotic factors besides vertebrate seed predation substantially limit
377 seedling establishment of most species in the first year. There have been recent suggestions for
378 managing herbivores in successional contexts to reduce effects on vegetation diversity and
379 abundance, for example by reintroducing carnivores (Huanca-Nuñez et al., 2023; Xu et al.,
380 2023). However, neither reintroducing predators nor excluding seed predators is likely to be
381 practical when (a) restoration sites are small and embedded within a fragmented landscape, and
382 (b) vertebrate seed predator assemblages are comprised of species with diverse sizes and life
383 histories. Direct seeding later-successional species has been suggested as a way to diversity
384 depauperate secondary forests (e.g. Bonilla-Moheno & Holl, 2010) and restoration plantings
385 (Sangsupan et al., 2018). Seed predation trials could help restoration practitioners to select tree
386 species that are most likely to survive predation for broadcast sowing efforts.

387 **Tables**

388

389 **Table 1.** Focal tree species added in exclosure experiments. Measurements are means (± 1 SD)
390 from a sample of >50 seeds per species.

391

Species (authority)	Family	Seed mass (g)	Seed width (mm)	Seeds per quadrat	Month collected	Removal survey period (weeks)
<i>Ocotea puberula</i> Nees	Lauraceae	1.1 \pm 0.2	10 \pm 1	7	Aug 2022	7
<i>Otoba novogranatensis</i> Moldenke	Myristicaceae	5.0 \pm 1.4	20 \pm 2	8	Apr 2022	8
<i>Pseudolmedia mollis</i> Standl.	Moraceae	5.7 \pm 1.4	21 \pm 2	11	Jun 2021	11
<i>Quercus benthamii</i> A. DC.	Fagaceae	10.1 \pm 2.6	25 \pm 2	7	Jul 2021	8

392

393 **Table 2.** Vertebrate seed predators detected removing seeds. We determined the status of each
 394 species as a potential seed disperser based on the literature. The relative activity index presented
 395 for restored (Rest.) forests and remnant (Rem) forests is based on the number of independent
 396 detections per 1000 trap nights across all sites of each habitat type.

397

Taxon	Common name	Possible seed disperser	Rest. RAI	Rem. RAI
Birds				
<i>Odontophorus gujanensis</i>	marbled wood-quail	No	5.1	105.0
<i>Tinamus major</i>	great tinamou	No	34.3	36.3
Mammals				
<i>Cuniculus paca</i>	Tepezcuintle	No	8.9	43.9
<i>Dasyprocta punctata</i>	Central American agouti	Yes	72.4	814.9
<i>Sciurus granatensis</i>	red-tailed squirrel	Yes	24.1	36.3
-----	unidentified mice/rats	Yes ^a	38.1	43.9
<i>Dicotyles tajacu</i>	collared peccary	No	0	7.6

398 ^a Note: To our knowledge, *Heteromys desmarestianus* is the only known scatterhoarding species
 399 of the 14 species of mice and rats documented in the region.

400 **Figures captions**

401 **Figure 1.** Map of study sites within Coto Brus, southern Costa Rica (8°45'34"N, 82°57'05.0"W).

402 Green circles represent restoration sites. Green circles with purple outlines are sites with both a
403 restored forest plot and a paired area of remnant forest.

404 **Figure 2.** Experimental design showing (a) plot-level design with one station distributed in each
405 quadrant of each 50 × 50 m restored forest plot and similarly distributed within remnant forest
406 patches (not pictured); and (b) station containing one seed addition quadrat with a 0.3-m tall
407 vertebrate enclosure (represented by a gray square) and two exposed seed addition quadrats
408 (green squares). At one station per plot, a camera trap was positioned to detect vertebrates
409 encountering experimental seeds in exposed quadrats.

410 **Figure 3.** Bipartite networks depicting vertebrate-seed encounters detected by camera trapping
411 of seed addition experiments in (a) remnant forest, and (b) restored forests. Note that each
412 encounter between a confirmed seed predator species and seed addition quadrat does not
413 necessarily represent an individual seed predation event. Species are ordered from left to right by
414 increasing mass (vertebrates not to scale). For full species names, see Table 1 (trees) and Table
415 S1 (vertebrates). Vertebrate silhouettes from PhyloPic (phylopic.org).

416 **Figure 4.** Percentage of (a) seeds remaining (i.e. not removed) 7-11 weeks post-addition and (b)
417 added seeds established as seedlings after 1 year, for all species, forest types, and enclosure
418 treatments. Points represent treatment means of plots (averaged over 4 stations) and lines
419 represent 1 SE. N = 6-8 restored forests, depending on response variable and species; n = 4
420 remnant forests for all species.

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435 **Disclosure statements**

436 **CONFLICT OF INTEREST**

437 No potential conflict of interest was reported by the authors.

438

439 **AUTHOR CONTRIBUTIONS**

440 FJ, KH, and RZ conceptualized the study. FJ and BR collected the data. FJ conducted analyses

441 and wrote the first draft. All authors reviewed and edited the manuscript.

442

443 **DATA AVAILABILITY STATEMENT**

444 The data that support the findings of this study are available in Dryad Digital Repository doi:

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1 **Supporting Information for:**

2 **Vertebrate seed predation can limit recruitment of later-successional species in tropical**
3 **forest restoration**

4
5 **Figure S1.** Example detections of vertebrate seed predators in baseline camera trapping

6 **Figure S2.** Litter depth in seed addition plots

7 **Table S1.** List of study sites

8 **Table S2.** Summary of linear mixed effects model comparing litter depth between enclosure
9 treatments and forest types

10 **Table S3.** Vertebrate species detected in baseline camera trapping

11 **Table S4.** Summary of binomial GLMM coefficients predicting the proportion of seeds
12 remaining after 7-11 weeks

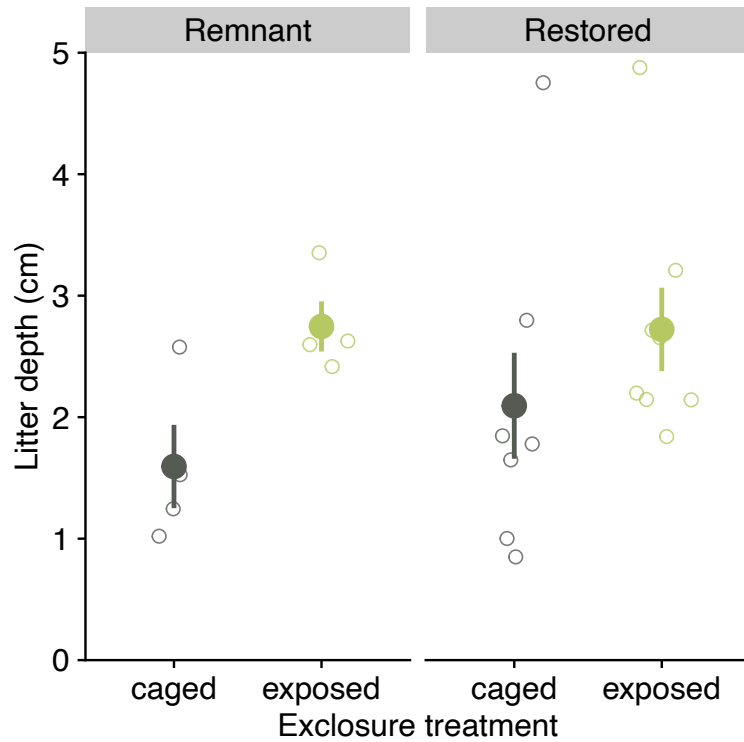
13 **Table S5.** Summary of binomial GLMM coefficients predicting the proportion of added seeds
14 established after one year

15 **Table S6.** Summary of binomial GLMM for *Quercus benthamii* seedling establishment

16



Figure S1. Camera trapping detected both granivorous birds and mammals at study sites: (a) *Dasyprocta punctata*, (b) *Odontophorus gujanensis*, and (c) *Dicotyles tajacu*.



20

21 **Figure S2.** Litter depth measured in seed addition quadrats in June 2022. Large points represent
 22 treatment-level means for each forest type and lines represent ± 1 SE. Smaller open circles
 23 represent plot-level means for each treatment. Litter depth was measured in June 2022 at five
 24 points within each seed addition quadrat and averaged by enclosure treatment \times plot. Exposed
 25 seed addition quadrats had significantly deeper leaf litter ($p < 0.001$), but there was no significant
 26 difference in depth between forest types ($p = 0.19$, Table S2).

27

28 **Table S1.** Study sites used in seed addition experiments. One site (HB) was lost to land use
 29 change in January 2022, so we set up additional seed addition plots at two additional restoration
 30 sites (GN and SC). Accordingly, sample sizes varied by species and stage of data collection
 31 (seed removal vs. seedling establishment). Additional information about individual sites is
 32 available from Holl et al. (2020).

Site code	Year planted	Paired remnant forest	Data availability			
			<i>Pseudolmedia & Quercus</i>		<i>Otoba & Ocotea</i>	
			Removal	Establishment	Tagging	Removal & Establishment
EC	2006	-	X	X	-	X
GN	2005	-	-	-	-	X
HB	2005	-	X	-	-	-
JG	2005	X	X	X	X	X
LL	2004	X	X	X	X	X
MM	2004	X	X	X	-	X
OM	2005	X	X	X	-	X
SC	2006	-	-	-	-	X
SG	2004	-	X	X	-	X
Total	9	4	7	6	2	8
sites						

34 **Table S2.** Summary of linear mixed effects model comparing litter depth between exclosure
 35 treatments and forest types. The model included station within site as a random intercept to
 36 account for grouping of seed addition quadrats within plots.

Coefficient	Estimate	95% CI¹	p-value
(Intercept)	1.6	0.99, 2.2	<0.001
Forest type: restored	0.59	-0.24, 1.2	0.19
Exclosure treatment: exposed	1.2	0.58, 1.7	<0.001
Interaction: restored * exposed	-0.47	-1.2, 0.23	0.2

¹CI = Confidence Interval

37

38 **Table S3.** Vertebrate species detected in baseline camera trapping (January- June 2021 and June
39 2022) in restored and remnant forests. Total survey effort was 1311 trap-nights (524 in remnant
40 forests, 787 in restored forests). Species are ranked by overall relative abundance index (RAI =
41 independent detections·100/trap-nights). Species subsequently detected interacting with
42 experimentally added seeds are in bold. Mammal species names follow the Mammal Diversity
43 Database (Burgin et al., 2018), and bird species names follow the American Ornithological
44 Society’s Checklist of North American Birds (Chesser et al., 2023).

Species	Relative abundance index	Total independent observations	Restored forest RAI	Remnant forest RAI
<i>Dasyprocta punctata</i>	369.2	484	72.4	814.9
<i>Leptotila cassinii</i>	126.6	166	91.5	179.4
<i>Didelphis marsupialis</i>	82.4	108	99.1	57.3
<i>Aramides cajaneus</i>	81.6	107	88.9	70.6
unidentified bird	72.5	95	55.9	97.3
<i>Dasypus novemcinctus</i>	58	76	63.5	49.6
<i>Nasua narica</i>	54.2	71	31.8	87.8
<i>Arremon aurantiirostris</i>	46.3	53	24.2	72.5
<i>Odontophorus gujanensis</i>	45	59	5.1	105
<i>Formicarius analis</i>	43.5	57	10.2	93.5
unidentified mouse/rat	40.4	53	38.1	43.9
unidentified species	38.1	50	29.2	51.5
<i>Philander opossum</i>	37.4	49	50.8	17.2
unidentified mammal	36.6	48	34.3	40.1
<i>Tinamus major</i>	35.1	46	34.3	36.3
<i>Sciurus granatensis</i>	29	38	24.1	36.3
<i>Catharus aurantiirostris</i>	28.4	34	16.4	43.9
<i>Geotrygon montana</i>	26.7	35	19.1	38.2
<i>Canis familiaris</i>	24.4	32	20.3	30.5
<i>Cuniculus paca</i>	22.9	30	8.9	43.9
unidentified bat	20.6	27	26.7	11.5
<i>Seiurus aurocapilla</i>	16	21	19.1	11.5
<i>Henicorhina leucosticta</i>	12.2	16	3.8	24.8
<i>Leopardus pardalis</i>	9.9	13	6.4	15.3

Species	Relative abundance index	Total independent observations	Restored forest RAI	Remnant forest RAI
<i>Marmosa mexicana</i>	9.9	13	11.4	7.6
<i>Momotus lessonii</i>	9.2	12	11.4	5.7
<i>Geothlypis formosa</i>	8.4	11	7.6	9.5
<i>Crypturellus soui</i>	7.6	10	6.4	9.5
<i>Eira barbara</i>	7.6	10	8.9	5.7
unidentified dove	7.6	10	7.6	7.6
unidentified passerine	7.6	10	2.5	15.3
<i>Gymnopithys bicolor</i>	6.9	9	6.4	7.6
<i>Catharus ustulatus</i>	6.1	8	5.1	7.6
<i>Tamandua mexicana</i>	5.3	7	3.8	7.6
<i>Eucometis penicillata</i>	4.6	6	6.4	1.9
<i>Myiothlypis fulvicauda</i>	4.6	6	7.6	0
<i>Opossum sp.</i>	4.6	6	6.4	1.9
<i>Cebus imitator</i>	3.8	5	5.1	1.9
<i>Procyon lotor</i>	3.8	5	6.4	0
<i>Turdus assimilis</i>	3.8	5	3.8	3.8
<i>Herpailurus yagouaroundi</i>	3.1	4	3.8	1.9
<i>Dicotyles tajacu</i>	3.1	4	0	7.6
<i>Basileuterus delatrii</i>	2.3	3	1.3	3.8
<i>Dendrocincla homochroa</i>	2.3	3	3.8	0
<i>Penelope purpurascens</i>	2.3	3	2.5	1.9
<i>Saltator maximus</i>	2.3	3	2.5	1.9
<i>Amazilia tzacatl</i>	1.5	2	2.5	0
<i>Attila spadiceus</i>	1.5	2	0	3.8
<i>Felis catus</i>	1.5	2	2.5	0
<i>Myrmeciza exsul</i>	1.5	2	0	3.8
<i>Rupornis magnirostris</i>	1.5	2	1.3	1.9
<i>Turdus grayi</i>	1.5	2	2.5	0
<i>Arremon brunneinucha</i>	0.8	1	0	1.9
<i>Arremon costaricensis</i>	0.8	1	0	1.9
<i>Buteo platypterus</i>	0.8	1	1.3	0
<i>Cardellina pusilla</i>	0.8	1	1.3	0
<i>Cathartes aura</i>	0.8	1	0	1.9
<i>Choloepus hoffmanni</i>	0.8	1	1.3	0
<i>Conepatus semistriatus</i>	0.8	1	1.3	0
<i>Manacus aurantiacus</i>	0.8	1	0	1.9
<i>Micrastur ruficollis</i>	0.8	1	1.3	0

Species	Relative abundance index	Total independent observations	Restored forest RAI	Remnant forest RAI
<i>Mniotilta varia</i>	0.8	1	1.3	0
<i>Phaethornis guy</i>	0.8	1	1.3	0
<i>Poliocrania exsul</i>	0.8	1	0	1.9
<i>Psarocolius decumanus</i>	0.8	1	1.3	0
<i>Sclerurus mexicanus</i>	0.8	1	1.3	0
<i>Setophaga petechia</i>	0.8	1	0	1.9
<i>Sylvilagus dicei</i>	0.8	1	1.3	0
unidentified possum	0.8	1	1.3	0

45

46 **Table S4.** Summary of binomial GLMM coefficients predicting the proportion of seeds
 47 remaining after 7-11 weeks. Coefficients are presented as log odds ratios. The reference levels
 48 for habitat type and enclosure treatment coefficients are remnant forest and caged treatments,
 49 respectively. The model includes tree species and station nested within site as random effects.

50
 51

Parameter	log(OR)^a	Std. error	z value	p-value
Intercept	4.38	0.69	6.35	<0.001
Habitat type: Restored	-0.87	0.30	-2.90	0.004
Enclosure: exposed	-4.03	0.28	-14.32	<0.001
Interaction: restored* exposed	0.58	0.32	1.82	0.07

^aOR = Odds Ratio

52

53 **Table S5.** Summary of binomial GLMM coefficients predicting the proportion of added seeds
 54 established after one year.

55

Parameter	log(OR)^a	Std. error	z value	p-value
Intercept	0.004	0.55	0.008	0.99
Habitat type: Remnant	-0.13	0.19	-0.70	0.48
Exclosure: exposed	-1.6	0.13	-15.76	<0.001
Interaction: Remnant * exposed	-0.44	0.21	-2.07	0.038

^aOR = Odds Ratio

56

57 **Table S6.** Summary of binomial GLMM for *Quercus benthamii* seedling establishment.

Parameter	log(OR)^a	Std. error	z value	p-value
(Intercept)	-0.89	0.25	-3.77	<0.001
Forest type: Remnant	0.18	0.28	0.66	0.51
Exclosure treatment: exposed	-1.20	0.30	-4.01	<0.001
Interaction: Remnant * exposed	-1.6	0.62	-2.54	0.01

^aOR = Odds Ratio

58

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