

1     **The value of trophic interactions for ecosystem function: dung**  
2             **beetle communities influence seed burial and seedling**  
3                     **recruitment in tropical forests**  
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## 24 Abstract

25 Anthropogenic activities are causing species extinctions, raising concerns about the  
26 consequences of changing biological communities for ecosystem functioning. To address  
27 this, we investigated how dung beetle communities influence seed burial and seedling  
28 recruitment in the Brazilian Amazon. First, we conducted a burial and retrieval experiment  
29 using seed mimics. We found dung beetle biomass had a stronger positive effect on the  
30 burial of large than small beads, suggesting that anthropogenic reductions in large-bodied  
31 beetles will have the greatest effect on the secondary dispersal of large-seeded plant  
32 species. Second, we established mesocosm experiments in which dung beetle communities  
33 buried *Myrciaria dubia* seeds to examine plant emergence and survival. Contrary to  
34 expectations, we found that beetle diversity and biomass negatively influenced seedling  
35 emergence, but positively affected the survival of seedlings that emerged. Finally, we  
36 conducted germination trials to establish the optimum burial depth of experimental seeds,  
37 revealing a negative relationship between burial depth and seedling emergence success.  
38 Our results provide novel evidence that seed burial by dung beetles may be detrimental for  
39 the emergence of some seed species. However, we also detected positive impacts of beetle  
40 activity on seedling recruitment, which are likely due to their influence on soil properties.  
41 Overall, this study provides new evidence that anthropogenic impacts on dung beetle  
42 communities could influence the structure of tropical forests, in particular their capacity to  
43 regenerate and continue to provide valuable functions and services.

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49 **Key words:** *plant recruitment; biodiversity-ecosystem functioning; soil; ecosystem processes;*  
50 *defaunation*

## 51 1. Introduction

52 Human activities over the past 500 years **have driven a dramatic decline in biodiversity** [1, 2].  
53 The loss of species is of concern for the maintenance of functioning ecosystems [3]. So too  
54 is the on-going decline in the abundances of individuals that remain. It is increasingly  
55 recognised that this erosion of biodiversity will lead to the breakdown of species interactions  
56 and a loss of associated ecosystem **functions and services** [3,4].

57 The geographic pattern of species loss is non-random [5], with tropical forests  
58 displaying the highest rates of declines in biodiversity [1], caused by unsustainable hunting  
59 in conjunction with habitat loss and modification [6-8]. Decreases in vertebrate populations  
60 within tropical forests are of particular concern because top-down trophic cascades can  
61 affect plants through changes in the abundance of frugivores, granivores and folivores [9].  
62 For example, in this edition, Bregman *et al.* (2016) [10] demonstrate that **landuse change**  
63 **negatively impacts primary seed dispersers, which could influence the long term**  
64 **regeneration of tropical forests**. However, most biodiversity-ecosystem function experiments  
65 focus on bottom-up processes governed by terrestrial plant communities, demonstrating that  
66 diversity is important for resource capture and ecosystem resilience [11-13]. We therefore  
67 have a poor understanding of **direct effects of diversity within higher trophic levels** or the  
68 indirect, cascading effects of biodiversity loss across trophic levels [but see 14]. There is  
69 mounting evidence that changes in forest vertebrate communities can lead to direct top  
70 down consequences for plant demography, community composition and diversity [15-22],  
71 with knock-on effects for forest services and resilience [23,24]. **However**, because the  
72 indirect, multitrophic consequences of changing mammal communities are rarely  
73 experimentally tested, we have limited understanding of the **ecosystem-wide** consequences  
74 anthropogenic impacts on tropical forests.

75 The secondary dispersal of seeds by dung beetles is an example of an indirect trophic  
76 interaction between vertebrates and plants, which likely impacts seedling recruitment [25].  
77 Seeds within mammalian dung are frequently relocated to beneath the soil surface because

78 dung beetles move and bury faeces for feeding and nesting purposes [26]. This can benefit  
79 seeds by placing them in a more suitable microsite for germination [27,28], avoidance of  
80 density dependent competition [29] and through escape from predation [27,30]. However, if  
81 seeds are placed too deep, burial by beetles can result in seed mortality [27,30,31];  
82 suggesting there exists a species specific optimal seed burial depth.

83         According to the International Union for Conservation of Nature (IUCN) Redlist,  
84 approximately 20% of mammals globally are considered vulnerable, endangered or critically  
85 endangered, with the highest numbers of declining species occurring within tropical forests  
86 [1,32]. Since dung beetles depend on mammalian faeces, this pervasive decline in mammal  
87 populations and biomass can cascade through ecosystems, reducing dung beetle body size  
88 and species richness [33]. At the same time, positive links have been established between  
89 dung beetle taxonomic and functional diversity and the burial and dispersion of seeds [34-  
90 36], and large-bodied beetles have a disproportionately important role in seed and dung burial  
91 [35,37]. Therefore, it is likely that top-down, cascading declines in dung beetle diversity and  
92 changes to community structure will impact the germination and establishment of  
93 secondarily dispersed seeds, with potential implications for forest regeneration and  
94 ecosystem resilience to environmental change. However, to our knowledge this has not yet  
95 been experimentally tested.

96         Therefore, in this study we investigate how dung beetle community composition  
97 (biomass, taxonomic and functional diversity) influences the burial, germination and survival  
98 of seeds in a tropical forest, and explore whether the presence of dung, and the burial  
99 depths of beetle dispersed seeds, influences seedling emergence. To do this, we carried out  
100 three sets of experiments, each testing a different hypothesis/prediction. First, because large  
101 bodied dung beetles are instrumental in the dispersal of large seeds [35], we predicted that  
102 large seeded species are more sensitive to reductions in dung beetle biomass and diversity  
103 than smaller seeds. To test this, we carried out an experiment in which beads (seed mimics)  
104 were buried by naturally assembled beetle communities. Second, because dung beetle  
105 diversity has been shown to positively influence the likelihood of bead burial and dispersion

106 throughout the soil profile [36], we used real seeds to test the hypothesis that beetle  
107 functional diversity and species richness positively influences seedling emergence and  
108 survival. This is because: (1) burial decreases seed predation [27,30]; and (2) the greater the  
109 dispersal distance of seeds from a central point, the higher the likelihood that each individual  
110 seed will be placed in its optimal species-specific microsite for recruitment. Finally,  
111 experiments were complemented by germination trials to establish the optimal burial depth  
112 for experimental seeds and allow interpretation of any patterns observed between beetle  
113 activity and seedling emergence/survival. We predicted that highest germination would occur  
114 in microsites near the surface (from 1cm to 4cm), deep enough to reduce predation, yet  
115 shallow enough to avoid soil depth preventing emergence following germination (c.f.  
116 [27,28]).

117

## 118 2. Methods

119

### 120 (a) Using seed mimics to examine burial

121 Experiments were conducted in the 17 000-km<sup>2</sup> Jari Florestal landholding, located in the  
122 State of Pará, north-eastern Brazilian Amazon (0°53S, 52°36W). Unlike many regions of the  
123 Amazon, the predominant anthropogenic disturbance in this area is forest clearance for  
124 *Eucalyptus* plantations rather than clearance for pasture land and cattle ranching. As such  
125 the region consists of a matrix of *Eucalyptus* plantations, regenerating secondary forests,  
126 and large areas of largely undisturbed primary *terra firme* rainforest that do not provide  
127 viable habitat for any domesticated ungulates. Within this landscape, experiments were  
128 established in three primary forests sites (see [36] for full site description).

129         During July and August 2012 we established a grid of thirty mesocosms, separated  
130 by 100m, at each experimental site (n = 90 in total). Mesocosms were created by burying  
131 nylon netting 10cm vertically into the soil in a 50cm x 50cm square (Appendix S1) and were

132 baited with 100g mixture of 50:50 human and pig dung containing 20 plastic seed mimics  
133 (beads) of 4 different sizes: 2 large (20mm diameter, 4.12g), 6 medium (10mm diameter,  
134 0.50g), 6 small (5mm diameter, 0.09g), and 6 very small (2mm diameter, 0.06g). The dung  
135 and beads were placed on the floor within the plots, protected from rain by a plastic cover  
136 and left open for beetle colonisation for between 12 and 24 hours. After baiting, mesocosms  
137 were closed using pegs to hold the netting together, ensuring beetles could not leave and  
138 preventing further colonisation by beetles that had not buried the dung. Each mesocosm  
139 also contained an internal, non-baited pit-fall trap (13.5cm width, 9cm depth), buried flush  
140 with the ground surface and filled with a salt-water solution. Internal traps were opened when  
141 mesocosms were closed to capture the beetle community that had buried the dung and  
142 beads following emergence from the soil. After closure, mesocosms were left for 7-14 days  
143 before the soil beneath the dung was destructively sampled to a depth of 50cm in search of  
144 the beads buried by beetles. This difference in time that mesocosms were left before  
145 sampling had no impact on the numbers of beads buried [36]. Internal pitfall traps were  
146 removed and beetles oven dried for laboratory processing (see [36] for detailed experimental  
147 design and rationale).

148

## 149 (b) Evaluating seedling emergence and survival

150 Following the procedure described above, in February 2014, we created a further 90  
151 mesocosms in one of the sites (0°38`46.418"S, 52°34`11.125"W) with clay textured Oxisols  
152 (mean clay content  $\pm$  SE: 67.3  $\pm$  1.5%, silt: 14.4  $\pm$  1%, sand: 14.1  $\pm$  1.1%). This site was  
153 selected because previous work demonstrated that dung beetle diversity strongly influenced  
154 the dispersal of seed mimics in this site compared with other sites in the region [36]. We  
155 therefore designed this experiment to investigate if the observed patterns between dung  
156 beetle diversity and the burial of seed mimics influence the success of real seeds. Each  
157 mesocosm was baited with 100g mixture of 50:50 human and pig dung containing two seeds

158 each of five animal-dispersed, Amazonian fruit species: *Genipa americana*, *Malpighia*  
159 *emarginata*, *Myrciaria dubia*, *Psidium guajava* and *Rubus chamaemorus*.

160           Dung and seeds were placed on the forest floor at the centre of the mesocosms  
161 between 07:00 and 09:00, protected from rain by a plastic cover. To enhance variation in the  
162 diversity of dung beetle communities, we randomly assigned mesocosms to one of three  
163 experimental treatments (n = 30 in each): control: baited and closed immediately, preventing  
164 any beetles from accessing dung and seeds; partial exclusion treatment: a 50cm x 50cm  
165 wire cage placed over the dung and seeds (mesh size 15mm x 8mm) within mesocosms;  
166 open treatment: baited and left open for colonisation by all beetles. This prevented the  
167 largest beetles from entering plots and created a greater spread in diversity between  
168 mesocosms, while maintaining naturally assembled communities (Appendix S2 for  
169 treatment effects on dung beetle communities). During the establishment of mesocosms,  
170 nine were baited each day for 10 days (n = 3 per treatment, per day). The partial exclusion  
171 and open treatments were left for 24 hours following baiting before closure.

172           Internal pitfall traps were opened when mesocosms were closed to capture the  
173 beetle community that had buried dung and seeds following emergence from the soil.  
174 Mesocosms were left closed for two weeks, during which time internal pitfall traps were  
175 emptied of beetles and refilled with saltwater once. After two weeks, we removed the pitfall  
176 traps and nylon netting covering mesocosms. The leaf litter and exposed soil was inspected  
177 to recover any beetles that remained within the mesocosms but hadn't fallen into the pitfall  
178 traps. All beetles recovered from within the mesocosms were dried and stored for laboratory  
179 processing. After baiting, mesocosms were monitored weekly for 18 weeks to assess  
180 emergence and survival of seedlings.

181

## 182 (c) Germination trials

183 To facilitate the interpretation of any patterns observed from the seed emergence and  
184 survival experiments in 2014, we created nine plots in the field to assess how burial depth  
185 and the presence of dung influenced emergence and survival of experimental seedlings. In  
186 each 120cm x 200cm plot we planted seeds at 10 different depths (n = 40 per species; n =  
187 200 seeds per plot): above the leaf litter, below the leaf litter, 1cm, 2cm, 3cm, 5cm, 7cm,  
188 10cm, 15cm and 20cm. At each depth, seeds were either planted alone or in the centre of a  
189 1g ball of dung (n = 2 per treatment, per depth). Plots were divided into 10cm<sup>2</sup> sections,  
190 seeds were assigned a depth x treatment (dung or alone) and placed randomly within the  
191 plots (n = 200 seeds x 9 plots). Following planting, plots were monitored weekly for 18  
192 weeks to assess the emergence and survival of seedlings.

193 Fifty-seven per cent of *M. dubia* seeds emerged from within mesocosms and 18%  
194 from within germination plots, compared to an emergence success of less than 10% and 5%  
195 from mesocosms and germination plots respectively for the other four species. Therefore,  
196 we focus results on only *M. dubia* (similar in dimensions to the medium bead used in burial  
197 trials: bead weight = 0.5g, width = 10mm, length = 10mm; *M. dubia* mean weight = 0.45g ±  
198 0.03g, mean width = 10.68mm ± 0.26mm, mean length = 13.76g ± 0.26g, calculated from 15  
199 seeds) because emergence of the other species was too low to allow analyses (Appendix S3  
200 for further explanation for exclusion of seed species). *M. dubia* (HBK) McVaugh, is a small,  
201 dicotyledonous tree, belonging to the *Myrtaceae* family that produces spherical fruits 2-5cm  
202 in diameter, each containing 2 seeds [38]. It is widely distributed across the north-eastern  
203 Brazilian Amazon [39].

204



#### 205 (d) Dung beetle traits and diversity metrics

206 We identified beetles to species using a reference collection at the Universidade Federal de  
207 Lavras, Brazil, and identification keys developed by T. A. Gardner and F. Z. Vaz-de-Mello.  
208 To calculate functional diversity, we used species median values of four continuous  
209 morphological traits: biomass (measured using a Shimatzu AY220 balance), biomass  
210 adjusted pronotum volume, biomass adjusted front leg area, back: front leg length (each  
211 measured using a Leica M250 microscope and Life Measurement software; Appendix S4);  
212 as well as three behavioural traits: nesting strategy (tunneller, roller, dweller [26]), diurnal  
213 activity (diurnal, nocturnal, crepuscular, or generalist) and diet (coprophagus or generalist).  
214 Categorical trait information was gathered from [40] and [41]. These seven traits were  
215 selected because they have been linked to dung beetle mediated seed dispersal [36]  
216 (Appendix S5 for details of the dung beetle communities and trait values).

217 We calculated species richness, total biomass, functional richness and the  
218 community weighted means (CWM) of the continuous traits (biomass, biomass adjusted  
219 pronotum volume, biomass adjusted front leg area, back: front leg length) for all mesocomms  
220 that contained beetles. Functional richness, is a multidimensional measure of the range of  
221 traits in a biological community [42] and was calculated using median biomass, biomass  
222 adjusted pronotum volume, biomass adjusted front leg area, back: front leg length, nesting  
223 strategy, diurnal activity. Community-weighted means describe the mean value of each trait  
224 in the communities, weighted by the relative abundances of the species carrying that trait  
225 [43]. Functional richness and CWM traits were calculated using the “FD” package in R 3.0.2  
226 [44,45].

227

#### 228 (e) Statistical analyses

229 Analyses were carried out in R version 3.0.2 [45]. Our first hypothesis was that large seeds  
230 are more sensitive to reductions in dung beetle biomass and diversity than smaller seeds. To

231 test this we used generalised linear mixed effects models (glmm) from the “lme4” package  
232 [46] to investigate if bead size, beetle community metric and the interaction between the two  
233 factors affected probability of bead buried (2012 experiment). Each community metric was  
234 included in a separate model and mesocosm was nested within site as random factors. Our  
235 second hypothesis was that dung beetle diversity positively influences the emergence and  
236 survival of real seeds. We used linear models (lm) to investigate if treatment (open or partial  
237 exclusion) succeeded in enhancing the variety in beetle community metrics across  
238 mesocosms (2014 experiment, Appendix S2). We then used glmms to assess how beetle  
239 community metrics within mesocosms influenced the probability of seed emergence and  
240 survival until the end of the 18-week experimental period. Mesocosm was included as a  
241 random factor. Our final goal was to assess the optimal burial depth of *M. dubia* seeds and  
242 to investigate if the presence of dung influences seedling emergence or survival. Here we  
243 used glmms to ascertain if burial depth, the presence of dung and the interaction between  
244 the two factors influenced probability that seeds emergence from the soil and subsequently  
245 survived until the end of the 18-week monitoring period. We then used glmms to investigate  
246 if the week that seedlings emerged influenced the likelihood that they survived until the end  
247 of the experimental period to ensure that any observed correlations between burial depth  
248 and seedling survival were not an artefact of the seedlings having emerged at different  
249 times. Germination plot was a random factor in lmers and glmms.

250         Within glmm models assessing the likelihood of bead burial, beads were assigned a  
251 1 if they were buried and a 0 if they remained on the soil surface; in seed emergence  
252 models, seeds were assigned a 1 if they emerged from the soil surface and a 0 if they did  
253 not; in models assessing the likelihood of survival, seedlings that emerged where assigned a  
254 1 if they survived until the end of the monitoring period and a 0 if they did not. As such a  
255 binary error distribution with a logit link function was specified for all glmms. All community  
256 metrics were log<sub>10</sub>-transformed to ensure models satisfied assumptions of normality. Models  
257 were created using all fixed terms and interactions, we then used a top-down approach to  
258 arrive at the best descriptive model [47] in which only significant terms ( $P < 0.05$ ) remained.

259 Chi-squared likelihood ratio tests (LRT) were used within the “drop1” function in R for glmm  
260 models and anovas for lm models to assess the loss of explanatory power following removal  
261 of an interaction or a single term predictor.

262

### 263 3. Results

264

#### 265 (a) Using seed mimics to examine burial

266 Bead size had a highly significant impact on the likelihood that dung beetles buried beads  
267 (LRT = 398.98, Df = 3,  $P < 0.0001$ ) and significantly affected the depth at which they were  
268 placed within the soil (LRT = 325.91, df = 3,  $P < 0.0001$ ). Both the proportion of beads buried  
269 and burial depth decreased with increasing bead size (Appendix S6). Dung beetle total  
270 biomass and CWM back: front leg lengths were the only community metrics that significantly  
271 affected probability of bead burial. Biomass had a consistent positive effect on the likelihood  
272 that beads of all sizes were buried (LRT = 4.53, df = 3,  $P = 0.033$ ). However, the effect was  
273 stronger for the burial of medium sized beads: probability of burial increased from around  
274 20% at the lowest biomass values to around 70% at the highest values for medium beads,  
275 compared to an increase from 70% to 90% for very small beads and a 60% to 80% increase  
276 for small beads (Fig. 1 (a)). There was a significant interaction between CWM back: front leg  
277 length and bead size (LRT = 9.23, df = 3,  $P = 0.026$ ). An increase in CWM back: front leg  
278 length had a negative effect on the likelihood that small and very small beads were buried (a  
279 reduction of 80% to 55% and 90% to 65%, respectively, but did not affect the probability that  
280 medium beads were buried (Fig. 1 (b)). The effect of beetle community metrics on the  
281 likelihood of burial of the large beads could not be assessed because too few were buried  
282 (<10%) to allow model testing.

## 283 (b) Evaluating seedling emergence and survival

284 Functional richness, species richness and total biomass had a significant negative effect on  
285 the likelihood of *M. dubia* emergence. Eighty per cent of seeds emerged from mesocosms  
286 displaying the lowest values for functional richness, species richness and total biomass,  
287 compared to around 20% emergence from mesocosm displaying the highest values for  
288 functional richness, species richness and total biomass. Community weighted mean  
289 biomass, pronotum volume, front leg area and back: front leg length had no significant effect  
290 on emergence success (Table 1; Fig. 2 (a) – (c)).

291 In contrast, CWM back: front leg length, total biomass and species richness had a  
292 significant positive effect on the likelihood that emerged seedlings survived until the end of  
293 the 18-week monitoring period (Fig. 2 (d) – (f)). The strongest predictor of seedling survival  
294 was CWM back: front leg length (Table 1): 0% of seedlings buried by beetle communities  
295 displaying the lowest CWM back: front leg length values survived until the end of the  
296 monitoring period, whereas 100% of seedlings within mesocosms with the highest values  
297 were alive at the end of the experiment. Functional richness, CWM biomass, CWM front leg  
298 area and CWM pronotum volume had no effect on seedling survival (Table 1), nor did the  
299 week that seedlings emerged from the soil surface (LRT = 1.19, d.f. = 1,  $p = 0.275$ ).

300

## 301 (c) Germination trials

302 Burial depth was the only factor that significantly influenced the likelihood of emergence  
303 (LRT = 69.4, D.f. = 9,  $P < 0.0001$ ); the presence of dung had no significant effect. Seeds that  
304 were buried below the soil surface were less likely to emerge as seedlings than those placed  
305 above or below the leaf litter: there was a 44.4% and 52.8% emergence rate for seeds  
306 above and below the litter respectively, compared to between 19.4% and 5.6% for seeds  
307 buried at 1cm and 20cm respectively (Fig. 3). No factor or interaction had a significant effect  
308 on the probability of seedling survival. Emergence week had no effect on the probability that

309 seedlings survived to the end the monitoring period ( $LRT = 2.8$ ,  $df = 1$ ,  $P = 0.0921$ ). No  
310 seeds emerged from mesocosms after week 16 or from germination plots later than week 14  
311 (Appendix S7). As such, we are confident that all emergence events were captured during  
312 the monitoring period.

313

## 314 4. Discussion

315 In this study we investigated the consequences of changes in dung beetle community  
316 composition (biomass, taxonomic and functional diversity) for secondary seed dispersal and  
317 the emergence and survival of tropical seedlings. We found a stronger positive effect of  
318 beetle biomass on the likelihood of burial for medium sized beads compared to smaller  
319 beads, suggesting that anthropogenic driven reductions large-bodied dung beetles [48] will  
320 have the greatest relative effect on the secondary dispersal of large-seeded plant species.  
321 Furthermore, we found a negative relationship between dung beetle species richness,  
322 functional richness and biomass, and the likelihood that seedlings emerged from the soil  
323 surface. These results suggest that secondary seed dispersal by dung beetles could inhibit,  
324 rather than promote the emergence of some tropical species. Conversely, we found that  
325 seedling survival was positively influenced by beetle species richness, biomass and the  
326 CWM of back: front leg length. It is worth noting here the possibility that unmeasured  
327 microsite variation could be driving or interacting with some of the reported significant  
328 correlations. Nevertheless, these results provide new evidence that changes in the richness  
329 and composition of dung beetle communities could impact seedling recruitment in tropical  
330 forests (here defined as seed germination and the short term survival of seedlings until the  
331 end of our experimental period), potentially affecting future vegetation composition. Since  
332 dung beetle communities are inherently linked to mammalian dung, our results suggest that  
333 changes in mammal communities, such as the loss of large bodied primates [49], caused by  
334 anthropogenic pressures could impact tropical forest regeneration through top-down trophic  
335 cascades involving below-ground fauna.

336           The relative effect of dung beetle biomass on the probability of seed mimic burial was  
337 strongest for medium beads. Previous work has demonstrated that large beetles are  
338 functionally more efficient in the removal of dung and seeds compared to smaller species  
339 and that they are instrumental in the movement of large seeds [35,37]. It is likely, therefore,  
340 that the stonger relationship we observed between biomass and medium bead burial,  
341 compared to small bead burial, is caused by the presence of large beetles in high biomass  
342 communities driving the burial of large seeds. This is important because large-bodied dung  
343 beetle species are known to be more prone to extinction and decline than smaller bodied  
344 speices [33,48]. These results therefore support our first hypothesis that changes in dung  
345 beetle community structure are likely to differentially affect the secondary dispersal of seeds  
346 depending on their size. This adds weight to suggestions that large seeded trees are most  
347 affected by the extinction of animal-plant interactions as a result of human pressures (c.f.  
348 [16]).

349           Secondary dispersal by dung beetles has been demonstrated on a number of  
350 occasions to be beneficial to buried seeds [27,28,50]. However, contrary to our predictions,  
351 we show that functional richness, species richness and total biomass of beetle communities  
352 are negatively correlated to the emergence success of seedlings, suggesting that dung  
353 beetle activity may be detrimental for some species. Previous beetle-mediated seed  
354 dispersal experiments in tropical forests demonstrate that burial depths of between 1cm and  
355 4cm result in increased germination success compared to seeds that remained on the soil  
356 surface or were buried to deeper depths [27,38]. We show that *M. dubia* emergence rates  
357 within germination plots were highest when seeds were placed either above or below the  
358 leaf litter, but immediately reduced by over 50% when seeds were buried within the soil  
359 profile. Therefore, it is likely that the negative relationship between beetle community  
360 attributes and emergence of *M. dubia* seeds is a consequence of higher biomass and  
361 diversity, resulting in higher rates of seed burial [c.f. 36] and net disadvantages to the fitness  
362 of this species. Furthermore, results from our bead burial and retrieval experiments  
363 demonstrate that small seeded species are buried deeper than larger seeds; given that only

364 large seeds have been shown to germinate from burial depths of 10cm or more [27], we also  
365 expect negative consequences of beetle activity for many smaller seeded species. It is  
366 therefore possible that seed burial by intact dung beetle communities may reduce the  
367 prevalence of small-seeded species, thus reducing competition experienced by larger seeds.

368 Seed predator escape is a key mechanism underpinning the increased germination  
369 success observed in seeds secondarily dispersed by dung beetles in tropical forests [27,28].  
370 We found no evidence for this process in this investigation. However, our experiments were  
371 carried out in a primary forest with relatively low hunting pressure, and a full complement of  
372 large mammals [49]. More heavily disturbed forests differ in that they can harbour large  
373 populations of seed predators and hence higher seed predation pressure [51,52]. If seed  
374 predation was sufficiently high, burial by beetles could impart net benefits rather than  
375 disadvantages to *M. dubia*. It is possible, therefore, that seed predator escape may be  
376 relatively more important in more heavily disturbed forests, and that this result  
377 underestimates the importance of dung beetle mediated seed burial in an increasing human-  
378 modified world. Furthermore, although *M. dubia* is a fleshy fruit dispersed by a wide range of  
379 forest vertebrates [53], it is also a riparian species and its seeds can be dispersed by water,  
380 which may explain its preference for being close to the soil surface. While these results  
381 highlight some interesting linkages across trophic levels, finding general patterns will require  
382 additional work using a broader range of plant species, and repeating the experiments in  
383 forests with differing levels of predation pressure.

384 We found a positive relationship between seedling survival and dung beetle total  
385 biomass, species richness and CWM back: front leg length. Results from our seed  
386 germination trials demonstrated that the presence of dung did not influence the survival of *M.*  
387 *dubia* seedlings. This suggests that the mechanisms driving increased seedling survival  
388 extend beyond simply the presence of dung surrounding seeds. There are myriad processes  
389 acting both above-ground and below-ground that influence whether a seedling lives or dies  
390 following germination [e.g. 54]. A plausible way in which beetles could influence seedling  
391 survival is through simultaneous effects on both soil resource (nutrients and water)

392 availability and the soil physical environment. Due to their small root system, recently  
393 emerged seedlings are reliant on the nutrient and water availability in their immediate  
394 surroundings [55]. Bang *et al.* (2005) [56] demonstrated that dung beetle activity had a  
395 positive effect on soil permeability in surface layers, which is positively associated to air and  
396 water movement, and greater soil pore space [57]. These soil characteristics could facilitate  
397 greater root and shoot growth. Furthermore, nitrogen is a mineral element that can become  
398 insufficient in seed reserves [58]. Dung beetles have been shown to positively influence  
399 rates of nitrogen (N) mineralisation and concentrations of inorganic N in soil, as well as the  
400 availability of other limiting nutrients such as phosphorus (P) and potassium (K) [59,60].  
401 Therefore, dung burial by beetles could concurrently alter soil biogeochemistry and physical  
402 structure so as to increase the availability of limiting nutrients, whilst facilitating the ease with  
403 which roots can access these resources. It is important to note, however, that past studies  
404 investigating dung beetle impacts on soil nutrient availability and physical structure have  
405 been exclusively carried out in grassland and heathlands, which differ in their soil properties  
406 to tropical forests [61,62]; hence, making inferences about the role of dung beetles in  
407 modifying tropical soils based on evidence from temperate systems is problematic. Future  
408 investigations are therefore needed to elucidate the small scale impact of dung beetles on  
409 tropical soils, where highly heterogeneous distributions in soil nutrients are important factors  
410 structuring plant communities [63].

411         The only dung beetle trait that was positively associated with seedling survival was  
412 the CWM of back: front leg length. The abundance of dwelling dung beetle species, which do  
413 not bury dung or seeds but feed and nest within the dung [26], within these communities was  
414 positively related to CWM back: front leg length (supplementary material, appendix S8); as  
415 such, an increase in the ratio between back and front leg lengths indicates an increase in the  
416 number of dwellers present. The burial of beads similar in size to *M. dubia* was low  
417 compared to smaller beads and was always unaffected by leg length. Therefore, it is unlikely  
418 that the relationship we found between seedling survival and CWM back: front leg length is a  
419 consequence of dwellers decreasing the likelihood that seeds are buried. Instead it is likely



420 that processing of dung on the soil surface increases with an increase in the abundance of  
421 dwelling species. This could give rise to similar processes described above, altering soil  
422 nutrient availability and physical environment in a way that provides benefits to seedling  
423 growth and survival. We are not aware of any studies to date that have investigated how the  
424 morphological traits of dung beetles influence soil properties and plant growth.

425

## 426 5. Conclusions

427 This investigation aimed to better understand the role of dung beetle communities in  
428 maintaining ecosystem functioning in tropical forests, through studying their impact on  
429 secondary seed dispersal and seedling establishment. Conceptual frameworks predict that  
430 large seeded species are most at risk from the negative impacts of defaunation due to the  
431 extirpation of their large-bodied primary dispersers [23,24]. Here, we demonstrate that large  
432 seeds may also be differentially vulnerable to the loss of their secondary dispersers through  
433 anthropogenic driven reductions in large bodied dung beetles [33,48]. However, our results  
434 also suggest that decreases in dung beetle biomass and diversity could result in net  
435 disadvantages to some small-seeded species because seed burial can negatively impact  
436 their emergence success. Furthermore, we present novel experimental evidence suggesting  
437 that dung beetle activity could modify conditions within the soil and/or dung in a way that  
438 promotes seedling survival. Combined, these results demonstrate the complexities of  
439 predicting how anthropogenic driven changes biological communities can cause top-down  
440 cascading effects on ecosystem functioning; point to new avenues for future experimental  
441 work into the mechanisms driving plant responses to shifts in the community composition of  
442 their secondary dispersers, through alteration of the soil environment; and demonstrate ways  
443 in which dung beetle activity could impact forest regeneration and future forest composition.  
444 We therefore provide further evidence of the value of biodiversity for the maintenance of  
445 ecosystem functions and self-sustaining natural systems.

446

447 **Ethics:** Sampling did not involve any endangered species and permission to collect  
448 zoological material was granted to JL. By the Instituto Brasileiro do Meio Ambiente dos  
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450 **Data accessibility:** Data can be accessed through Dryad (doi:10.5061/dryad.d20g3)

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463

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631 **Table 1.** Generalised linear mixed effects model outputs to assess the influence of dung beetle  
 632 community attributes on the probability of seed emergence (left section) and seedling survival until the  
 633 end of the 18-week experimental period (right section). Dung beetle community attributes that  
 634 significantly affected emergence or survival ( $P < 0.005$ ) are highlighted in bold.

635

glmm(seed emergence ~ beetle community)	LRT	df	<i>P</i>	glmm(seedling survival ~ beetle community)	LRT	df	<i>P</i>
<b>Functional richness</b>	<b>6.3</b>	<b>1</b>	<b>0.0124</b>	<b>CWM back: front leg length</b>	<b>8.4</b>	<b>1</b>	<b>0.0038</b>
<b>Total biomass</b>	<b>5.7</b>	<b>1</b>	<b>0.017</b>	<b>Total biomass</b>	<b>6.5</b>	<b>1</b>	<b>0.0107</b>
<b>Species richness</b>	<b>4.6</b>	<b>1</b>	<b>0.0326</b>	<b>Species richness</b>	<b>3.9</b>	<b>1</b>	<b>0.0495</b>
CWM biomass	0.3	1	0.6119	CWM front leg area	1.8	1	0.18
CWM pronotum volume	0.1	1	0.7924	CWM biomass	1.3	1	0.2598
CWM front leg area	0.1	1	0.7416	CWM pronotum volume	0.9	1	0.3373
CWM back: leg length	0	1	0.9733	Functional richness	0.7	1	0.3994

636

637

638 **Figure 1.** Effects of dung beetle total biomass (a) and CWM back: front leg length (b) on the probability  
 639 of seed mimic burial. Very small beads (left panels), small beads (middle panels) and medium beads  
 640 (right panels). Significance determined by generalised linear mixed effects models. Predicted values  
 641 (solid black lines) ± SE (ribbons) are displayed along with individual seeds (black points), which were  
 642 either buried (1) or remained on the soil surface (0).

643



644 **Figure 2.** Significant negative effect of dung beetle functional richness (a), total biomass (b) and species  
645 richness (c) on the probability of seed emergence (top panels) and the significant positive effect of  
646 community weighted mean (CWM) back: front leg length (d), total biomass (e), and species richness  
647 (e) on the likelihood that emerged seedlings survived until the end of the 18-week experimental period  
648 (bottom panels). Significance was determined by generalised linear mixed effects models. Predicted  
649 values (solid black lines)  $\pm$  SE (ribbons) are displayed along with individual seeds (black points, jittered  
650 to avoid overlap), which either emerged (1) or did not emerge (0); and survived (1) or died after  
651 emergence (0).

652

653 **Figure 3.** Percentage of *M. dubia* that emerged from the soil surface after being experimentally planted  
654 to ten different depths,  $n = 36$  at each depth; left panel) and percentage of emerged *M. dubia* seedlings  
655 at each burial depth that survived until the end of the 18-week experimental period (right panel). The  
656 soil surface is shown with a horizontal dashed line.

657

658