The value of trophic interactions for ecosystem function: dung beetle communities influence seed burial and seedling recruitment in tropical forests

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Abstract

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

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

1. Introduction

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

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

The secondary dispersal of seeds by dung beetles is an example of an indirect tropic interaction between vertebrates and plants, which likely impacts seedling recruitment [25]. Seeds within mammalian dung are frequently relocated to beneath the soil surface because dung beetles move and bury faeces for feeding and nesting purposes [26]. This can benefit seeds by placing them in a more suitable microsite for germination [27,28], avoidance of density dependent competition [29] and through escape from predation [27,30]. However, if seeds are placed too deep, burial by beetles can result in seed mortality [27,30,31]; suggesting there exists a species specific optimal seed burial depth.

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

Therefore, in this study we investigate how dung beetle community composition (biomass, taxonomic and functional diversity) influences the burial, germination and survival of seeds in a tropical forest, and explore whether the presence of dung, and the burial depths of beetle dispersed seeds, influences seedling emergence. To do this, we carried out three sets of experiments, each testing a different hypothesis/prediction. First, because large bodied dung beetles are instrumental in the dispersal of large seeds [35], we predicted that large seeded species are more sensitive to reductions in dung beetle biomass and diversity than smaller seeds. To test this, we carried out an experiment in which beads (seed mimics) were buried by naturally assembled beetle communities. Second, because dung beetle diversity has been shown to positively influence the likelihood of bead burial and dispersion throughout the soil profile [36], we used real seeds to test the hypothesis that beetle functional diversity and species richness positively influences seedling emergence and survival. This is because: (1) burial decreases seed predation [27,30]; and (2) the greater the dispersal distance of seeds from a central point, the higher the likelihood that each individual seed will be placed in its optimal species-specific microsite for recruitment. Finally, experiments were complemented by germination trials to establish the optimal burial depth for experimental seeds and allow interpretation of any patterns observed between beetle activity and seedling emergence/survival. We predicted that highest germination would occur in microsites near the surface (from 1cm to 4cm), deep enough to reduce predation, yet shallow enough to avoid soil depth preventing emergence following germination (c.f. [27,28]).

2. Methods

(a) Using seed mimics to examine burial

Experiments were conducted in the 17 000-km^2 Jari Florestal landholding, located in the State of Pará, north-eastern Brazilian Amazon (0º53S, 52º36W). Unlike many regions of the Amazon, the predominant anthropogenic disturbance in this area is forest clearance for *Eucalyptus* plantations rather than clearance for pasture land and cattle ranching. As such the region consists of a matrix of *Eucalyptus* plantations, regenerating secondary forests, and large areas of largely undisturbed primary *terra firme* rainforest that do not provide viable habitat for any domesticated ungulates. Within this landscape, experiments were established in three primary forests sites (see [36] for full site description).

During July and August 2012 we established a grid of thirty mesocosms, separated by 100m, at each experimental site (n = 90 in total). Mesocosms were created by burying nylon netting 10cm vertically into the soil in a 50cm x 50cm square (Appendix S1) and were baited with 100g mixture of 50:50 human and pig dung containing 20 plastic seed mimics (beads) of 4 different sizes: 2 large (20mm diameter, 4.12g), 6 medium (10mm diameter, 0.50g), 6 small (5mm diameter, 0.09g), and 6 very small (2mm diameter, 0.06g). The dung and beads were placed on the floor within the plots, protected from rain by a plastic cover and left open for beetle colonistation for between 12 and 24 hours. After baiting, mesocosms were closed using pegs to hold the netting together, ensuring beetles could not leave and preventing further colonisation by beetles that had not buried the dung. Each mesocosm also contained an internal, non-baited pit-fall trap (13.5cm width, 9cm depth), buried flush with the ground surface and filled with a salt-water solution. Internal traps were opened when mesocosms were closed to capture the beetle community that had buried the dung and beads following emergence from the soil. After closure, mesocosms were left for 7-14 days before the soil beneath the dung was destructively sampled to a depth of 50cm in search of the beads buried by beetles. This difference in time that mesocosms were left before sampling had no impact on the numbers of beads buried [36]. Internal pitfall traps were removed and beetles oven dried for laboratory processing (see [36] for detailed experimental design and rationale).

(b) Evaluating seedling emergence and survival

Following the procedure described above, in February 2014, we created a further 90 mesocosms in one of the sites (0°38`46.418"S, 52°34`11.125"W) with clay textured Oxisols (mean clay content ± SE: 67.3 ± 1.5%, silt: 14.4 ± 1%, sand: 14.1 ± 1.1%). This site was selected because previous work demonstrated that dung beetle diversity strongly influenced the dispersal of seed mimics in this site compared with other sites in the region [36]. We therefore designed this experiment to investigate if the observed patterns between dung beetle diversity and the burial of seed mimics influence the success of real seeds. Each mesocosm was baited with 100g mixture of 50:50 human and pig dung containing two seeds each of five animal-dispersed, Amazonian fruit species: *Genipa americana, Malpighia emarginata, Myrciaria dubia*, *Psidium guajava* and *Rubus chamaemorus.*

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

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

(c) Germination trials

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

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

(d) Dung beetle traits and diversity metrics

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

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

(e) Statistical analyses

Analyses were carried out in R version 3.0.2 [45]. Our first hypothesis was that large seeds are more sensitive to reductions in dung beetle biomass and diversity than smaller seeds. To test this we used generalised linear mixed effects models (glmm) from the “lme4” package [46] to investigate if bead size, beetle community metric and the interaction between the two factors affected probability of bead buried (2012 experiment). Each community metric was included in a separate model and mesocosm was nested within site as random factors. Our second hypothesis was that dung beetle diversity positively influences the emergence and survival of real seeds. We used linear models (lm) to investigate if treatment (open or partial exclusion) succeeded in enhancing the variety in beetle community metrics across mesocosms (2014 experiment, Appendix S2). We then used glmms to assess how beetle community metrics within mesocosms influenced the probability of seed emergence and survival until the end of the 18-week experimental period. Mesocosm was included as a random factor. Our final goal was to assess the optimal burial depth of *M. dubia* seeds and to investigate if the presence of dung influences seedling emergence or survival. Here we used glmms to ascertain if burial depth, the presence of dung and the interaction between the two factors influenced probability that seeds emergence from the soil and subsequently survived until the end of the 18-week monitoring period. We then used glmms to investigate if the week that seedlings emerged influenced the likelihood that they survived until the end of the experimental period to ensure that any observed correlations between burial depth and seedling survival were not an artefact of the seedlings having emerged at different times. Germination plot was a random factor in lmers and glmms.

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

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

3. Results

(a) Using seed mimics to examine burial

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

(b) Evaluating seedling emergence and survival

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

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

(c) Germination trials

Burial depth was the only factor that significantly influenced the likelihood of emergence (LRT = 69.4, D.f. = 9, $P < 0.0001$); the presence of dung had no significant effect. Seeds that were buried below the soil surface were less likely to emerge as seedlings than those placed above or below the leaf litter: there was a 44.4% and 52.8% emergence rate for seeds above and below the litter respectively, compared to between 19.4% and 5.6% for seeds buried at 1cm and 20cm respectively (Fig. 3). No factor or interaction had a significant effect on the probability of seedling survival. Emergence week had no effect on the probability that seedlings survived to the end the monitoring period (LRT = 2.8, df = 1, *P* = 0.0921). No seeds emerged from mesocosms after week 16 or from germination plots later than week 14 (Appendix S7). As such, we are confident that all emergence events were captured during the monitoring period.

4. Discussion

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

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

Secondary dispersal by dung beetles has been demonstrated on a number of occasions to be beneficial to buried seeds [27,28,50]. However, contrary to our predictions, we show that functional richness, species richness and total biomass of beetle communities are negatively correlated to the emergence success of seedlings, suggesting that dung beetle activity may be detrimental for some species. Previous beetle-mediated seed dispersal experiments in tropical forests demonstrate that burial depths of between 1cm and 4cm result in increased germination success compared to seeds that remained on the soil surface or were buried to deeper depths [27,38]. We show that *M. dubia* emergence rates within germination plots were highest when seeds were placed either above or below the leaf litter, but immediately reduced by over 50% when seeds were buried within the soil profile. Therefore, it is likely that the negative relationship between beetle community attributes and emergence of *M. dubia* seeds is a consequence of higher biomass and diversity, resulting in higher rates of seed burial [c.f. 36] and net disadvantages to the fitness of this species. Furthermore, results from our bead burial and retrieval experiments demonstrate that small seeded species are buried deeper than larger seeds; given that only large seeds have been shown to germinate from burial depths of 10cm or more [27], we also expect negative consequences of beetle activity for many smaller seeded species. It is therefore possible that seed burial by intact dung beetle communities may reduce the prevalence of small-seeded species, thus reducing competition experienced by larger seeds.

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

We found a positive relationship between seedling survival and dung beetle total biomass, species richness and CWM back: front leg length. Results from our seed germination trials demonstrated that the presence of dung did not influence the survival of *M. dubia* seedlings. This suggests that the mechanisms driving increased seedling survival extend beyond simply the presence of dung surrounding seeds. There are myriad processes acting both above-ground and below-ground that influence whether a seedling lives or dies following germination [e.g. 54]. A plausible way in which beetles could influence seedling survival is through simultaneous effects on both soil resource (nutrients and water) availability and the soil physical environment. Due to their small root system, recently emerged seedlings are reliant on the nutrient and water availability in their immediate surroundings [55]. Bang *et al.* (2005) [56] demonstrated that dung beetle activity had a positive effect on soil permeability in surface layers, which is positively associated to air and water movement, and greater soil pore space [57]. These soil characteristics could facilitate greater root and shoot growth. Furthermore, nitrogen is a mineral element that can become insufficient in seed reserves [58]. Dung beetles have been shown to positively influence rates of nitrogen (N) mineralisation and concentrations of inorganic N in soil, as well as the availability of other limiting nutrients such as phosphorus (P) and potassium (K) [59,60]. Therefore, dung burial by beetles could concurrently alter soil biogeochemistry and physical structure so as to increase the availability of limiting nutrients, whilst facilitating the ease with which roots can access these resources. It is important to note, however, that past studies investigating dung beetle impacts on soil nutrient availability and physical structure have been exclusively carried out in grassland and heathlands, which differ in their soil properties to tropical forests [61,62]; hence, making inferences about the role of dung beetles in modifying tropical soils based on evidence from temperate systems is problematic. Future investigations are therefore needed to elucidate the small scale impact of dung beetles on tropical soils, where highly heterogeneous distributions in soil nutrients are important factors structuring plant communities [63].

The only dung beetle trait that was positively associated with seedling survival was the CWM of back: front leg length. The abundance of dwelling dung beetle species, which do not bury dung or seeds but feed and nest within the dung [26], within these communities was positively related to CWM back: front leg length (supplementary material, appendix S8); as such, an increase in the ratio between back and front leg lengths indicates an increase in the number of dwellers present. The burial of beads similar in size to *M. dubia* was low compared to smaller beads and was always unaffected by leg length. Therefore, it is unlikely that the relationship we found between seedling survival and CWM back: front leg length is a consequence of dwellers decreasing the likelihood that seeds are buried. Instead it is likely that processing of dung on the soil surface increases with an increase in the abundance of dwelling species. This could give rise to similar processes described above, altering soil nutrient availability and physical environment in a way that provides benefits to seedling growth and survival. We are not aware of any studies to date that have investigated how the morphological traits of dung beetles influence soil properties and plant growth.

5. Conclusions

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

Ethics: Sampling did not involve any endangered species and permission to collect zoological material was granted to JL. By the Instituto Brasileiro do Meio Ambiente dos Recursos Naturais Renováveis (IBAMA).

Data accessibility: Data can be accessed through Dyrad (doi:10.5061/dryad.d20g3)

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

glmm(seed emergence ~ beetle community)	LRT	df	*P*	glmm(seedling survival ~ beetle community)	LRT	df	*P*
Functional richness	**6.3**	**1**	**0.0124**	**CWM back: front leg length**	**8.4**	**1**	**0.0038**
Total biomass	**5.7**	**1**	**0.017**	**Total biomass**	**6.5**	**1**	**0.0107**
Species richness	**4.6**	**1**	**0.0326**	**Species richness**	**3.9**	**1**	**0.0495**
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

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

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

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