Upward movement of buried seeds: another ecological role of dung beetles promoting seedling establishment

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Abstract: Dung beetles are known to perform important ecological functions, such as secondary seed dispersal of vertebrate-defecated seeds. We found that dung beetles also move buried seeds upwards, with positive consequences for seedling establishment. In the Lacandon rain forest of southern Mexico we conducted field experiments to address three questions: (1) What proportions of different-sized seeds buried by dung beetles are exhumed by them? (2) Does upward relocation of seeds caused by dung beetle activity promote seedling establishment? (3) Does recurrent beetle activity increase seedling establishment? Using 4-mm, 8-mm and 12-mm beads as seed mimics, embedded in howler-monkey dung, we found that 2–6% of buried beads were later exhumed by beetles, with smaller beads exhumed more often. In small plots (N = 100) where beetles were allowed to bury dung and seed rain was excluded, seedling establishment was over three times higher compared with plots without beetle activity. In plots (N = 8) where we placed dung on four occasions in 1 mo, seedling establishment was more than twice as high compared with plots with single-time dung placement. We believe that our findings open up interesting research opportunities to help further elucidate this newly discovered ecological function of dung beetles.

Key Words: Lacandon rain forest, Scarabaeinae, seed dynamics, seed fate, soil seed bank

INTRODUCTION

After dispersal a seed may follow many possible fate paths depending on the biotic and abiotic factors it encounters (Vander Wall *et al.* 2005). Seeds deposited on the soil surface can germinate, die or be secondarily dispersed. When secondary dispersal involves seed burial, seeds become part of the underground seed bank (the concept of seed bank also includes seeds on the surface and in the leaf litter; Simpson *et al.* 1989), thus we use the term 'underground' to characterize the portion of the seed bank that is buried. Seeds can be buried by biotic agents (e.g. fossorial animals), abiotic agents (e.g. rain) or simply by gravity (Beattie & Culver 1982, Marthews *et al.* 2008).

Buried seeds remain viable during a variable period of time, depending on morpho-physiological seed traits (e.g. latency), microclimatic conditions (e.g. light, humidity) and the action of predators/pathogens (Dalling *et al.* 2011, Garwood 1989, Wang *et al.* 2013). It is well established

that buried seeds are better protected from detection by seed predators than seeds on the surface (Crawley 2000, Hulme 2002). Yet, the effects of other factors influencing survival of buried seeds, as well as those affecting seed germination and seedling emergence, are more unpredictable and context-specific (Dalling 2005, Fenner 2000, Pearson *et al.* 2002).

In tropical rain forests it has been shown that buried seeds, in particular those found at depths > 5 cm, may not encounter adequate conditions for germination (Pearson *et al.* 2002), or may not be able to emerge as seedlings after germination has occurred (Andresen & Levey 2004, Dalling 2005). It has been suggested that these seeds may need to be brought back to the surface, or shallower depths, for germination and seedling establishment to occur (Donath & Eckstein 2012, Grant 1983, Putz & Appanah 1987). It is to be expected that many of the animals responsible for burying seeds through their burrowing behaviour may also play a role in moving buried seeds upwards (Donath & Eckstein 2012). Through upward relocation, seeds may be brought back to the surface, which we call seed exhumation, or may

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be brought closer to the surface, remaining buried at shallower depths.

Dung beetles (Coleoptera: Scarabaeinae) constitute an abundant and diverse insect taxon in tropical forests, and are known to play important ecological functions (see Nichols *et al.* 2008). In the last decade several studies have shown that rain-forest dung beetles bury and/or move horizontally many vertebrate-defecated seeds (Andresen & Feer 2005). It has also been shown that this secondary seed dispersal by dung beetles can have a positive effect on seedling establishment through reduced predation of buried seeds (Andresen 2001, Andresen & Levey 2004) and/or a reduction in the spatial aggregation of seedlings (Lawson *et al.* 2012). More recently, one study also concluded that by burying seeds, dung beetles may play a major role in structuring tropical soil seed banks (Feer *et al.* 2013). Finally, we know that, as they dig tunnels to bury faeces, dung beetles excavate a large amount of soil (Braga *et al.* 2013). Yet, the question remains: Are dung beetles, through their tunnelling and soil excavation behaviour, also moving buried seeds upwards, possibly enhancing seedling establishment?

The main objective of this study was to address this general question. With this purpose we designed field experiments to test the following hypotheses: (1) Some of the seeds buried by dung beetles are later exhumed by them, depending on seed size; (2) Through their dung-burying activity, dung beetles cause the upward movement of seeds present in the underground seed bank, which in turn promotes seedling establishment; (3) The frequency of dung beetle activity will be positively associated with seedling establishment.

METHODS

Study site

This study was carried out between September 2011 and September 2013 in the Montes Azules Biosphere Reserve (16°07′58″N, 90°56′36″W), located in the Lacandon rain-forest region in the Mexican state of Chiapas. This reserve covers an area of 3310 km^2, most of which corresponds to conserved tropical rain forest (Holdridge 1967). Mean annual temperature and precipitation are 24 °C and 3000 mm, respectively (Medellín 1994). More information on the study site can be found elsewhere (Medellín 1994).

Seed exhumation

We established 12 experimental stations in three groups of four stations. In each group, stations were placed in the corners of a 50 × 50-m square; distance among groups

was > 500 m. In each station we placed 50 g of fresh dung (we used the dung of the howler monkey, *Alouatta pigra* Lawrence) containing spherical plastic beads used as artificial seeds (Andresen 2002). In each dung pile we introduced 50 small beads (4 mm), 20 medium beads (8 mm) and 10 large beads (12 mm). Each station was checked after 48 h, when all dung had been removed by dung beetles. We collected and counted all beads that remained on the surface. All beads that were not found on the surface were assumed to have been buried by dung beetles underneath the dung source, although some beads might have been buried at some distance from the station.

The whole experimental setup was repeated in three time periods, t_1, t_2, t_3, with 4 mo between t_1 and t_2 and 8 mo between t_2 and t_3 (due to logistical reasons we were unable to have equal time intervals), always using the same locations. Beads were of different colours: purple in t_1, pink in t_2 and white in t_3. Four months after t_3 we again placed 50 g of dung in each station, but without beads (t_4). Seed exhumation was quantified in periods t_2 (February 2012), t_3 (October 2012) and t_4 (February 2013). In a given time period t_i (where i = 2, 3 or 4) we counted the number of beads, of those that had been buried in previous time periods (t_{i-1} and/or earlier periods), that we found in the loose soil excavated by dung beetles. Thus, in t_2 we quantified exhumation of beads that had been buried during t_1; in t_3 we quantified exhumation of beads buried during t_1 and/or t_2; finally, in t_4 we quantified exhumation of beads buried during t_1, t_2 and/or t_3.

Seedling establishment

To assess the effect of the soil-tunnelling activity of dung beetles, and of the frequency of this activity, on the establishment of seedlings from seeds naturally present in the soil seed bank we carried out two complementary experiments. Experiment 1 was designed to address both questions by comparing three treatments simultaneously (no beetle activity vs. single-time beetle activity vs. recurrent beetle activity). However, during the first months after setting up Experiment 1 we observed very few seedlings establishing and thus decided to increase sample size by setting up Experiment 2. Yet, the large scale of this second experiment did not allow us to include the 'recurrent beetle activity' treatment (due to the large amount of monkey dung needed).

Experiment 1. We established 1 × 1-m plots with all sides surrounded by a wire mesh (2 mm mesh size) 30 cm high and buried 5–10 cm into the ground (the mesh was buried to make sure that dung beetles started building their tunnels inside the experimental plots, so that all excavated soil would be within plots). We had a total of eight groups of three plots each, distributed in eight forest locations or blocks (N = 8), with a distance of 2 m between plots

in a block and 50 m between blocks. All seedlings found inside the plots were removed. Plots within a block were randomly assigned to three treatments: (1) single-time placement of 100 g of fresh howler monkey dung (divided into three piles to mimic natural defecation patterns); (2) repeated placement of 100 g of dung, once every week, for a total of four times; and (3) control, no dung added. All seeds were removed from dung used in this experiment, except for seeds of *Cecropia obtusifolia* Bertol. which could not be removed due to their small size. Thus, *C. obtusifolia* seedlings establishing in experimental plots were excluded from analyses. During 2 d the tops of all plots were left open to allow dung beetle activity. Thereafter, all tops were covered using the same type of mesh; this was done to avoid the arrival of new seeds, and to prevent access to herbivores. Plots were checked every 2 wk for 12 mo. During every check we counted, identified and marked all seedlings of woody plant species. When possible, using several information sources (literature, internet, experts, direct measurements) we classified seedlings according to life form (tree, shrub, liana), primary seed dispersal syndrome (zoochory, anemochory, barochory) and seed size category (<5 mm and ≥5 mm).

Experiment 2. We established small circular plots, 0.17 m² in area, by burying plastic cylinders (we used 25-cm tall buckets with bottoms removed) 5–10 cm into the soil. All seedlings found inside plots were removed. We placed 100 pairs of plots along existing trails; distance between plots in a pair was 0.5 m and distance between pairs was 30 m. Half the pairs were set out in October 2012 and the other half in February 2013. Plots in each pair were assigned randomly to two treatments: (1) single-time placement of 50 g of seedless monkey dung, and (2) control, no dung added. As in Experiment 1, plots were left open for 2 d to allow dung beetle activity; thereafter they were covered with mosquito net. Plots were checked for seedling establishment every 2 wk for 6 mo.

Data analyses

To analyse the effect of seed size on the proportion of seeds exhumed by dung beetles we used a generalized linear model with artificial seed size as the independent variable. Exhumation data obtained in the three periods were pooled for analyses. A quasi-binomial error structure was used due to overdispersion of data (Crawley 2007).

To analyse the effect of dung beetle activity and its frequency on seedling establishment (Experiment 1) we used two generalized linear models with treatment as fixed factor with three levels (repeated dung, single-time dung, no dung). A quasi-Poisson error structure was used for each of the dependent variables: 'number of species' and 'number of individuals'. Post hoc comparisons between pairs of treatments were performed using the HSD Tukey

test. To analyse paired data from Experiment 2 (single-time dung vs. no dung), for the same dependent variables as in Experiment 1, we used the non-parametric Wilcoxon test for paired comparisons. G-tests were used to test for independence between seed size of established seedlings (<5 mm and ≥5 mm) and experimental treatments.

Adequacy of error structures used in the generalized linear models was corroborated with residual analyses (Crawley 2007). Analyses were carried out in R version 2.15.3 (R Development Core Team. http://www.R-project.org/).

RESULTS

Seed exhumation

Dung beetles buried an average of 68% ± 30% (mean ± SD) of the artificial seeds (pooling all sizes) placed inside dung piles. Dung beetles exhumed 5.4% ± 1.06% of previously buried beads. As expected, smaller beads had a higher probability of being exhumed by dung beetles compared with larger beads (6.1% for small beads, 4.9% for medium beads and 2.4% for large beads; $F_{1,34} = 8.52$, $P = 0.006$; Figure 1).

Seedling establishment

Experiment 1. Pooling the 24 plots of 1 m² we recorded the establishment of 269 seedlings of 27 woody plant species, in 1 y (Appendix 1). Additionally, 130, 27 and 36 seedlings of *C. obtusifolia* established in the repeated dung, single-time dung and no dung treatments, respectively, but were not included in analyses. Both the mean number of individuals and mean number of species per plot were affected by treatment (individuals: $F_{2,7} = 12.5$, $P < 0.001$; species: $F_{2,7} = 6.53$, $P < 0.001$; Figure 2). Post hoc tests revealed that values were significantly higher in plots with repeated dung placement, when compared with the treatment with single-time dung placement (individuals: $N = 8$, $Z = -3.49$, $P = 0.001$; species: $N = 8$, $Z = -2.98$, $P = 0.008$) and the control treatment (individuals: $N = 8$, $Z = 4.24$, $P < 0.001$; species: $N = 8$, $Z = 2.98$, $P < 0.001$). No significant differences were found between the latter two treatments (individuals: $Z = 0.990$, $P = 0.579$; species: $Z < 0.001$, $P = 1$; Figure 2). Seed size (two categories: <5 mm and ≥5 mm) of established seedlings was independent of treatment (species, $G = 0.047$, df = 2, $P = 0.977$; individuals, $G = 3.15$, df = 2, $P = 0.207$).

Experiment 2. In the 200 plots of 0.17 m² pooled together we recorded the establishment of 187 seedlings of 26 woody plant species, in 6 mo (Appendix 2). Contrary to the results in the previous experiment, having increased statistical power in this experiment, we found increased
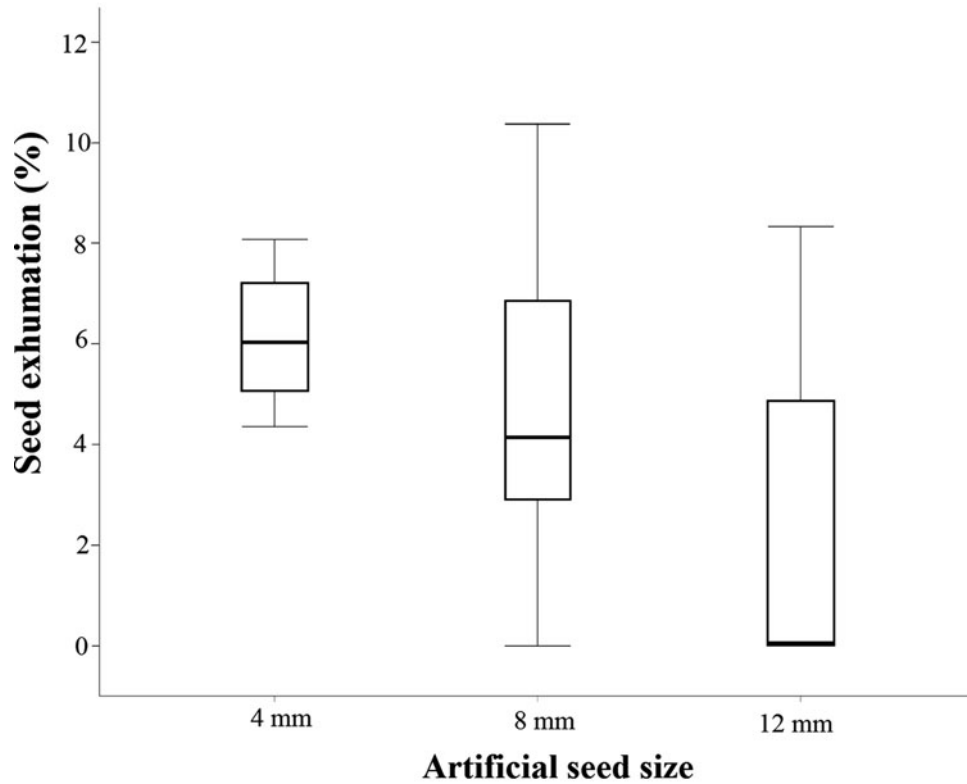
Figure 1. Percentage of plastic beads used as artificial seeds, of three different sizes, exhumed by dung beetles (N = 12 for each size). Boxes represent the interval between the 25th and 75th percentiles; also shown are the positions of the medians. The whiskers indicate the minimum and maximum values observed.
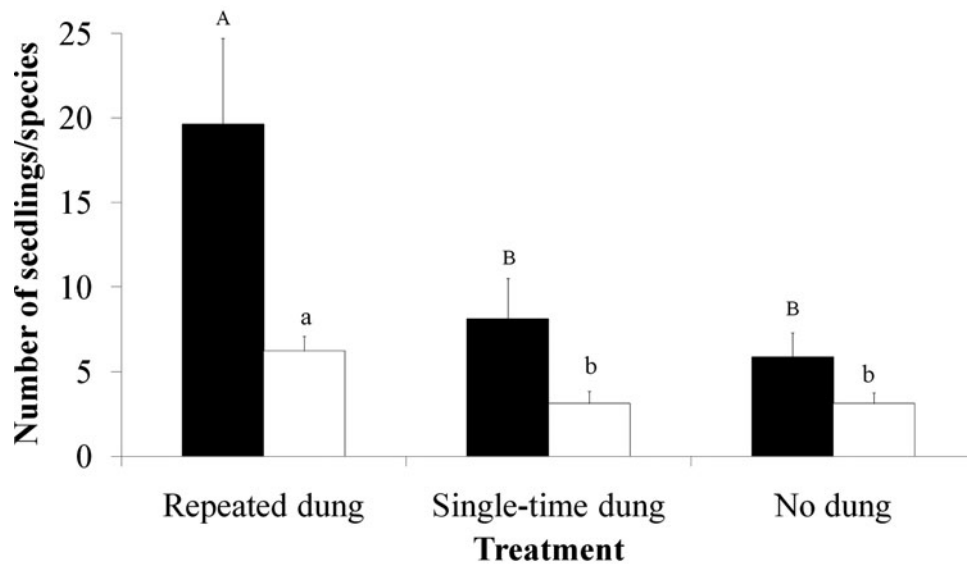
Figure 2. Mean number of seedling individuals (black bars) and species (white bars) in 1-m^2 plots with three different treatments: repeated placement of 100 g of dung (once a week for 4 wk), single-time placement of 100 g of dung, and no dung placement. Error bars represent 1 SE. Different letters above bars indicate statistical differences with the HSD Tukey post hoc tests; uppercase letters are for number of individuals and lowercase letters for number of species.
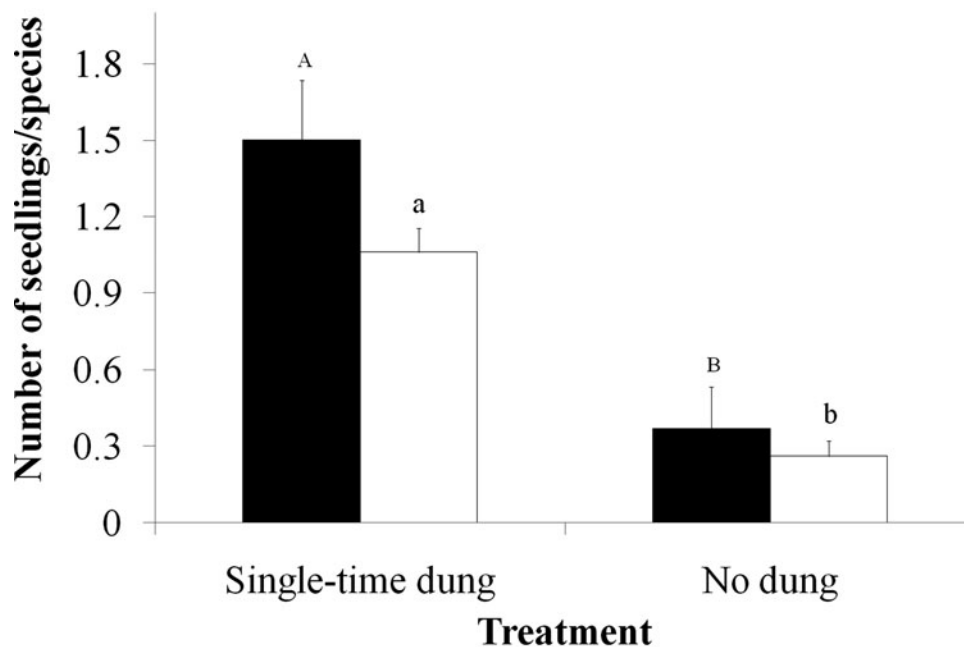
Figure 3. Mean number of seedling individuals (black bars) and species (white bars) in 0.17-m^2 plots with two different treatments: single-time placement of 50 g of dung, and no dung placement. Error bars represent 1 SE. Different letters above bars indicate statistical differences based on the Wilcoxon paired test; uppercase letters are for number of individuals and lowercase letters for number of species.

seedling establishment in plots with dung beetle activity than in plots without it (individuals: Z = −5.38, N = 100, P <0.001; species: Z = −5.27, N = 100, P <0.001; Figure 3). As in Experiment 1, seed size of established seedlings was independent of treatment (species, G = 0.123, df = 1, P = 0.726; individuals, G = 0.377, df = 1, P = 0.539).

DISCUSSION

Seed bank dynamics involves processes that are crucial for plant regeneration (Thompson 2000), with important practical implications for management and restoration (Garwood 1989, Skoglund 1992). With our study we have shown that dung beetles, through their dung-processing behaviour of building underground tunnels, actively participate in seed bank dynamics, not only by burying seeds, but also by moving buried seeds upwards. We propose that upward relocation of buried seeds be added to the large list of ecological functions performed by dung beetles in tropical rain-forest ecosystems (Nichols *et al.* 2008).

In some instances the upward relocation of seeds brings seeds back to the surface, resulting in seed exhumation. Every time rain-forest dung beetles bury a given amount of dung, they excavate more than twice that amount of soil (Braga *et al.* 2013), which would be expected to contain at least some seeds in most cases (Dalling *et al.* 1994).

Although the percentage of artificial seeds exhumed estimated in our study was low (≤6%), this represents a conservative figure. We only measured exhumation for those beads buried immediately underneath the experimental station, and we assumed that all buried beads had been buried there. However, it is most likely that roller beetles incorporated some of the artificial seeds into their dung balls and buried them at some distance away from the experimental station. Thus our estimate of the total number of seeds buried underneath each station was likely overestimated, and consequently our percentages of seeds exhumed were likely underestimated.

Seed exhumation was negatively associated with seed size, as previously documented for seed burial by dung beetles (Andresen & Feer 2005, Culot *et al.* 2009). However, in the case of upward seed relocation it may occur that, rather than actively being excluded by dung beetles as in the case of seed burial, the inherent size of larger seeds might hamper their upward movement through soil layers during the tunnelling activity of dung beetles. To more accurately describe and quantify seed exhumation, future studies could carry out experiments in which known numbers of beads are buried by the researcher at known depths, and in which the activity of dung beetles is restricted to a certain area. Also, use of a broader range of seed sizes, in particular small ones (1–3 mm), would be very informative. Future studies would also need to compare the role played by dung beetles in the upward relocation of buried seeds, relative to other

soil-dwelling organisms. Finally, future studies on seed exhumation would need to assess the increased risk of seed predation due to seed exposure on the surface. Indeed, the best-documented advantage of secondary seed dispersal by beetles is that seeds buried by them suffer very low rates of seed predation by granivores, compared with seeds on the surface (Santos-Heredia *et al.* 2010). So, seed exhumation might have a high associated cost in terms of seed survival, one that remains to be quantified.

However, exhumed seeds represent only a proportion of all seeds moved upwards by dung beetles, and likely a small proportion, with most upward-relocated seeds remaining buried close to the surface. Yet, future studies would need to corroborate this by conducting careful experiments in which the downward and upward movements of seeds along soil profiles, as a consequence of dung beetle tunnelling activity, is measured with more detail. Although in our study we did not quantify the exhumed and relocated seed bank directly, we did so indirectly by quantifying seedling establishment, which provides a conservative estimate of actual seed bank abundance and composition (Dalling *et al.* 1994). We found that significantly more individuals and species established as seedlings in plots in which dung beetles had buried dung and excavated soil, compared with plots where beetles had not been active (Appendix 2, Figure 3).

Studies on the ecology of tropical soil seed banks have shown that soil depth tends to be negatively associated with seed viability (Pouvelle *et al.* 2009) and seedling emergence (Dalling *et al.* 1994), and that many seeds need to be in shallower depths in order to encounter conditions that trigger germination and/or allow seedling emergence (Donath & Eckstein 2012, Grant 1983, Pearson *et al.* 2002, Putz & Appanah 1987). Upward relocation by dung beetles may be particularly important for small seeds, which are often buried more deeply (Andresen & Feer 2005, Feer *et al.* 2013), and which have greater depth constraints for seed germination and seedling emergence (Dalling 2005).

Our study also showed that in specific rain-forest areas in which dung beetle activity occurs repeatedly over time, seedling establishment is further increased, in comparison to areas in which dung beetle activity is less frequent (Appendix 1, Figure 2). Recurrent dung beetle activity is expected to occur in areas which receive an equally recurrent input of dung. Many mammal species defecate repeatedly in the same sites, a pattern known as 'latrine behaviour' (Irwin *et al.* 2004). Among Neotropical frugivorous mammals, primates and tapirs are well known for this behaviour (Fragoso *et al.* 2003, González-Zamora *et al.* 2012), and studies focusing on the consequences of primary seed dispersal by these mammals often show increased seedling establishment of the dispersed plant species in these sites (Bravo 2012, Russo & Augspurger 2004).

Such increased seedling establishment is primarily caused through the continuous seed deposition by the frugivorous mammals. However, our results suggest that dung beetle activity may also in part be responsible for facilitating such increased seedling establishment in recurrent defecation sites of frugivorous mammals. Indeed, results of a recent study suggest that the combined effect of primary seed dispersal by primates and secondary seed dispersal by dung beetles is actively shaping seed bank structure and dynamics (Feer *et al.* 2013). That study also found that dung beetle activity was significantly higher in sites in which monkey defecation was more frequent. Our results on seedling establishment further suggest that dung beetle activity will independently enhance seedling establishment through upward relocation of seeds present in the underground seed bank, i.e. even in defecation sites of non-frugivorous mammals where seed input is not increased through primary seed dispersal. This remains to be tested through future studies.

In conclusion, our study shows that dung beetles play yet another important ecological role through their dung-relocation behaviour, namely the upward movement of buried seeds. In turn, upward seed movement is likely to be an important process in the dynamics of seed banks, with relevant consequences for plant populations and communities. We believe that our study opens up a promising avenue for future research in our way to attaining a more detailed understanding of all the possible outcomes of the interactions between seeds and dung beetles, and the overall role of this insect group in plant regeneration. Finally, as dung beetles are increasingly being used as a model taxon in studies assessing the effects of biodiversity loss on ecosystem functions and services (Braga *et al.* 2013, Dangles *et al.* 2012), we propose that assessment protocols could easily incorporate the measurement of this new ecological function.

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Appendix 1. Total number of seedlings established in 1-m^2 plots (N = 8 plots per treatment) with three different treatments: repeated placement of 100 g of dung (once a week for 4 wk), single-time placement of 100 g of dung, and no dung placement. Information on life form, primary seed dispersal syndrome and seed size (width) are given for each species, when available. For those species, for which we could not measure seeds directly or obtain measurements from other sources, we provide two broad categories (<5 mm and ≥5 mm) based on the information available for the particular genera in our study region.

Species	Repeated dung	Single-time dung	No dung	Life form	Dispersal	Seed size (mm)
Acalypha sp. 1	4			Shrub	Barochory	<5
Ardisia sp. 1	2			–	Zoochory	<5
Arrabidaea sp. 1	5	1	1	Liana	Anemochory	≥5
Bignoniaceae		2	1	–	–	–
Brosimum lactescens (S. Moore) C.C. Berg	6			Tree	Zoochory	7
Ceiba pentandra (L.) Gaertn.	1			Tree	Anemochory	≥5
Celtis iguanaea (Jacq.) Sarg.	3		1	Liana	Zoochory	6
Cissus microcarpa Vahl	3	4	6	Liana	Zoochory	7
cf. *Combretum*	14	10	2	Liana	Anemochory	–
Davilla sp. 1	12	10		Liana	Zoochory	≥5
Dialium guianense (Aubl.) Sandwith	4			Tree	Zoochory	4
Erythrina sp. 1	3	5	2	Tree	Barochory	<5
Eugenia sp. 1	7	2	8	Tree	Zoochory	13
Gliricidia sp. 1	4		2	Tree	Barochory	<5
Guarea excelsa Kunth	3	7	2	Tree	Zoochory	5
Malpighiaceae	2		2	–	–	–
Inga pavoniana G. Don	2			Tree	Zoochory	5
Ochroma pyramidale (Cav. ex Lam.) Urb.	3			Tree	Anemochory	<5
Passiflora sp. 1	34	2	8	Liana	Zoochory	<5
Paullinia sp. 1	7	2		Liana	Zoochory	9
Psychotria sp. 1	6	3		Shrub	Zoochory	2
Rinorea sp. 1	8	2	5	Shrub	Barochory	2
Serjania sp. 1	16	12	6	Liana	Anemochory	2
Spondias mombin L.	1			Tree	Zoochory	12
Trophis racemosa (L.) Urb.	4	3	1	Tree	Zoochory	5
Virola guatemalensis (Hemsl.) Warb.	1			Tree	Zoochory	17
Vochysia guatemalensis Donn. Sm.	2			Tree	Anemochory	5
Total number of individuals	157	65	47			
Total number of species	26	14	14			

Appendix 2. Total number of seedlings established in 0.17-m^2 plots (N = 100 plots per treatment) with two different treatments: single-time placement of 50 g of dung, and no dung placement. Information on life form, primary seed dispersal syndrome and seed size (width) are given for each species, when available. For those species, for which we could not measure seeds directly or obtain measurements from other sources, we provide two broad categories (<5 mm and ≥5 mm) based on the information available for the particular genera in our study region.

Species	Single-time dung	No dung	Life form	Dispersal	Seed size (mm)
Acalypha sp.1	8	1	Shrub	Barochory	<5
Arrabidaea sp.1	4	6	Liana	Anemochory	≥5
Calophyllum brasiliense Cambess.	5		Tree	Zoochory	13
Castilla elastica Sessé	1		Tree	Zoochory	5
Celtis iguanaea (Jacq.) Sarg.	12	3	Liana	Zoochory	6
Cissus microcarpa Vahl	2		Liana	Zoochory	7
Combretum sp. 1	4	2	Liana	Anemochory	≥5
Davilla sp. 1	3		Liana	Zoochory	≥5
Dialium guianense (Aubl.) Sandwith	4	1	Tree	Zoochory	4
Erythrina sp. 1	7	1	Tree	Barochory	<5
Eugenia sp. 1	31	11	Tree	Zoochory	13
Eugenia sp. 2	3		Tree	Zoochory	≥5
Eugenia sp. 3	2		Tree	Zoochory	≥5
Gliricidia sp. 1	9		Tree	Barochory	<5
Guarea excelsa (Aubl.) Sandwith	1	1	Tree	Zoochory	5
Hirtella americana L.	1		Tree	Zoochory	4
Malpighiaceae	5		–	–	–
cf. *Tapirira*	6	4	–	–	–
Passiflora sp. 1	14	7	Liana	Zoochory	<5
Passiflora sp. 2	1		Liana	Zoochory	<5
Posoqueria latifolia (Rudge) Schult.	3		Tree	Zoochory	10
Rinorea sp. 1	4		Shrub	Barochory	2
Schizolobium parahyba (Vell.) S.F. Blake	2		Tree	Anemochory	2
Trophis racemosa (L.) Urb.	8		Tree	Zoochory	5
Virola guatemalensis (Hemsl.) Warb.	1		Tree	Zoochory	17
Vochysia guatemalensis Donn. Sm.	9		Tree	Anemochory	5
Total number of individuals	150	37			
Total number of species	26	10			