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1 **Monkey and dung beetle activities influence soil seed bank structure**

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1 Abstract

2 We investigated the influence of Neotropical dung beetles on soil seed bank structure after
3 primary dispersal by the red howler monkey (*Alouatta seniculus*). We collected seeds from
4 soil samples (up to 15 cm depth) in defecation versus control areas and showed that seed
5 number and diversity increased with monkey frequentation. Seed numbers decreased with
6 depth. Seed viability, ascertained from seed coat integrity, decreased with depth and was
7 higher in sites rarely visited by monkeys compared to control areas or sites frequently visited
8 by monkeys. In field experiments, we incorporated plastic beads (1.3 – 5.8 mm) to fresh dung
9 and monitored bead fate: the proportion of beads found in the soil top 10 cm increased with
10 bead size and this effect was more pronounced in sites more frequently visited by monkeys.
11 The same conclusions were drawn by comparing the beads found in the topsoil and the beads
12 found deeper. We explored bead processing behaviour in several tunneller and roller species
13 by performing experiments involving one species at a time. We showed that selectivity was
14 highly variable: bead exclusion from dung reserves was higher in small than in large beetle
15 species, higher for large than for small beads. Differences in selectivity between medium and
16 small beads decreased for greater per-capita resource, an effect which was more pronounced
17 with small species and with rollers. These results support a major role of dung beetles in soil
18 seed bank structure and dynamics. They reveal interesting interspecific variability within the
19 dung beetle community, a complex interplay with primary dispersal, and a possible role of
20 competition between dung beetles calling for more refined investigations.

21

22 *Key words:* French Guiana; Dung beetles; Pioneer species; Howler monkey; Soil seed bank.

23

1 INTRODUCTION

2 The seed dispersal process commonly comprises two phases (diplochory), each involving a
3 different dispersal agent. In tropical forests, frugivorous animals like monkeys can promote
4 long distance escape away from the parent plant, thus acting as major seed primary dispersers.
5 Secondary dispersers like dung beetles or other insects relocate the dung deposited by
6 frugivorous mammals, dispersing seeds at short range, a process which can lower seed
7 mortality by reducing aggregation or by placing seeds in favourable microsites for
8 germination (Engel 2000, Wenny 2001, Vander Wall and Longland 2004). Soil seed bank
9 structure and dynamics are influenced both by primary and secondary dispersers (Dalling
10 2005). Dung produced by frugivorous animals often contains large quantities of small seeds,
11 many of which are from pioneer plant species in the Neotropical region. Hence, diplochory
12 appears as one of the major biotic processes involved in the early regeneration of tropical
13 forests.

14 Red howler monkeys act as important primary dispersers. They promote local
15 concentration of small seeds through their highly variable site-specific defecation behaviour,
16 this variability being possibly due to the occurrence of monkey visits, but also to a differential
17 in the activity of secondary dispersers (Julliot et al. 2001, Pouvelle et al. 2009). Dung beetles
18 are ubiquitous in tropical forests and play an important role in seed secondary dispersal.
19 According to their food relocation behaviour they either bury seeds directly below dung
20 deposits as tunnellers or dwellers, or move them away in dung balls as rollers (Andresen and
21 Feer 2005). Experiments with Neotropical dung beetles have shown that smaller seeds are
22 buried in greater amount and at greater depth than larger seeds (review in Andresen and Feer
23 2005). Dwellers bury seeds just below the soil surface (Vulinec 2002). Tunnellers bury seeds
24 in larger proportion than rollers, the latter group being never observed to bury seeds larger
25 than 5 mm in length (Vulinec 2002). Finally, larger beetles bury seeds at greater depth than
26 smaller beetles (Vulinec 2000). Burial protects seeds from terrestrial predators like rodents
27 and places seeds in safe and fertile sites for seedling establishment (Andresen and Levey
28 2004, Dos Santos Neves et al. 2010). It has recently been demonstrated that dung beetles
29 reduce the spatial aggregation of tropical seedling which may enhance their survival (Lawson
30 et al 2012). Dung beetles have been shown to be highly active as they are able to process
31 dung and its content in a few hours (Feer 1999). Because of their rapidity and abundance,
32 dung beetles are likely highly effective agents of secondary dispersal.

33 In the present study, we set out to investigate the origins of soil seed bank variability
34 in relation to monkey and dung beetle activity. Identifying the factors responsible for such a

1 variability is crucial to assess its impact on seed fate (mortality, competition for germination
2 and recruitment), and ultimately on forest dynamics. Focusing on the system primate-beetle is
3 interesting to assess the potential adaptability of beetles to resource availability.
4 Understanding this system is particularly timely in the current context of threat on
5 biodiversity, particularly on primates.

6 Possible and mutually non-exclusive causes for seed accumulation may be recurrent
7 monkey frequentation (Muñoz Lazo et al. 2011), modifications (saturation or increase) of
8 dung beetle activity, greater activity of tunnellers or dwellers compared to rollers (tunnellers
9 being less efficient in dispersing seeds away from dung deposits), and strong selectivity in
10 seed exclusion from dung reserves (Feer 1999).

11 First, we question whether increased monkey frequentation translates into differences
12 in the structure of soil seed bank. Potential differences may result from differences in activity
13 of monkeys, dung beetles, or other dispersers or consumers. If such differences have already
14 been observed in previous studies (Pouvelle et al. 2009), it is crucial to examine the existence
15 of potential differences in this study before testing the implication of dung beetles. We
16 analysed soil seed bank structure – number of seeds, species richness, seed viability at various
17 depths – in sites differing by monkey frequentation.

18 Second, we question whether dung beetles are similarly effective at all sites or more
19 active in sites more often frequented by monkeys. Differences in activity may result from
20 differences in beetle assemblages (in number of species, species identity, and/or number of
21 individuals) or from an increased activity shown by all individuals (due to a higher
22 temperature or a higher stimulation triggered by cues delivered by dung or seeds for instance).
23 It is crucial to examine the existence of potential differences in activity before questioning
24 some of their possible origins. Hence, in a field experiment using artificial beads, we tracked
25 beads translocated by dweller or tunneller species to estimate the relative topsoil activity of
26 these functional groups in sites differing by monkey frequentation.

27 Third, we question whether dung beetle activity (quantity of dung processed) and
28 selectivity (seed exclusion from processed dung – efficiency of seed dispersal according to
29 seed size) varies with beetle dung relocating behaviour (tunneller versus roller), beetle size
30 and dung availability (per capita resource). We chose the most abundant or specialised species
31 and conducted experiments in which we placed a variable number of individuals of the same
32 species in containers provided with dung and artificial beads. The analysis of dung processing
33 and bead dispersal allowed us to estimate beetle activity and potential effects of intraspecific
34 competition on seed dispersion patterns.

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METHODS

1) Study site and species

This study was conducted at the Nouragues Research Station (French Guiana), located 100 km south of Cayenne (4°5'N, 52°41'W, alt. 110 m a.s.l.) in a 1000 km² wilderness reserve dominated by tropical rain forest (Charles-Dominique 2001). The average annual rainfall is 2990 mm and the mean temperature is 26.3 °C (Grimaldi and Riéra 2001). The dominant vegetation type is a high mature forest with canopy at 30-35 m (Poncy et al. 2001). The howler monkey (*Alouatta seniculus* L.) is the dominant primate in the study area, feeding on ripe, fleshy fruits and foliage (Julliot and Sabatier 1993, Simmen et al. 2001). Among the 97 plant species which constitute its diet, fruits of 21 species have seeds of ≤ 0.1 g, of which 10 make 21.6 percent of the monkey diet (Julliot 1994). Monkeys rest or sleep in particular tree crowns, some of them regularly or seasonally used for several years, while others are used more erratically (Julliot 1996a). They generally defecate after resting, scattering their dung on the ground over about 10 m², enriching the soil microsite with seeds which accumulate over the course of time (Julliot et al. 2001). The majority of seeds remain viable once they have transited through howler guts (Julliot 1996b, Pouvelle et al. 2009). Besides seed concentration, the input of dung enriches soil nutrients particularly in the areas where defecation occurs more frequently (Feeley 2005, Dos Santos Neves et al. 2010).

The local dung beetle community shows a high species diversity (79 species attracted to howler monkey dung; Feer 2000, F. Feer unpubl. data). Species are specialized according to diet, diel activity rhythm and dung-processing behaviour (see species checklist and ecological characteristics in Feer and Pincebourde 2005).

2) Effect of monkey frequentation on soil seed bank structure – soil sampling

To explore the impact of monkey defecation activity on seed secondary dispersal by the dung beetle community, we sampled the soil seed bank in October and November 2007 at eight sleeping sites visited by howler monkeys. Sites were scattered around a 13 ha area and were at least 30 m apart. Based on tree cartography and field inspection, we checked that there was no treefall gap, no *Cecropia* or *Ficus* spp. adult tree within 50 m of site and control areas. This ensured that (1) there was no direct influence of gaps on the study sites and (2) the presence in the soil seed bank of small seeds from dominant plant species was essentially due

1 to dispersal. Sites were categorised in two groups according to the number of monkey visits
2 we were able to observe during the months of October-November in 2006 and in 2007:

- 3 - Sleeping sites rarely frequented by monkeys (freq-, $N = 6$) received one or two visits
4 in two years (one or none in 2006 and one visit in 2007);
- 5 - Sleeping sites often frequented by monkeys (freq+, $N = 2$) received at least four visits
6 in two years (at least two in 2006 and two visits in 2007). The maximal number of
7 visits observed was seven.

8 Control areas were never visited by monkeys (control) in 2006 and 2007. A control area was
9 arbitrarily defined 15 m east of a sleeping site, thereby outside the defecation area but in
10 similar vegetation and soil conditions. In statistical analyses, a control area and its associated
11 defecation area were considered as belonging to the same “site”.

12 We defined sampling areas in the morning shortly after a defecation event was spotted.
13 We first determined the centre of the defecation/control area which we used as the centre of a
14 2x2m square. We labelled 9 sampling points for each area: the centre of the area, as well as 8
15 points along the perimeter of the square, 1m apart from one another. We took topsoil samples
16 from the 9 sampling points within each area 48 hours after defecation events when all dung
17 seemed to have been processed by dung beetles. At each sampling point, six successive layers
18 were dug with a 5-cm-diameter drill: the first 5 layers were 2 cm thick while the last one was
19 5 cm thick. Digging deeper than 15 cm was uneasy because of the presence of numerous tree
20 roots and deeper burial depths are considered much less effective in terms of regeneration
21 potential (Dalling et al. 1994). Soil samples of the same depth layer were pooled over the 9
22 sampling points, transferred to plastic bags and sieved at 0.1 mm under tap water later on
23 during the same day. Seeds, intact or not, were rapidly sorted and sealed in black plastic bags
24 to avoid light-favoured (photoblastic) germination. Once back at the laboratory, plant species
25 were identified at the species level whenever possible, using the laboratory seed collection
26 from French Guiana and species lists for the Guianan rain forest established by Favrichon
27 (1994). Seeds were kept in a fresh state, and thoroughly inspected with forceps under a
28 dissecting microscope. Coat inspection was used to score seed viability (Borza et al. 2007):
29 viable (intact and firm coat) or non viable (void, tunnelled or damaged coat).

31 **3) Dung beetle activity at sites differing in monkey frequentation – field experiments** 32 **with beads**

33 Fresh monkey dung was used for experiments set out in 10 defecation areas: the 8 previously
34 selected and two newly discovered areas both from freq+ category (4 freq+ sites and 6 freq-

1 sites). Preliminary experiments conducted in five sampling areas showed that when an
2 enclosure prevented dung beetles from processing dung during 48 h, dung went mouldy
3 without any change in structure and without any sign of activity from other agents. As a
4 consequence, we did not perform control experiments with enclosures systematically in
5 association with each defecation area. Round plastic beads were used as seed mimics (e.g.
6 Andresen 2002). Seed artefacts were preferred to real seeds to prevent confusion with seeds
7 naturally present in dung. We used a mixture of beads of three different diameters: small (1.3-
8 1.9 mm; $N = 200$ per replicate), medium (3.3-3.7 mm; $N = 80$) and large (4.8-5.8 mm; $N =$
9 10). On the day monkeys defecated in a specific area, we placed 80 g of fresh dung with
10 embedded beads randomly on the ground within the defecation area but outside the area for
11 soil sampling. We placed these pseudo-defecations between 7.00 and 9.30 AM, shortly after
12 the monkey visit, to mimic exact conditions of site use by these primates. We estimated the
13 proportion of dung buried or removed 12 and 24 hours after the beginning of the experiment.
14 Soil samples were taken 48 h after dung deposition. At each sampling point we sampled soil
15 layers at 1, 2, 4 6 8 and 10 cm depth within a 23 cm diameter circular area. Beads were
16 counted by sieving soil samples to calculate the proportion of beads buried by beetles at the
17 different depths. Only few beads were visible on the soil surface. We considered that beads in
18 the 0-1 cm layer were processed mostly by dwellers (Vulinec 2000, F. Feer personal
19 observation) whereas the remaining beads were processed by tunnellers. Missing beads were
20 buried deeper than 10 cm or moved by rollers away from the area surveyed.

21

22 **4) Relative activity and selectivity of the most abundant beetle species – container** 23 **experiment with artificial beads**

24 We selected six dung beetle species among those most frequently captured in pitfall traps
25 baited with howler monkey dung (see Feer 2000) and/or most frequently observed in howler
26 monkey defecation areas or perching on leaves nearby (Feer, pers. obs. since 1995). As beetle
27 activity with respect to dung increases with beetle size (Vulinec 2000), we disregarded some
28 species that were more abundant but smaller, retaining only species longer than 7 mm. We
29 thus selected the three rollers *Hansreia affinis* (9.2 mm length), *Canthon bicolor* (10.2 mm)
30 and *Glaphyrocantion vulcanoae* (12.6 mm) and the three tunnellers *Canthidium cf onitoides*
31 (7.3 mm), *Oxysternon durantoni* (16.4 mm) and *Dichotomius boreus* (23.7 mm) (see species
32 ecological characteristics in Feer and Pincebourde 2005).

33 To test for interspecific variability in dung processing and potential differences in
34 selectivity (active exclusion of beads from processed dung resource), we performed a series of

1 experiments (from 2 to 4 per species) on each species separately. We placed 2 to 7 individuals
2 in a mesh-covered 30-cm-diameter cylindrical container filled with soil (20 cm deep for
3 tunnellers; 10 cm deep for rollers) and with fresh dung with plastic beads (40-50 g dung with
4 100 small, 40 medium and 5 large beads for tunnellers; 30 g dung with 50 small, 30 medium
5 and 5 large beads for rollers). Containers were left aside for 72 h. They were subsequently
6 excavated centimeter by centimeter for buried beads and dung balls were examined for
7 incorporated beads. Rollers were disturbed by the small size of the containers and did not
8 bury all of the balls. We estimated the proportion of processed dung by weighing dung
9 reserves and remaining dung.

10

11 **5) Statistical analysis**

12 Data were analysed using generalized linear mixed models. Such models are widely
13 recommended in ecology as they provide a flexible and robust approach for analysing non-
14 normal data when random effects are present (Bolker *et al.* 2009). Depending on which
15 variable we tested, we took a Poisson or a binomial structure for the dependent variable. With
16 mixed models, it is possible to separate fixed effects from random effects. Fixed effects are
17 biologically relevant predictor variables which permit to extract a general “principle”. For
18 instance, we tested depth as a fixed factor to examine the vertical structure of seed soil bank
19 and the general rule of how seeds (numbers, richness, viability) varied with depth.

20 Conversely, random effects are designed to capture the undesirable variability intrinsic
21 to protocol design but of no particular general value. For instance, we repeatedly sampled the
22 same site or container at different depths. Site (or container) had to be taken as a random
23 effect to account for these repeated observations, and for the natural variability among sites
24 which was not interesting as a rule (sites had no value in themselves as they would change if
25 we were to conduct the experiment again). For container experiments, the experiment (that is
26 the container itself) was taken as the random effect. For soil samples and field experiments
27 with plastic beads, we tested either site (mean value varying randomly among sites) or depth
28 within site (mean value and relationship with depth varying randomly among sites) as a
29 random effect. As explained in detail by Bolker *et al.* (2009), taking a given factor as a random
30 effect and a fixed factor allowed to part its variability into undesired (due to variations of soil
31 between sites, the variation of seeds with depth may vary randomly between sites) and
32 relevant variation (general effect common to all sites).

33 Concerning soil sampling, the variable to explain was seed number, species richness
34 and seed viability (proportion of viable seeds). As fixed effects, we tested the interactions

1 between seed depth (taken as mean layer depth, analysed in logarithm), and the monkey
2 frequentation effect (three levels: control, freq- and freq+). For the latter effect, we built two
3 independent contrasts: the first tested the difference between control and monkey sites
4 (control < [freq-, freq+]); the second tested monkey frequentation (freq- < freq+). We
5 weighted species richness by the number of seeds found in the soil layer to correct for biases
6 due to seed abundance.

7 Concerning the field experiment with beads, we analysed the proportion of beads
8 found at different depths. First, we included all five 2 cm thick layers to explore bead vertical
9 distribution. Second, we contrasted the first centimetre (0-1 cm) and the rest of the soil
10 column (1-10 cm) to gain insights about seed vulnerability to predation or infection. Despite
11 being processed by dwellers, seeds near the soil surface remain more vulnerable to predation
12 than deep-buried seeds (Andresen and Levey, 2004). As fixed effects, we tested the
13 interactions between bead depth (taken as mean layer depth, analysed in logarithm), monkey
14 frequentation effect (two levels: freq- and freq+) and bead size (small, medium and large with
15 two contrasts: M < S and L < [M, S]).

16 Concerning the experiment with containers, we first analysed the ratio of the
17 proportion of beads in the processed dung to the proportion of beads in the dung delivered.
18 Decreasing values corresponded to increasing selectivity (exclusion of beads from the
19 processed dung). Second, we analysed the depth at which beads were buried. As fixed effects,
20 we tested the interactions between functional group (roller or tunneller), species body size
21 (length in mm), proportion of dung processed, per-capita resource (computed as the ratio of
22 dung delivered to the number of individuals), and bead size (small, medium and large with
23 two contrasts (M < S and L < [M, S])). We only tested one to three-way interactions as more
24 complex models could not yield any sound biological interpretation.

25 We used a maximum likelihood approach and minimization of Akaike's Information
26 Criteria (AIC) to select the best statistical models according to the parsimony principle,
27 considering that two models differing by less than two AIC units are statistically
28 indistinguishable, as currently accepted (Burnham and Anderson 1998). We corrected AIC
29 values for potential residual overdispersion and small differences between the number of
30 parameters estimated and the number of observations (Bolker et al. 2009). We first selected
31 the random effect via AIC minimization based on the full model, as suggested by Bolker *et al.*
32 (2009). Once the random effect was selected, we selected fixed effects using the same
33 method. Coefficients and standard errors were computed using a restricted maximum
34 likelihood approach and factor significance was tested using Wald z tests (Bolker et al. 2009).

1 All statistics were performed using R version 2.11.1 (copyright 2008, The R Development
2 Core Team).

3

4 **RESULTS**

5

6 **1) Influence of monkey frequentation on soil seed bank structure**

7 Soil samples contained a total of 1922 seeds from 72 plant species (Online Table 1). Monkey
8 frequentation affected seed numbers, species richness, and seed viability. These variables
9 exponentially decreased with depth. Seed number and species richness increased with monkey
10 frequentation (control < freq- < freq+, Table 1, Figure 1). Compared to control sites, sites
11 visited by monkeys had a smoother decrease in seed number and species richness with depth.
12 Yet, the decrease in seed number – but not in species richness – was marginally steeper in
13 sites often visited by monkeys compared to sites rarely visited (more seeds in top layers and
14 less in deeper layers).

15 We observed that seeds buried deeper had a lower viability. Seed viability decreased
16 exponentially when depth increased. Seed viability was similar in control areas and in sites
17 often visited by monkeys (control < freq+, $P = 0.83$) while it was lower than in sites rarely
18 visited by monkeys (Table 1, Figure 1, [control, freq+] < freq-, $P < 0.001$), suggesting that
19 monkey activity could have positive or negative effects on seed viability depending on its
20 intensity.

21

22 **2) Influence of monkey frequentation on dweller and tunneller activity**

23 Between 50% and 95 % of dung was buried 12 hours after deposition, and 100 % disappeared
24 after 24 h. A total of 59 % of beads (total $N = 2,900$) were found in the top 10 cm in the area
25 surveyed around dung deposits and resulted from tunneller and dweller burying activity. The
26 remaining 41% were either buried more deeply by tunnellers or translocated away by rollers.
27 In the top 10 cm of soil, the proportion of buried bead varied with bead size, depth (five
28 levels) and monkey site frequentation. This proportion increased with bead size (bead size
29 effect, $P < 0.001$, online Table 2). It exponentially decreased when depth increased (depth
30 effect, $P < 0.001$), a variation that was more pronounced for larger beads (depth x bead size
31 effect, $P < 0.01$). The difference in proportion between small and medium beads was more
32 pronounced in sites often visited by monkeys (frequentation x bead size effect, $P < 0.001$), and
33 this effect faded with depth (frequentation x depth x bead size effect, $P < 0.001$).

1 Comparing the proportion of beads buried superficially by dwellers and to that buried
2 by tunnellers yielded similar results. We found a higher proportion of beads near the surface
3 than deeper in the soil (more than 50% of beads found; $P < 0.001$) and this difference increased
4 with bead size ($P < 0.001$). The difference between small and medium beads was more
5 pronounced in sites more often visited by monkeys ($P < 0.001$). The lack of interaction
6 between depth and frequentation suggested that dwellers and tunnellers were similarly
7 affected by site frequentation by monkeys.

10 3) **Relative activity and selectivity of most abundant beetle species**

11 Beetle selectivity for seed size (inversely related to proportion of bead retrieved) depended on
12 all possible triple interactions between beetle size, bead size, functional group and proportion
13 of resource available per capita. For a given body size, rollers and tunnellers did not show any
14 difference in how selective they were when in the presence of seed artefacts (beads). Beetles
15 were less selective for small than medium beads (bead size effect, $P < 0.001$, online Table 2),
16 and for large than (small and medium) beads (bead size effect, $P < 0.01$). Difference in
17 selectivity between large and smaller beads decreased in larger beetles (beetle size x bead size
18 effect, $P < 0.001$, Fig. 2), an effect which faded for greater per-capita resource (beetle size x
19 bead size x part, $P < 0.001$). Difference in selectivity between medium and small beads
20 decreased for greater per-capita resource (bead size x part effect, $P < 0.001$, Fig. 3), an effect
21 which faded in larger beetles (bead size x part x beetle size effect, $P < 0.001$). Variation in
22 selectivity between medium and small beads faded with increasing per-capita resource, more
23 strongly in tunnellers than in rollers (bead size x part x mode effect, $P < 0.001$). Finally,
24 selectivity between medium and small beads faded with increasing beetle size, more strongly
25 in tunnellers than in rollers (bead size x mode x beetle size, $P < 0.001$).

26 Between 21.2 percent ($N = 33$ balls, *H. affinis*) and 33.3 percent ($N = 9$ and $N = 15$ for
27 *G. vulcanoae* and *C. bicolor*, respectively) of dung balls made by roller species contained
28 beads. A higher proportion of balls contained natural seeds (91.2 %, $N = 57$), which were
29 smaller in size than small beads (*Ficus* spp.) or than medium beads (*Cecropia obtusa*, *C.*
30 *sciadophylla*). The largest seed species found in balls was *Bagassa guianensis* (4.0 mm).

31 The depth at which tunnellers buried beads varied with bead size and beetle size.
32 Smaller beads were buried at greater depths (bead size effect, $P < 0.001$, online Table 4).
33 Larger beetles buried beads at greater depths ($P < 0.001$). The difference in burial depth
34 between large beads and smaller beads decreased as beetle size increased ($P < 0.001$). The

1 largest species *D. boreus* was the less selective, burying all beads at high depth levels (total
2 average: $13.6 \pm \text{CI } 0.6$ cm), with maximum depth reaching the bottom of the container,
3 whereas the smaller *O. durantoni* buried large beads less deeply than other beads ($13.4 \pm \text{CI}$
4 0.8 cm). The smallest species *C. onitoides* ($4.3 \pm \text{CI } 1.2$ cm) did not bury large beads and
5 buried medium beads at lower depth levels than small beads.

6

7 **DISCUSSION**

8 **Impact of monkey frequentation**

9 We confirm that endozoochory by the red howler monkey can result in high local
10 concentrations of small seeds. Small seeds are significantly more abundant and diverse in
11 defecation areas than in control areas, a result that agrees with previous studies (Julliot 1992,
12 Pouvelle et al. 2009). More interestingly, we first show that site frequentation by monkeys
13 increases seed accumulation. Such an accumulation unlikely results from a saturation of
14 activity by dung beetle assemblages as all deposited dung was processed quickly at all sites; if
15 it were the case, we would have found a much pronounced accumulation of seeds at the soil
16 surface, a situation that has been observed in forests where beetle fauna is impoverished
17 (Larsen *et al.* 2005).

18 Monkey frequentation has negative effects on seed viability: seed viability is higher at
19 sites rarely used by monkeys compared to control areas or sites frequently used by monkeys.
20 Given that seed viability slowly decreases with time, frequent dung inputs should induce an
21 increase in the proportion of viable seeds. Yet, reduced viability suggests negative effects of
22 high seed density and/or high local concentrations of dung. Such negative effects may be
23 caused by fungal infection, which occurs despite protective structures which enhance seed
24 longevity in the soil seed bank (Lobova et al. 2003). Fungal infection decreases seed survival
25 for *Cecropia* spp. (Dalling et al. 1998) and is more pronounced for *Ficus maxima* seeds
26 embedded in howler monkey faeces than for seeds outside (Jones 1994). Seed viability
27 decreases with depth, as shown in a previous study (Pouvelle et al. 2009), probably due to the
28 lesser renewal rate of seeds in deeper soil layers. By contrast, Dalling et al. (1998) showed
29 that deeply buried seeds survive longer than seeds located near soil surface, likely because of
30 a lower pathogen activity at greater depths. Yet, their experiments were conducted in the
31 absence of dung and do not tell anything about the possible influence of gut and dung
32 components on seed fate.

33

1 **Rapid and monkey-dependent activity of dung beetles**

2 Our field experiments with beads confirm that dung beetles rapidly process large amounts of
3 dung (within 24h, see also Feer 1999). As this effect is not observed when dung beetles are
4 excluded, we can conclude that they are the dominant agent affecting small seed fate after
5 primary dispersal. Other agents likely play a minor role in small seed processing. Rainfall
6 may bury small seeds but only at shallow depths ca 1 cm and slowly in a few weeks time
7 (Marthews et al. 2008). Earthworms active in latrines (Pouvelle et al. 2008, Dos Santos Neves
8 *et al.* 2010) are known to ingest and move small seeds in tropical grasslands (Decaëns et al.
9 2003). Although their contribution to seed movements is unknown in rainforests, it should be
10 slow compared to burial by beetles. Litter ants, known to process small seeds from bird and
11 primate dung (Pizo et al. 2005), likely process only small amounts of dung.

12 Small beads are moved or buried more often and more deeply than large beads, in
13 accordance with previous observations on seeds (Estrada and Coates-Estrada 1991, Sheperd
14 and Chapman 1998, Andresen 1999, 2002). Hence, dung beetles are key contributors to the
15 presence of small seeds of pioneer monkey-dispersed tree species in deep soil strata. The
16 experiment with beads reveals that differences in retrieval and burial rates between small and
17 medium beads are more pronounced in the most often visited areas, the first evidence of an
18 influence of monkey frequentation on beetle activity. This pattern may emerge from different
19 non-exclusive processes: (1) regular dung deposition may produce more attractive
20 components to roaming beetles, (2) beetles may more actively explore areas more often
21 frequented by monkeys, (3) beetle assemblages may be spatially structured, with higher
22 densities and functional diversity in areas with more frequent (and thus predictable) resource
23 availability. These results call for more research concerning beetle population structuring,
24 behaviour and activity patterns. Flying dung beetles have been seen following monkey troops
25 (Vulinec and Quintero cited in Tirado Herrera et al. 2002), a strategy that fosters more
26 efficient food location for these highly specialised animals. Yet, no study has explored the
27 existence of vertebrate-dependent spatial organisation in beetle populations.

28

29 **Dung beetle selectivity in seed processing**

30 Dung beetles show a significant selectivity in their processing of artificial seeds, more
31 actively rejecting larger-sized beads. Selectivity depends on beetle body size, smaller species
32 being more selective than larger species, a result which confirms previous studies (Estrada
33 and Coates-Estrada 1991, Andresen 1999, Feer 1999). In French Guiana rainforest, rollers are
34 in average smaller than tunnellers (see Feer and Pincebourde 2005). Although for a given

1 body size, tunnellers and rollers do not show any difference in selectivity, we suggest that, as
2 a whole, rollers are likely more selective than tunnellers. A higher selectivity by rollers has
3 been detected experimentally (Vulinec 2002, Slade et al. 2007), likely in relation with a
4 relatively small (dung ball mass: beetle size) ratio (Hanski and Cambefort 1991, F. Feer
5 unpublished data). As selectivity depends on body size, we predict that large tunnellers bury a
6 larger number of seeds at higher depths than small tunnellers. The potential for emergence of
7 light-demanding tree species with photoblastic germination, such as *Cecropia* spp., is limited
8 to the upper layer of the soil (Pearson et al. 2003). Hence, we can speculate that large
9 tunnellers put seeds of those species in less favourable conditions for germination than all
10 other dung beetles. By contrast, dung processed by dwellers is very unlikely processed by
11 other beetles. Hence, by maintaining seeds near the soil surface, dwellers put them in
12 favourable conditions for further germination.

13 We show for the first time that selectivity increases when resources available per
14 individual decrease, suggesting that beetles perceive the level of potential intraspecific
15 competition (as estimated by the per capita resource) and adapt their behaviour accordingly.
16 In a context of more intense intra-specific competition, individuals avoid incorporating
17 unpalatable items into their dung reserve, a behaviour that improves dung nutritional value.
18 Whatever their dung processing behaviour (roller or tunneller), smaller species show more
19 pronounced changes in their seed exclusion behaviour. For a given body size and a given
20 decrease in per-capita resource, rollers get more selective than tunnellers and exclusion
21 concerns a larger range of seed sizes. Altogether, our results suggest that rollers likely entail
22 higher costs than tunnellers in carrying non-palatable items in their dung balls, either because
23 of carrying energetic costs or fitness consequences for offspring. Surprisingly, intense
24 competition does not lead to hastened dung processing but to more careful dung processing,
25 with more active exclusion of unpalatable seeds, a behaviour which likely improves the
26 quality of dung balls at the expense of time saving. We can speculate that in a context of high
27 competition, adults may favour the quality of food provisioning to offspring at the expense of
28 offspring number (number of balls produced). To date, several studies suggest that
29 exploitative and interference competition is quite intense in tropical dung beetle communities
30 (Peck and Forsyth 1982, Gill 1991) which differ from temperate communities (Finn and
31 Gittings 2003). Experimental studies demonstrate that some species have the potential for
32 fierce interspecific competition for food (Giller and Doube 1989, review in Hanski and
33 Cambefort 1991). Specific tests are required to assess the importance and modalities of intra
34 and interspecific competition in tropical rainforest dung beetle communities.

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As a conclusion, dung beetles appear able to adapt their activity to resource temporal and spatial availability (sensitivity to monkey activity, to intraspecific competition). The exact and complex mechanisms leading to this flexibility remain to be studied. Yet, we can predict that any limitation of primate density induced by forest fragmentation or hunting is expected to impact beetle community, to disturb the diplochory process and thereby the dynamics of small-seeded tree species.

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Figure legends

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Figure 1. Species richness, seed number and seed viability (proportion of viable seeds) in the topsoil in sites frequently used (Freq+), rarely used (Freq-) by red howler monkeys (black circles) and in control areas never visited by monkeys (open circles). The curve is obtained with values predicted by the best model (see methods) and data points are observed values. Mean values and standard error bars are presented. A small amount of noise has been added to depth data in order to visualize overlapping points.

Figure 2. Selectivity (100 - proportion of beads retrieved in dung beetle reserves in percent) as a function of bead size (small, medium and large circle: S, M and L beads), dung beetle functional group (open circles: tunnellers; black circles: rollers) and body size. The graph shows the values predicted by the model.

Figure 3. Selectivity (100 - proportion of beads retrieved in dung beetle reserves in percent) as a function of bead size (small, and medium circle: S and M beads), dung beetle functional group (open circles: tunnellers; black circles: rollers) and resource per capita (dung delivered per individual).

Table 1. Monkey frequentation (Freq+, Freq- or Control) and depth effect (6 levels) on seed number, species number and seed viability. Tests were performed with GLMM models. Random effects are site and depth for seed number and species number, site for seed viability. ~: $0.05 < p < 0.075$, ***: $p < 0.001$.

Effect	Parameter estimate (\pm 1 SE)	Wald statistics (z)
Species richness		
Depth	-0.39 (\pm 0.05)	-7.35***
Freq- < Freq+	0.32 (\pm 0.03)	10.91***
Control<(Freq- ,Freq+)	0.08 (\pm 0.01)	8.16***
Depth x Freq- < Freq+	0.03 (\pm 0.03)	0.95
Depth x Control<(Freq- ,Freq+)	0.07 (\pm 0.01)	6.35***
Number of seeds		
Depth	-0.82 (\pm 0.10)	-8.29***
Freq- < Freq+	1.14 (\pm 0.08)	14.99***
Control<(Freq- ,Freq+)	0.36 (\pm 0.03)	12.95 ***
Depth x Freq- < Freq+	-0.11 (\pm 0.06)	-1.80 ~
Depth x Control<(Freq- ,Freq+)	0.07 (\pm 0.02)	3.34***
Proportion of viable seeds		
Depth	-0.24 (\pm 0.06)	-4.23***
Freq- < Freq+	-0.47 (\pm 0.13)	-3.74***
Control<(Freq- ,Freq+)	0.17 (\pm 0.05)	3.56***

Figure 1.

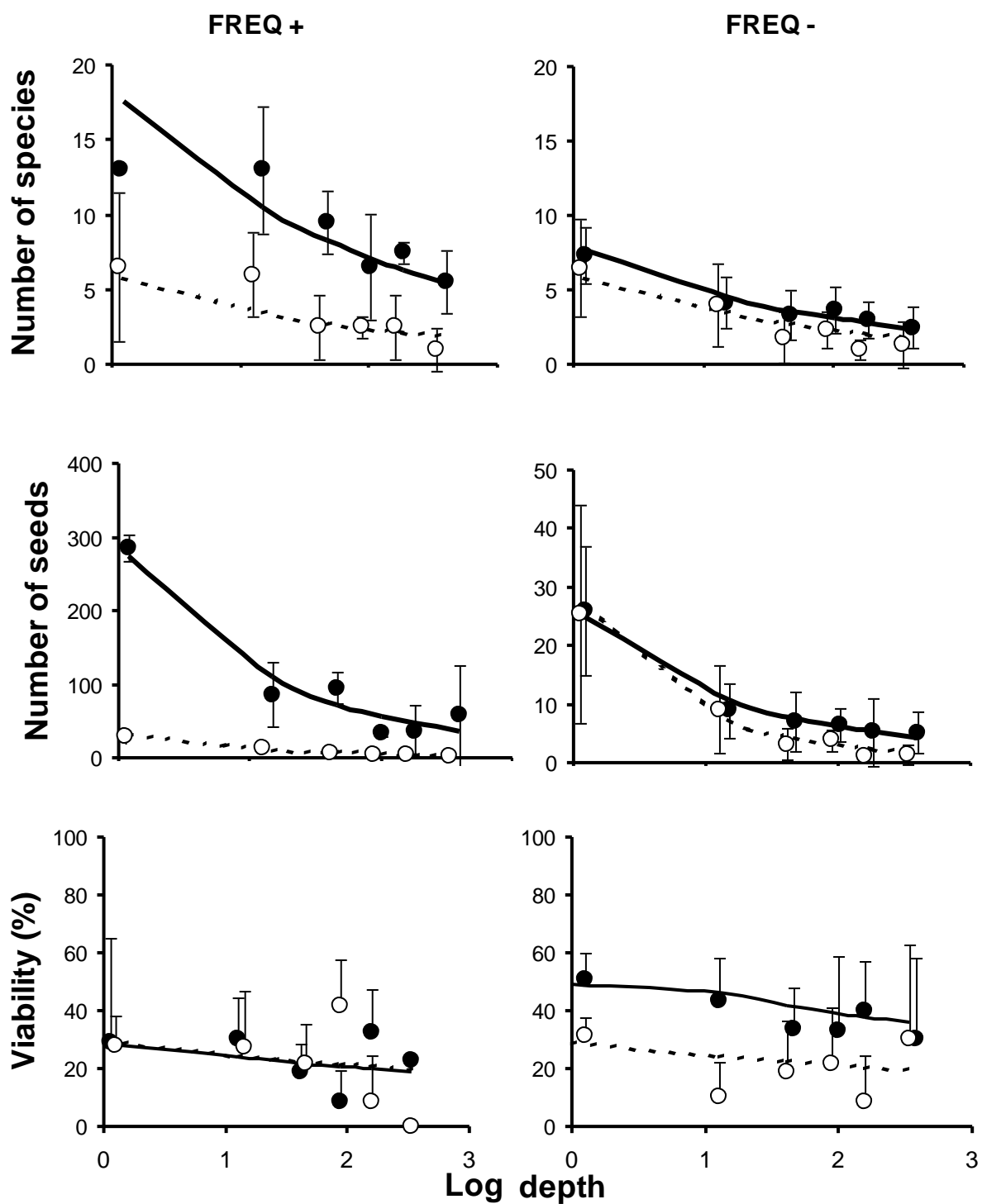


Figure 2

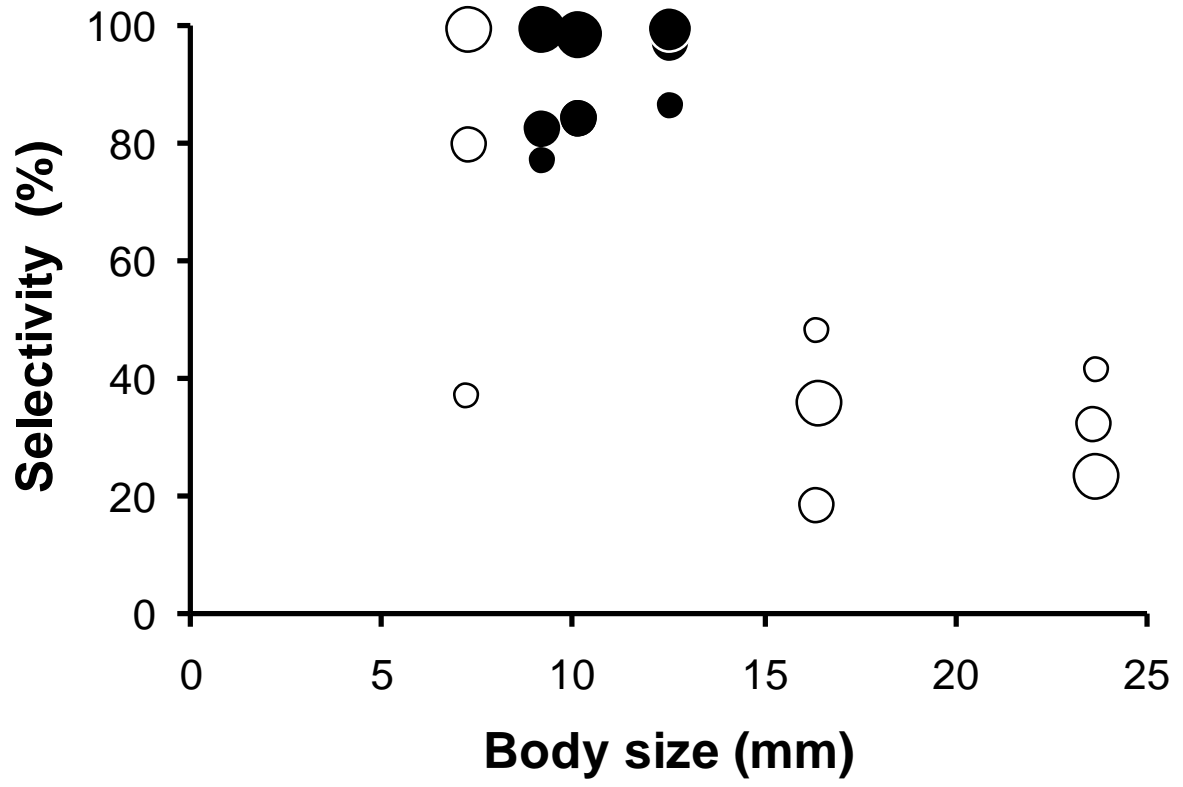


Figure 3

