Assessing the role of a mammalian frugivorous species on seed germination potential depends on study design: A case study using wild samango monkeys

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ABSTRACT

Frugivory and seed dispersal contribute to the maintenance and regeneration of plant communities through transportation of seeds and enhancing germination through seed processing mechanisms. The effects of mammalian frugivore seed processing mechanisms on seed germination are generally well studied and the potential benefits include disinhibition (pulp removal), scarification (gut passage) and fertilisation (from faecal matrix). Nevertheless, our review found that there is bias in the comparative treatments included in seed dispersal studies through exclusion of entire fruit control groups and the fertiliser effect. In this study, we aimed to address such bias by using ecologically relevant experiments to investigate the influence of seed processing mechanisms on germination probability and latency of selected locally abundant fleshy-fruiting plant species, common in the diet of samango monkeys (*Cercopithecus albogularis schwarzi*), and who are seed-spitters (disinhibition) and seed-swallowers (gut-passage and fertiliser effects). We designed experiments to isolate the cumulative effects of seed processing mechanisms and tested the effects of five treatments and one control treatment (entire fruit). We further assessed if exclusion of ecologically relevant seed treatments or relevant controls would affect our interpretation of the impact of the disperser on seed germination. Comparing gut passage and disinhibition indicated negative effects, whereas comparing gut passage and entire fruit controls indicated neutral effects in one species. Compared with gut passage alone, the fertiliser effects indicated positive or neutral benefits on germination probability. Our study demonstrated that the impacts of mammalian frugivores on germination may be under- or over-estimated in ecological literature where relevant treatments and meaningful controls are excluded.

KEYWORDS

Control; Entire Fruit; Gut passage; Frugivore; Dispersal; Seed handling; Scarification

1. INTRODUCTION

Frugivory and seed dispersal form an important mutualistic relationship (Farwig and Berens 2012). Across many habitats, plants rely on frugivores to disperse seeds away from the parent (Janzen 1970; Connell 1971) and enhance germination and seedling establishment (Terborgh et al. 2002; Traveset et al. 2007). Germination experiments have been vital in understanding the role that vertebrate dispersers play in seed dispersal effectiveness (Schupp et al. 2010) and thus the contribution of frugivory to the maintenance and regeneration of plant communities (Janzen 1970; Howe and Smallwood 1982; Traveset et al. 2007). The effectiveness of a disperser depends on the quality of dispersal (the probability of seed germination and survival to reproduction) and quantity of seeds dispersed (Schupp 1993). The quality of dispersal refers to any potential enhancement of germination through seed processing mechanisms, such as passage through the gut of the disperser (Schupp 1993; Schupp et al. 2010. Traveset 1998), which are extrinsic to deposition into microsites favourable for germination and survival.

The effects of frugivore seed processing mechanisms on seed germination are well studied (Traveset 1998; Traveset et al. 2007; Fuzessy et al. 2016) and directly affect seed germination in three ways (Traveset and Verdú 2002). Firstly, removal of fruit pulp (*the disinhibition effect*) releases seeds from chemicals that inhibit germination and decrease the risk of fungal-mediated mortality (Traveset and Verdú 2002). Secondly, passage through the gut or fine oral processing can enhance germination by breaking seed dormancy through mechanical and chemical scarification of the seed coat (*the scarification effect*) which, in addition to releasing the seed from inhibiting chemicals through pulp removal, may increase its permeability to water and gases (Traveset and Verdú 2002; Baskin and Baskin 2014). Thirdly, in mammals the faecal matrix can enhance seed germination through a fertilising effect, or can have a lethal effect by facilitating the growth of fungus and/or bacteria *(the fertiliser effect)* (Traveset and Verdú 2002; Traveset et al. 2007). The majority of studies focus on the effect of gut passage, reporting an increase in the number of seeds germinating and decrease in latency to germinate (time from seed deposition to germination) (e.g. Petre et al. 2015; Muñoz-Gallego et al. 2019). However, despite previous calls for ecologically valid comparative experiments to ascertain the effect of processing mechanisms and the importance of entire fruits as controls (e.g. Samuels and Levy 2005), there is still a prominent bias in the comparative treatments included in seed dispersal studies (Fuzessy et al. 2016).

It has been 14 years since Samuels and Levey (2005) posed the question *“Do germination experiments answer the questions they ask?*”, highlighting the importance of choosing appropriate treatments to answer questions about the effectiveness of gut passage on seed dispersal. At that time 77% of studies used the comparison between germination of gut-passed and manually extracted seeds in their study design (Samuels and Levy 2005). We therefore conducted a review of the literature since 2005, restricting our search to mammals only, to assess whether studies are now appropriately designed to answer such questions. We used the following search terms in a Web of Science topic search (which includes title, abstract and keywords) for the period January 2005 to May 2019: GERMINATION, SEED, EXPERIMENT OR DISPERS*, "GUT PASSAGE" OR "SEED HANDLING" OR SPIT*. Whilst noting that the review was not exhaustive, the search generated 212 papers in total, 69 of which investigated the likelihood of seed germination following spitting by, or passage through the gut of, mammal seed dispersers (Supplementary Information Table A).

Of these 69 studies, 62% used the disinhibition effect, rather than entire fruit, as the control treatment. Of the 59 studies investigating dispersal of fleshy-fruit seeds, 76% omitted entire fruit controls. Of the 66 studies investigating dispersal via gut passage 76% excluded the fertiliser effect of faecal matrix. Furthermore, only 9% of studies included manual, chemical and/or mechanical scarification of de-pulped seeds along-side gut-passed seeds, which would isolate the effect of scarification processes. Without differentiating each fundamentally different mechanism, the absolute effect of frugivores on seed germination is confounded (Samuels and Levey 2005; Robertson et al. 2006; Baskin and Baskin 2014) and these studies are weakened in their conclusions. Therefore, it is important to consider all processes and include entire fruits as a control group to estimate describe the role of frugivores in seed dispersal.

An additional issue is that many experiments are conducted *ex-situ* in laboratories (e.g. Kankam and Oduro 2012; Lessa et al. 2013), growth chambers and incubators (e.g. Carvalho-Ricardo et al., 2014; Maldonado et al., 2018), while few studies (e.g. Chen et al., 2016; Sánchez de la Vega and Godínez-Alvarez, 2010) conduct germination experiments in the field where natural environmental conditions fluctuate. Experiments conducted in controlled conditions often report that the effect of gut passage on seed germination is consistently higher than controls, whilst in field experiments, studies most often report no significant difference (Fuzessy et al. 2016), highlighting the importance of well-designed and controlled *in situ* studies.

Therefore, the aim of our study was two-fold. Firstly, we investigated the effect of seed processing mechanisms by a mammalian disperser on seed germination using all ecologically relevant seed treatments and entire fruit controls. Secondly, we assessed if a biased study design, excluding one or more of those treatments, would under- or over-estimate such a role. Specifically, how would our interpretation of the role of our frugivore in seed dispersal change if we excluded entire fruits as control groups and/or the fertiliser effect from our experiments, as is often the case in the literature. We predicted that, compared with entire fruit controls, seeds processed by a mammalian frugivore would demonstrate increased germination potential. We predicted that by excluding entire fruit controls and the fertiliser effect from our analyses, we would underestimate the role samango monkeys have in enhancing seed germination potential as we would be excluding a baseline from which to measure the magnitude of the effect and ecologically relevant seed processing mechanisms, namely the disinhibition effect and the faecal matrix.

In this study we focussed on a species of guenon monkey, the samango monkey (*Cercopithecus albogularis schwarzi*), as they make an ideal model species for seed dispersal studies for several reasons. Firstly, primates can consume larger fruits and seeds than most birds (Chapman and Onderdonk 1998; Balcomb and Chapman 2003), process fruit in different ways, and can be classified as seed-swallowers, seed-spitters and seed-destroyers (Corlett and Lucas 1990). The majority of primates species are seed swallowers (Lambert 1999; Clark et al. 2002; Dominy and Duncan 2005), however guenons are unique in that they display multiple seed-processing mechanisms. They are generally seed-swallowers of seeds smaller than 4 mm (on longest axis), seed-spitters of seeds larger than 4 mm (Lambert 1999; Gross-Camp and Kaplin 2011; Linden et al. 2015) and seed destroyers as they can crush seeds during oral processing of fruits and often consume unripe fruits (Wrangham et al. 1998; Lambert 1999). Mechanical and chemical scarification of seeds during oral processing may have similar effects to the scarification effects during gut passage, though to a lesser degree (Anzures-Dadda et al. 2016), or may have only a disinhibitory effect through pulp removal.

Secondly, guenons have a broad distribution from Ethiopia to South Africa (Lawes 1990; Grubb 2003) and are highly frugivorous, with more than 50% of their diet consisting of fruit (Lawes 1991; Chapman et al. 2002). In South Africa, compared with other South African primates, samango monkeys spend most of their time in and are highly dependent on, high-canopy evergreen forest (Linden et al. 2015). Samango monkeys are the only large-bodied arboreal species that feed on medium to large sized fruits in the canopy layer of South Africa’s evergreen forests (Coleman and Hill 2014; Linden et al. 2015, 2016). As such, the loss of samango monkeys could have negative cascading effects on the forests in which they reside.

We designed our germination experiments to isolate the effects of processing mechanisms on germination success. We selected five locally abundant fleshy-fruiting plant species that represented different seed processing mechanisms and were common in the diet of samango monkeys (Coleman 2013; Linden et al. 2015). The five species were three *Ficus* spp. which we assessed at genus level and two fleshy-fruiting tree species, *Searsia chirindensis* (red currant) and *Syzygium cordatum* (water berry), which we assessed at species level. We assessed the effects of five treatments that represented biological processes and one control treatment (entire fruit). The treatments were the disinhibition effect (simulating spitting and isolating pulp removal), scarification effect (simulating gut passage and isolating the mechanical effects of gut passage), gut passage effect (collected from faeces and isolating the chemical effect of gut passage) and the fertiliser effect (sown in faeces and isolating the effect of the faecal matrix). We also tested the added effect of seed desiccation following the disinhibition effect for one species, to simulate dispersal into dry habitat.

2. METHODOLOGY

2.1 Field Experiments

2.1.1 Study Area

We conducted germination trials between January and May 2018 at the Lajuma Research Centre (29°26'E, 23°01'S) in the far western Soutpansberg mountain range of South Africa's Limpopo Province. The Soutpansberg has an altitudinal range between 1150 m to 1750 m and local climate conditions are mesothermal with distinct cool dry winter (April to September) and warm wet summer (October to March) seasons (Munyati and Kabanda 2009). Vegetation at the study site is characterised by a complex mosaic of vegetation types including Limpopo Mistbelt Forest, Soutpansberg Moist Mountain Thickets and Soutpansberg Mountain Bushveld (Maltitz et al. 2003; Mostert et al. 2008). The south-facing ridges of the mountain range receive orographic and increased localised rainfall (Mostert 2006) which support high-canopy evergreen forests at their base (Linden et al. 2015). Away from the ridges, the evergreen forest transitions into semi-deciduous woodland, thicket, grassland and intersecting riverine forest (Maltitz et al. 2003; Mucina and Rutherford 2006).

2.1.2 Study Species

In this study we followed two groups of well habituated samango monkeys, "Barn Group", comprising approximately 40 individuals and "House Group", comprising approximately 60-70 individuals (Emerson et al.

2011; Coleman and Hill 2014; Nowak et al. 2014), between December 2016 and May 2017. Both groups comprised one resident male, multiple females, and their sub-adult and infant offspring. Between March and May, four to six bachelor males joined both groups intermittently for the mating season. The monkeys have been studied since 2004 (Ian Gaigher, pers. comm.) and we were able to follow them at a minimum distance of 5 m, using binoculars when they were high in the canopy.

For germination experiments, we selected fruiting tree species based on samango monkey feeding preferences in the preceding years (2015 – 2017 inclusive). We calculated the annual proportion of fruit species in the diet using data collected by the Primate and Predator Project, as described by Coleman (2013), subsequently selecting the ten most consumed fruit species, in terms of proportion of time spent feeding. We also took into account the ways in which the monkeys disperse the seeds, as well as the fruiting season of the trees, ensuring we were able to collect sufficient quantities of monkey-dispersed seeds and fresh ripe fruits for controls and manipulated treatments. Based on these criteria, we selected three *Ficus* species, *F. burkei* (common wild fig), *F. craterostoma* (forest fig), *F. sur* (broom-cluster fig), and two other species, *Searsia chirindensis* (red currant, formerly *Rhus*) and *Syzygium cordatum* (water berry) (Table 1). We could only identify *Ficus* spp. seeds recovered from faecal samples to genus level due to the high similarity and very small (< 2 mm) seed size. However, we are confident that only these three species of *Ficus* appeared in faeces, as there were no other *Ficus* species present in the area. Because we were not able to distinguish between *Ficus* spp. seeds we pooled all *Ficus* spp. for subsequent analyses. All three species were monoecious (hermaphrodite) (van Noort and Rasplus 2020) and we assigned seeds and entire fruits randomly to each treatment. We observed the primary method of dispersal by samango monkeys for *Ficus* spp. and *S. chirindensis* seeds to be via ingestion and defecation. Monkeys dropped partially consumed fruit under the parent tree and, to a lesser degree, spat out seeds either under the parent tree, or away from it following storage in cheek pouches (Linden et al., 2015). Monkeys spat *S. cordatum* seeds under the parent tree as they consumed the fruit, and occasionally away from the parent tree following storage in cheek pouches. Seeds of *Ficus* spp. and *S. chirindensis* are orthodox and can withstand desiccation whilst *S. cordatum* seeds are recalcitrant and are sensitive to desiccation (Roberts 1973; Royal Botanic Gardens Kew 2017).

Species [a]	Plant and Dispersal Traits			Mean Dimensions (mm ± SD)			Published Seed Data		
Scientific Name (Family) Common Name | Local Venda Name	Habitat [b]	Typical Fruiting Period [b]	Samango Dispersal (% Diet) [c]	Fruit H x W	Seed H x W	$\bar{x}$ no. seeds	Desiccation Tolerance	Germination	
								Time (days)	Proportion (%)
Ficus burkei (Miq.) Miq. (Moraceae) Common wild fig | Muumo	woodland / wooded grassland	year-round	Ingest / Spat (9.3%)	12.1 x 10.6 (± 1.7 x 1.8)		122 (± 44)			
Ficus craterostoma Warb. ex Mildbr. & Burret (Moraceae) Forest fig | Tshikululu	evergreen forest (strangler fig)	Aug - Dec (variable)	Ingest / Spat (8.7%)	14.1 x 12.1 (± 0.9 x 1.1)	genus $\bar{x}$ 1.3 x 0.8 (± 0.1 x 0.1)	180 (± 59)	Orthodox [d]	42 – 70 [d]	64 – 100 [d]
Ficus sur Forssk. (Moraceae) Broom cluster fig | Muhuyu	forest / riverine fringes / open woodland	Sep - Mar (variable)	Ingest / Spat (9.4%)	29.4 x 33.2 (± 4.2 x 6.1)		795 (± 319)			
Searsia chirindensis [i] (Baker f.) Moffett (Anacardiaceae) Red currant | Muvhadela-phanga	open woodland / mountain scrub / forest	Nov - Feb	Ingest / Spat (7.1%)	3.8 x 4.1 (± 0.4 x 0.5)	3.4 x 4 (± 0.5 x 0.4)	1	Orthodox [e]	35 – 56 [f]	not available
Syzygium cordatum Hochst. Ex C.Krauss (Myrtaceae) Water berry | Mutu	occurs near water in a variety of habitats	Dec - April	Spat (4.3%)	13.1 x 9.1 (± 1.3 x 1.1)	8.9 x 6.6 (± 1.6 x 1.1)	1	Recalcitrant [g]	18 – 40 [h]	90 - 99 [h]

[a] *Ficus* spp. seeds and entire fruit controls were selected randomly from these three species as we were unable to identify seeds retrieved from faecal samples to species

[b] Coates-Palgrave 2002

[c] 2015 – 2017, based on data collected as described in Coleman 2013

[d] Royal Botanic Gardens Kew 2017 Data only available for *Ficus sur*; 100% of 52 known taxa of genus *Ficus* are Orthodox.

[e] Royal Botanic Gardens Kew 2017 Data not available for *S. chirindensis*; 100% of 8 other *Searsia* taxa are Orthodox.

[g] Royal Botanic Gardens Kew 2017

[h] Wilson and Downs 2012 Seeds soaked 24 h prior to sowing

[i] Formerly *Rhus chirindensis* Moffett 2007

[f] South African National Biodiversity Institute (SANBI) 2008

Table 1 Plant and samango monkey dispersal traits, seed morphology and germination information reported in the literature for species used in our germination experiments

2.1.3 Germination Experiments

2.1.3.1 Seed Treatments

We applied five seed treatments that isolated specific biological processes, and a control (Control) of entire fruits for each seed species (Table 2). The control, in which seeds were left unchanged, allowed us to isolate the effect of the second treatment and fully evaluate the effect of seed handling mechanisms on germination (Samuels and Levey 2005; Baskin and Baskin 2014). The second treatment replicated the disinhibition effect (Disinhibition) of pulp removal either in the mouth or following gut passage, and allowed us to isolate the effects of seed-spitting and the chemical and/or mechanical effects of gut passage in proceeding treatments (Traveset and Verdú 2002; Samuels and Levey 2005; Fuzessy et al. 2016). The third treatment isolated mechanical scarification (Scarification) during oral processing and passage through gut from the first treatment and additional chemical scarification in the third treatment (Baskin and Baskin 2014). The fourth treatment was the gut passage effect (Gut-Passage), which allowed us to isolate the chemical processes and transit time that alter the seed during gut passage from the disinhibition and mechanical scarification effects of the second and third treatments respectively (Traveset and Verdú 2002; Samuels and Levey 2005). The fifth treatment was the fertiliser effect (Fertiliser) which allowed us to isolate the effect of the faecal matrix and measure the gut passage effect separately (Traveset and Verdú 2002). We only used the fourth and fifth treatments with *Ficus* spp. *S. chirindensis* seeds, as *S. cordatum* were not swallowed by samango monkeys. Lastly, the sixth treatment measured the added effect of desiccation following disinhibition on *S. cordatum* seeds (Desiccation), which we used to simulate dispersal into unfavourable dry habitats, with a potentially negative effect on germination, as these seeds are recalcitrant (Roberts 1973; Royal Botanic Gardens Kew 2017). Hereafter, we refer to treatments as Control, Disinhibition, Scarification, Gut-Passage, Fertiliser and Desiccation.

Treatment / Effect	Description / Seed Source	Species	Trials / Seeds in Each Nursery		
			Number of Trials	Length (days)	Seeds / Trial [a]
Control	Entire fruit, no treatment / fresh ripe fruit collected from different trees for each trial	*Ficus* spp.	5	105	40 fruits
		Searsia chirindensis	5	62	20 fruits
		Syzygium cordatum	4	102	20 fruits
Disinhibition	Manual removal of seed from pulp and sown within 24 h / fresh ripe fruit collected from different trees for each trial	*Ficus* spp.	5	105	40
		Searsia chirindensis	5	62	20
		Syzygium cordatum	4	102	20

Scarification	Manual removal of pulp and seed coat damaged by rubbing once along 100 mm length of medium-coarse sandpaper / fresh ripe fruit collected from different trees for each trial	*Ficus* spp.	5	105	40
		Searsia chirindensis	5	62	20
		Syzygium cordatum	4	102	20
Gut-Passage	Seeds collected from fresh faeces after passage through the monkeys' gut and washed in untreated water	*Ficus* spp.	5	105	40
		Searsia chirindensis	5	62	20
Fertiliser	Seeds left in freshly collected faecal matrix after passage through the monkeys' gut, and sown into the soil	*Ficus* spp.	5	105	20
		Searsia chirindensis	4	62	16 - 20
Desiccation	Seeds collected after being spat out by the monkeys and stored for 14 days	*Syzygium cordatum*	4	102	20

[a] *Ficus* spp. seeds were sown five per cell to control for hollow seeds used by fig wasps and we counted each cell as one seed.

Table 2 Seed processing treatments of selected samango monkey-dispersed fruit species used in our germination experiments, including the number of seeds/fruits sown and number and duration of trials.

2.1.3.2 Seed Collection

We collected seeds used for Gut-Passage and Fertiliser treatments from fresh faecal samples and seeds for Desiccation treatment as the monkeys spat them out. To do this, we followed the samango monkey groups two days each week per group from dawn at their sleep site, until dusk when they settled at their sleep site. We collected fresh faecal samples from all age and sex classes *ad libitum* in separate plastic bags, noting the date and time of deposition on the bag. Faecal samples were stored overnight in dark sealed plastic boxes to keep them cool and processed early the following day. For the Fertiliser treatment, we visually inspected faecal samples for *Ficus* spp. or *S. chirindensis* seeds, removed all other seeds and divided the faecal sample into portions, ensuring identical numbers of sub-samples were used in each nursery. For *S. chirindensis*, we ensured seeds from faecal samples were sown in sufficient faecal matter to bury the seed into. Faecal samples not used in the Fertiliser treatment were gently washed, removing digesta and air-dried in a tent for 48 h. Once dry, we collected all entire and undamaged seeds from each sample, identified and counted them. We could only identify *Ficus* spp. to genus level, due to the high similarity and very small (< 2 mm) seed size. As *Ficus* spp. and *S. chirindensis* seeds are orthodox, we were able to store these seeds in paper bags in a sealed dark plastic box until needed, which we did for a maximum of ten days prior to sowing for the Gut-Passage treatment (Baskin and Baskin 2014).

We collected monkey-dispersed *S. cordatum* seeds immediately after being spat out, storing them for fourteen days also in paper bags in a sealed dark plastic box prior to sowing for the Desiccation treatment. These seeds are recalcitrant and we stored them prior to sowing to simulate dispersal into dry habitats, which the monkeys did in their cheek pouches. We collected entire fresh mature fruits from monkey feeding trees, which we used for Control, Disinhibition and Scarification treatments, processing them no longer than 24 h after collection. We removed seeds from fruit pulp by hand and left seeds to air-dry at room temperature for 24 – 48 h (Baskin and Baskin 2014), although seeds removed from *S. cordatum* fruit pulp were air-dried for a maximum of 24 h to avoid desiccation. We scarified *S. chirindensis* and *S. cordatum* seeds by rubbing them once along a 15 x 15 cm piece of medium-coarse sandpaper (Kimura and Islam 2012). Due to their small size, we scarified *Ficus* spp. seeds by placing all seeds used per trial into a small plastic tub with a piece of the same sandpaper glued to each end and shaking the tub in a circular motion for 5 min.

2.1.3.3 Germination Trials

We constructed two germination nurseries in an open area where there was no shading effect from trees or other structures. We built a wooden frame and covered one with 80% shade cloth (shaded) and the other with 20% shade cloth (unshaded) to simulate the difference in light conditions (hereafter Shading) within and outside of continuous canopy cover into which the monkeys may disperse seeds respectively. Within each nursery, we recorded temperature (ºC) and relative humidity (%) at 30 min intervals using Easy-Log USB 2+ data loggers (Lascar Electronics, Salisbury, UK) placed in the centre 1 m above ground level. We collected topsoil from a transitionary zone between the evergreen forest and semi-deciduous woodland. We collected sufficient topsoil for all trials at once, in order to homogenise the substrate between trials and eliminate inconsistencies in soil texture, porosity and chemical composition between trials. Soil pH was analysed by the Agricultural Research Council, Institute for Soil, Climate and Water, Pretoria, South Africa, and was 6.76 and 6.52 in the shaded and unshaded nursery respectively. We sieved the soil using a 1 mm mesh to remove large particles, seeds and plant matter, mixed it with quarry sand (5-parts soil to 1-part sand), and stored it in a covered black plastic container until required.

We sowed seeds in polystyrene germination trays (Plasgrow, Mpumalanga, South Africa), each containing 200 30 x 30 mm cells with a depth of 50 mm. We sowed one seed, faecal sample portion (Fertiliser) or entire fruit (Control) per cell (Table 3) at a depth of 15 mm, except *Ficus* spp., for which we sowed five seeds per cell to control for those used by fig wasps (Serio-Silva and Rico-Gray 2002), counting each cell as one seed. We sowed entire *Ficus* spp. fruits at depths according to their individual size (Table 1), allowing for a covering of 15 mm of soil. For all species, we marked the placement of the seed with a toothpick and undertook daily removal of seedlings that were not at that location. We left seedlings that emerged next to the toothpick to grow until we could identify them as our focal seed.

We used a nested experimental design whereby trays and cells in each nursery mirrored each other in terms of species, treatments, faecal samples and positioning within the nursery, placing the germination trays on wooden pallets 50 cm above the ground. We used one tray per trial in each nursery, with all seeds used for simulated treatments originating from the same source tree in each trial. Primate dispersed *Ficus* spp. and *S. chirindensis* seeds came from different faecal samples for each trial, although it was not always possible to use a single faecal sample per trial, as they did not always contain sufficient quantities of seeds. We evenly watered trays in both nurseries daily using a hosepipe and water piped directly from a stream in the forest, until the soil was wet to the touch. Soil within the shaded nursery remained permanently moist for the duration of the germination trials, whilst within the unshaded nursery, soil dried out daily between watering. We monitored germination daily and considered the seed to have germinated upon first emergence of the cotyledon from the soil. Each trial was terminated after the maximum published germination time (Table 1) plus either 10% or 14 days with no new germination (Table 2). We did not determine the viability of ungerminated seeds after each trial.

2.2 Data Analysis

We conducted separate analyses for each species using Generalised Linear Mixed-Effects Models (GLMMs) in R version 3.5.1. (R Core Team 2018) using the lme4 package (Bates et al. 2015). Recent reviews recommend GLMMs as the preferred mode of analysis for germination data (Sileshi 2012), as the model allows analysis of nested designs such as our dichotomous shade effect, as well as allowing for non-normal distributions of the response variable and random effect specification (Schupp 1993; Bolker et al. 2009). In all cases, we modelled seed treatment (four or five levels depending on species) and the interaction between treatment and shading (two levels, shaded and unshaded) as fixed effects, to evaluate separately the effects of each treatment on the probability of germination (binomial) and germination latency (number of days taken to germinate). We included trial nested in shading (or nursery) as a random effect to control for spatiotemporal variation across trials, as we could not sow all trials at the same time due to accessible fruit availability across the study site. We verified parsimony of the model against a reduced model omitting the interaction term, followed by a null model of the random effect only using a likelihood ratio test. We assessed optimal model adequacy by examining standard residuals vs. fitted residuals and graphical distribution of errors and conducted Wald Chi-Square tests to determine the significance of the fixed effects at $p = 0.05$. To isolate the effects of the different treatments on seed germination, we conducted Least-Squares Means analysis of the final model using the emmeans package (Lenth 2018) and performed pairwise Tukey' HSD comparisons using the multcomp package (Hothorn et al. 2008), to compare treatments with control seeds.

3. RESULTS

3.1 Probability of Germination

We had a total germination success of 57.1% in *Ficus* spp., 25.3% in *S. chirindensis* and 53.9% in *S. cordatum* seeds. There was no interaction effect between shading and treatment on the probability of germination in all three fruit-tree species (Table 3). However, there was a significant effect of seed processing treatment on the probability of germination (Table 3) and significant pairwise differences between treatments (Fig. 1). All pairwise comparisons with Control were significant except for Fertiliser in *Ficus* spp. and Gut-Passage and Fertiliser in *S. chirindensis*. Non-significant pairwise comparisons are given in Supplementary Information Table B.

For both *Ficus* spp. and *S. chirindensis* seeds, our analyses indicated Disinhibition and Scarification treatments had the highest probability of germination, both of which were significantly higher than Controls, but not different from each other (Tukey's HSD test $Z = 1.091$, $p = 0.81$ and $Z = -3.03$, $p = 0.99$, respectively). In *Ficus* spp. seeds, Gut-Passage significantly reduced the probability of germination from Controls, and Fertiliser significantly increased the probability of gut passed seeds germinating, however our analyses indicated this was not significantly different from Controls (Tukey's HSD test $Z = 2.12$, $p = 0.21$). For *S. chirindensis*, we found neither Gut-Passage nor Fertiliser significantly affected the probability of seeds germinating compared with Controls (Tukey's HSD test $Z = -0.95$, $p = 0.87$ and $Z = -1.42$, $p = 0.6$ respectively), and Fertiliser had no additional benefits to Gut-Passage (Tukey's HSD test $Z = -0.56$, $p = 0.98$). In *S. cordatum*, our analyses indicated Disinhibition significantly increased the probability of germination compared with Controls, although our analyses also indicated a considerable reduction in the benefit of Disinhibition following Desiccation. Compared with Disinhibition, Scarification reduced the probability of germination, although not significantly (Tukey's HSD test $Z = 2.48$, $p = 0.05$).

	GLMM Wald Chi-square Test			
Species	Effect	x^2	df	*p*-value
Ficus spp.	Shading : Treatment	10.02	5	0.07
	Treatment	112.14	4	<0.001
Searsia chirindensis	Shading : Treatment	2.65	5	0.75
	Treatment	35.49	4	<0.001
Syzygium cordatum	Shading : Treatment	2.67	4	0.16
	Treatment	61.88	3	<0.001

	Significant Tukey's HSD Pairwise Comparisons			
	Effect Comparison	Estimate ±SE	*Z*-value	*p*-value
Ficus spp.	Control – Disinhibition	-0.7 ±0.17	-4.2	<0.001
	Control – Scarification	-0.52 ±0.16	-3.27	0.009
	Control – Gut-Passage	0.99 ±0.15	6.5	<0.001
	Disinhibition – Gut-Passage	1.7 ±0.16	9.45	<0.001
	Disinhibition – Fertiliser	0.99 ±0.16	6.32	<0.001
	Scarification – Gut-Passage	1.52 ±0.17	8.77	<0.001
	Scarification – Fertiliser	0.81 ±0.15	5.45	<0.001
	Gut-Passage – Fertiliser	-0.71 ±0.14	-4.98	<0.001
Searsia chirindensis	Control – Disinhibition	-1.6 ±0.41	-3.86	<0.001
	Control – Scarification	-1.66 ±0.42	-3.98	<0.001
	Disinhibition – Gut-Passage	1.14 ±0.31	3.65	0.002
	Disinhibition – Fertiliser	0.92 ±0.31	2.96	0.02
	Scarification – Gut-Passage	1.2 ±0.32	3.8	0.001
	Scarification – Fertiliser	0.99 ±0.32	3.12	0.01
Syzygium cordatum	Control – Disinhibition	-7.2 ±1.52	-4.75	<0.001
	Control – Scarification	-3.75 ±0.68	-5.54	<0.001
	Control – Desiccation	-2.06 ±0.65	-3.17	0.007
	Disinhibition – Desiccation	5.25 ±1.38	3.72	<0.001
	Scarification – Desiccation	1.69 ±0.28	5.94	<0.001

Table 3 Model output of the GLMM Wald Chi-square test on the effect of treatment and the interactive effect of shading (shaded/unshaded) and treatment, with Tukey's HSD pairwise comparison tests on the probability of germination for selected samango monkey-dispersed fruit species' seeds. Treatments: Control, entire fruit, no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage (*Ficus* spp. and *S. chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp. and *S. chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).

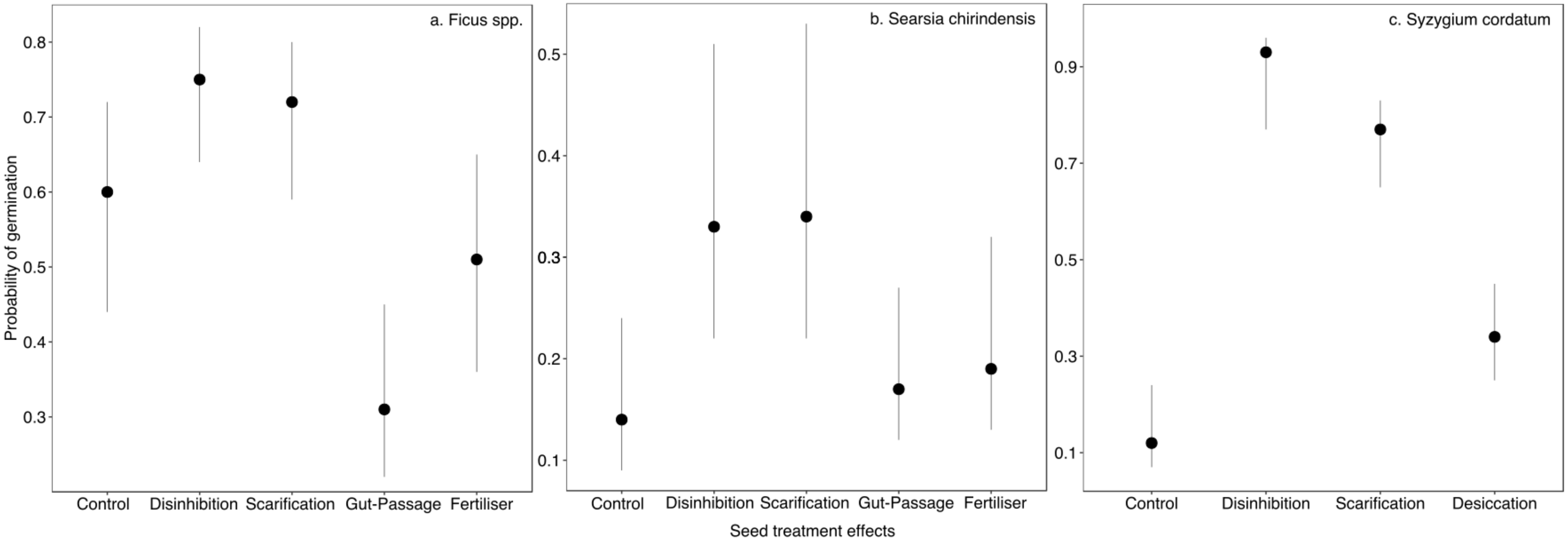

Fig. 1 Least squares means probability of germination (± 95% CI) of selected samango monkey-dispersed fruit species' seeds subject to different processing treatments. Treatments: Control, entire fruit no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation, disinhibition *plus* desiccation.

3.3 Germination Latency

We observed similar germination patterns between all treatments in *Ficus* spp. and *S. chirindensis* seeds, with 50% germination occurring between 25 – 30 and 18 – 20 days respectively (Fig. 2). We observed similar patterns in germination between two pairs of treatments in *S. cordatum*, with 50% germination occurring after 21 and 16 days in Disinhibition and Scarification treatments respectively, and after 57 and 45 days in Controls and Desiccation treatments respectively (Fig. 2).

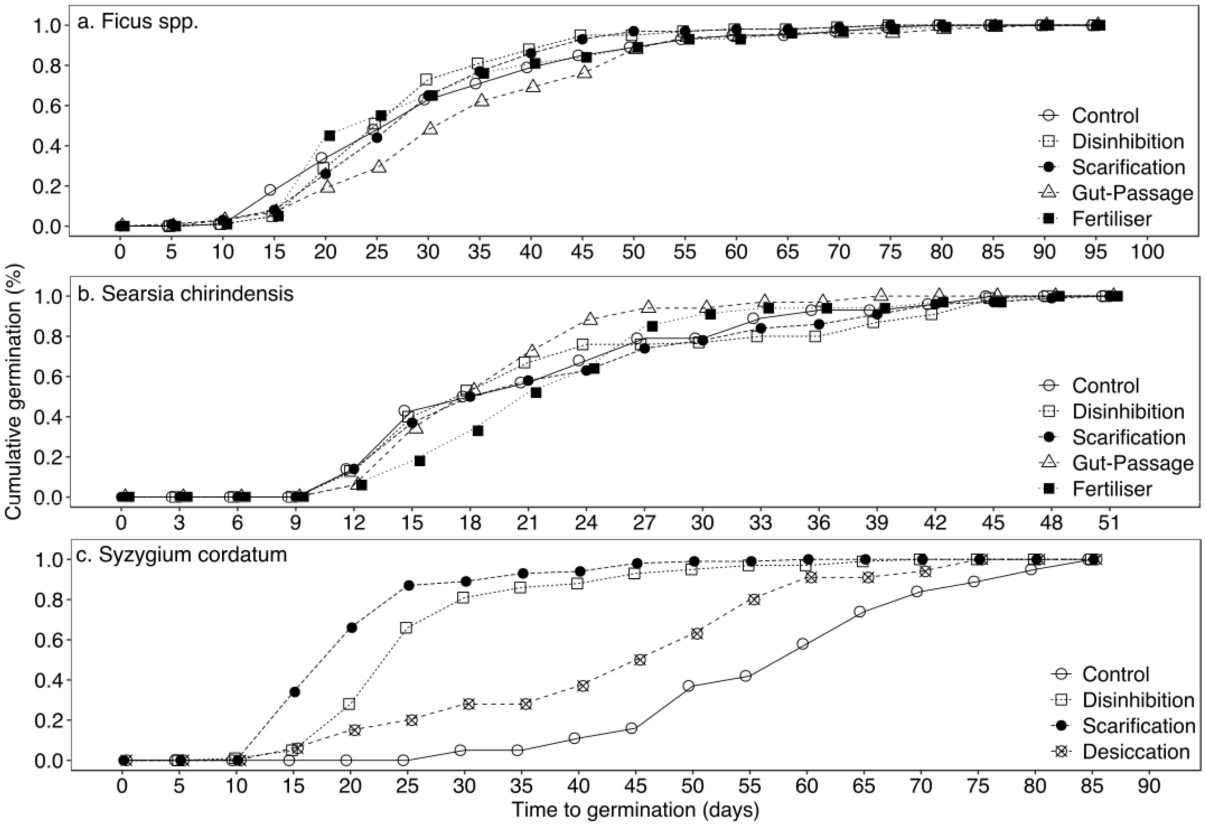

Fig. 2 Cumulative germination (%) of selected samango monkey-dispersed fruit species' seeds subject to different processing treatments. Treatments: Control, entire fruit no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation, disinhibition *plus* desiccation

Our analyses indicated significant interaction effects between treatment and shading on germination latency for *Ficus* spp. and *S. cordatum* seeds (Table 4); however, we found no significant effect of shading on germination latency within treatments in either of these species (Fig. 3). There was no significant interaction effect between shading and treatment in *S. chirindensis.* Non-significant pairwise comparisons are given in Supplementary Information Table B.

	GLMM Wald Chi-square Test			
Species	Effect	x^2	df	*p*-value
Ficus spp.	Shading : Treatment	2192.6	5	<0.001
	Treatment	3376.8	5	<0.001
Searsia chirindensis	Shading : Treatment	9.22	5	0.1
	Treatment	12.38	4	0.01
Syzygium cordatum	Shading : Treatment	12.3	4	0.02
	Treatment	142.52	3	<0.001

		Significant Tukey's HSD Pairwise Comparisons			
	Shading	Effect Comparison	Estimate ±SE	*Z*-value	*p*-value
Ficus spp.	Shaded	Control – Gut-Passage	-8.35 ±1.97	-4.23	<0.001
		Disinhibition – Gut-Passage	-6.51 ±1.91	-3.42	0.02
		Gut-Passage – Fertiliser	6.4 ±2.05	3.12	0.04
	Unshaded	Control – Disinhibition	5.81 ±1.61	3.62	0.008
		Control – Scarification	6.79 ±1.61	4.23	<0.001
		Disinhibition – Gut-Passage	-7.61 ±1.96	-3.9	0.003
		Scarification – Gut-Passage	-8.62 ±1.96	-4.4	<0.001
	Unshaded : Shaded [a]	Not Significant			
Searsia chirindensis		Shading not significant			
		Control – Gut-Passage	0.32 ±0.09	3.35	0.007
Syzygium cordatum	Shaded	Control – Disinhibition	32.63 ±7.37	4.57	<0.001
		Control – Scarification	41.97 ±7.35	7.71	<0.001
		Disinhibition – Scarification	8.35 ±1.23	6.81	<0.001
		Disinhibition – Desiccation	-14.2 ±7.35	2.68	<0.001
		Scarification – Desiccation	-22.55 ±7.35	2.6	<0.001
	Unshaded	Control – Disinhibition	32.07 ±5.75	5.57	<0.001
		Control – Scarification	36.63 ±5.74	6.38	<0.001
		Disinhibition – Scarification	4.56 ±1.45	3.12	0.03
		Disinhibition – Desiccation	-18.95 ±3.43	-5.53	<0.001
		Scarification – Desiccation	-23.5 ±3.43	-6.86	<0.001
	Unshaded : Shaded [a]	Control – Disinhibition	34.6 ±6.06	5.71	<0.001
		Control – Scarification	42.95 ±6.03	7.12	<0.001
		Control – Desiccation	20.4 ±6.48	3.15	0.03
		Disinhibition – Control	-31.1 ±7.63	-4.08	<0.001
		Disinhibition – Scarification	10.87 ±2.35	4.64	<0.001
		Disinhibition – Desiccation	-11.67 ±3.34	-3.5	0.008
		Scarification – Control	-35.65 ±7.61	-4.68	<0.001
		Scarification – Desiccation	-16.23 ±3.31	-4.9	<0.001
		Desiccation – Disinhibition	21.48 ±3.92	5.48	<0.001
		Desiccation – Scarification	29.82 ±3.87	7.71	<0.001

[a] The first treatment listed was in unshaded conditions

Table 4 Model output of the GLMM Wald Chi-square test on the effect of treatment and the interactive effect of shading (shaded/unshaded) and treatment, with significant Tukey's HSD pairwise comparison tests on germination latency for selected samango monkey-dispersed fruit species' seeds. Treatments: Control, entire fruit, no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage (*Ficus* spp.

and *S. chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp. and *S. chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).

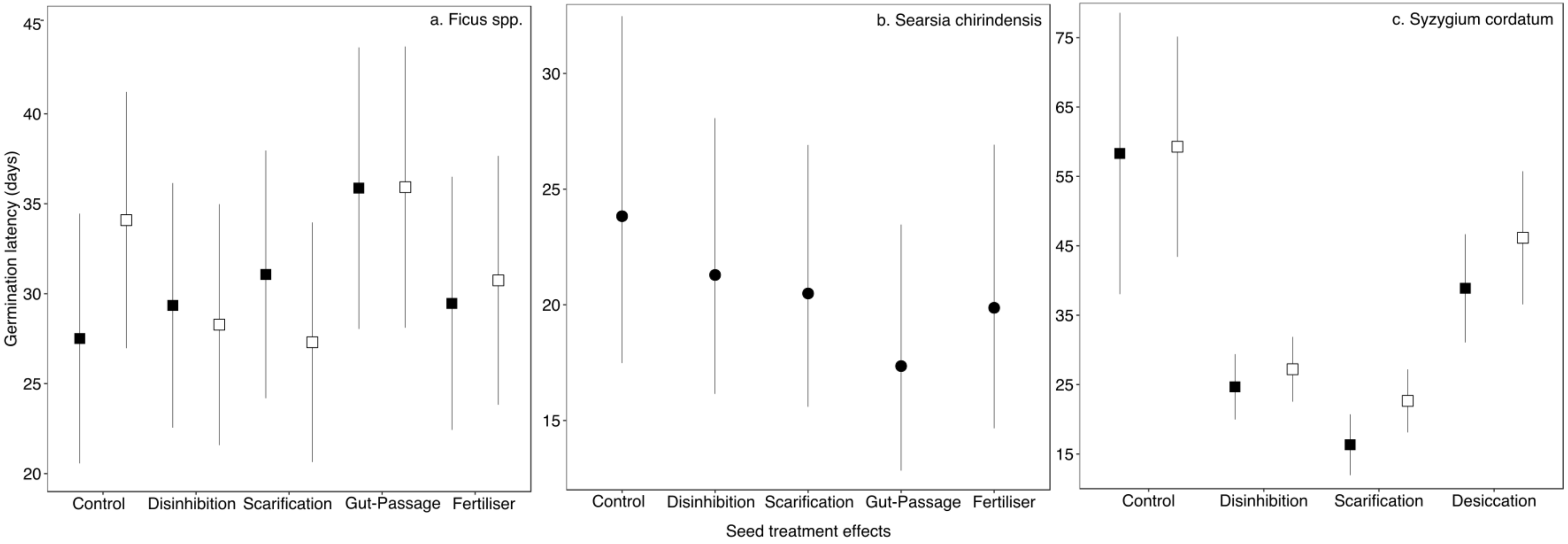

Fig. 3 Least squares means germination latency (± 95% CI) of selected samango monkey-dispersed fruit species' seeds subject to different processing treatments. Treatments: Control, entire fruit no treatment; Disinhibition, effect of pulp removal; Scarification, disinhibition *plus* mechanical scarification of seed coat; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification; Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix; Desiccation, disinhibition *plus* desiccation. Interactive effect of treatment & shading: ● no significant interacting effect; ■significant interactive effect shaded, □ significant interactive effect unshaded).

In shade, our analyses indicated that in *Ficus* spp., Gut-Passage significantly increased germination latency compared with Disinhibition and Fertiliser treatments and Controls. Unshaded, our analyses indicated that both Disinhibition and Scarification significantly reduced germination latency compared with the other treatments and Controls. We found no significant interacting effects between treatment and shading for *S. chirindensis* seeds, and our analyses indicated a significant difference in germination latency between Controls and Gut-Passage, the latter of which germinated fastest (Table 4). All three treatments reduced germination latency of *S. cordatum* seeds compared with Controls, which we found took the longest to germinate. Our analyses indicated that Scarification significantly reduced germination latency compared with all other treatments, with those sown in shade germinating fastest.

3.4 Exclusion of Ecologically Relevant Seed Treatments

To identify if exclusion of ecologically relevant seed treatments or relevant controls would affect our interpretations of the impact of the disperser on seed germination, we considered the positive, negative or neutral effects our disperser had on the probability of seed germination (Table 5). Firstly, we considered exclusion of entire fruit control groups (Controls), instead assessing the difference in probability of germination between manual removal of seeds from mature fruits (Disinhibition) and seeds either removed from faecal samples (Gut-Passage, *Ficus* spp. and *S. chirindensis*), or collected after being spat out (Desiccation, *S. cordatum*). Our analysis indicated negative disperser effects in each of these comparisons as compared with Disinhibition the probability of germination in seeds from Gut-Passage and Desiccation treatments was significantly lower. When we included Controls, disperser effects were positive (Disinhibition and Desiccation) for *S. cordatum*, positive (Disinhibition) and neutral (Gut-Passage) for *S. chirindensis*, and positive (Disinhibition) and negative (Gut-Passage) for *Ficus* spp. seeds. Secondly, we considered the disperser effect when we include the effect of the faecal matrix (Fertiliser) in species with gut-passed seeds. Our analysis indicated neutral effects when compared with both Controls and Gut-Passage in *S. chirindensis*, and neutral and positive effects when compared with Controls and Gut-Passage respectively in *Ficus* spp. seeds.

Species	Comparison	GLMM Tukey's HSD Pairwise Comparisons			Impact on Germination
		Z-Value	*p*-Value	Difference	
Exclusion of Entire Fruit Control and Fertiliser Effect					
Ficus spp.	Disinhibition – Gut-Passage	9.45	<0.001	- 44.28 %	Negative
Searsia chirindensis	Disinhibition – Gut-Passage	3.65	0.002	- 16.2 %	Negative
Syzygium cordatum	Disinhibition – Desiccation	3.72	<0.001	- 59.46 %	Negative
Inclusion of Entire Fruit Control					

Ficus spp.	Control – Disinhibition	-4.2	<0.001	+ 15.57 %	Positive
	Control – Gut-Passage	6.5	<0.001	- 28.71 %	Negative
Searsia chirindensis	Control – Disinhibition	-3.86	<0.001	+ 19.22 %	Positive
	Control – Gut-Passage	-0.95	0.87	+ 3.02 %	Neutral[a]
Syzygium cordatum	Control – Disinhibition	-4.75	<0.001	+ 81.59 %	Positive
	Control – Desiccation	-3.17	0.007	+ 22.1 %	Positive
Inclusion of Fertiliser Effect					
Ficus spp.	Control – Fertiliser	2.12	0.21	- 8.84 %	Neutral[a]
	Gut-Passage – Fertiliser	-4.98	<0.001	+ 19.87	Positive
Searsia chirindensis	Control – Fertiliser	-1.42	0.6	+ 4.9 %	Neutral[a]
	Gut-Passage – Fertiliser	-0.56	0.98	+ 1.88 %	Neutral[a]

[a] Non-significant difference

Table 5 Comparison between exclusion/inclusion of entire fruit control groups and/or the fertiliser effect, using model output of the GLMM Tukey's HSD pairwise comparison tests on the probability of germination for selected samango monkey-dispersed fruit species' seeds. Treatments: Control, entire fruit, no treatment; Disinhibition, effect of pulp removal; Gut-Passage, disinhibition *plus* mechanical *and* chemical scarification during gut passage (*Ficus* spp. and *S. chirindensis*); Fertiliser, gut passage effect *plus* fertiliser effect of the faecal matrix (*Ficus* spp. and *S. chirindensis*); Desiccation, disinhibition *plus* desiccation (*S. cordatum*).

4. DISCUSSION

In a review conducted in 2005, 77% of germination studies omitted using entire fruits as controls in experiments assessing the effect of gut passage on seed germination (Samuels and Levey 2005). In our review of mammalian germination studies, 76% of studies used manually extracted seeds as controls demonstrating a lack of progression. Furthermore, 76% of studies excluded the fertiliser effect and only 9% included mechanical scarification of de-pulped seeds along-side gut-passed seeds. Without isolating each fundamentally different mechanism, the absolute effect of frugivores on seed germination is confounded (Samuels and Levey 2005; Robertson et al. 2006; Baskin and Baskin 2014) and these studies are weakened in their conclusions. Our analyses indicated that samango monkeys are effective in the qualitative side of seed dispersal, as seed processing mechanisms had a positive and/or neutral influence on germination potential of seeds compared with entire fruits where seeds were untreated. As is widely reported (Robertson et al. 2006), removal of seeds from fruit pulp (*the disinhibition effect*) had the greatest positive effect on the probability of germination in each of the selected fruit species we assessed. Excluding entire fruit as control groups from our analyses altered the conclusions drawn from our data, thus underestimating the influence seed handling by samango monkeys has on seed germination. As such, our results concur with others, and reiterate the need for inclusion of all ecologically relevant treatments that simulate dispersal modes of frugivores, to estimate accurately their role in seed dispersal (Samuels and Levey 2005; Baskin and Baskin 2014; Fuzessy et al. 2016).

4.1 Effect of samango monkeys on seed germination

The disinhibition effect had a significant positive influence on the probability of germination compared with control groups of entire fruits in each of the fruit species we assessed, suggesting that as seed-spitters samango monkeys have an important role in their dispersal. This was most noticeable in *S. cordatum* where we observed samango monkeys to disperse seeds via spitting as they were consuming fruits in the parent tree or following storage in cheek pouches. Our results are similar to previous research conducted on *Syzygium legatii* (mountain water-berry) seeds spat out by samango monkeys (B. Linden, unpublished data), as well as studies on *Syzygium* spp. in other taxa (e.g. Albert et al., 2013; Gross-Camp and Kaplin, 2005; Sengupta et al., 2014). The role of samango monkeys in *S. cordatum* seed dispersal may also be greatly dependent on where they deposit seeds, as shown by the significant reduction in the probability of germination of seeds we subjected to desiccation, compared to freshly de-pulped seeds. As recalcitrant seeds are sensitive to desiccation (Roberts 1973), it may be that *S. cordatum* relies on seed-spitters to remove pulp and that germination must occur soon after before seeds desiccate. Mature *S. cordatum* trees are typically located close to water and dispersal under or close to parent trees, where the environment can slow the desiccation process, may confer an advantage to seeds. Compared with the disinhibition effect, we found scarification to have a non-significant negative effect on the probability of germination and a significant reduction on germination latency in *S. cordatum*. Damage to the protective seed coat through scarification or removal has been shown to reduce cumulative germination in *S. cordatum* (Prins and Maghembe 1994). Guenon cheek pouches contain a high level of α-amylase, a saliva enzyme involved in pre-digestion breakdown of starch (Murray 1975), which may reduce the need for high-molar mastication and therefore scarification damage to seeds in soft-pulped fruits. The significant benefits in increased germination and reduced latency to *S. cordatum* seeds through pulp removal are likely to help these seeds germinate and establish during the wet season. This is further evident in the significantly reduced benefits we observed when we dried spat seeds prior to sowing

While the disinhibition effect had positive effects on germination in each of the species we assessed, the gut passage effect was negative in *Ficus* spp. and neutral in *S. chirindensis*. In *Ficus* spp., the gut passage effect significantly reduced the probability of germination, and increased germination latency, more than every other treatment. The effect of gut passage on *Ficus* spp. seeds reported in the literature are highly variable, with positive (e.g. Chen et al., 2017; Figueiredo, 1993; Mosallam, 1996; Oleksy et al., 2017), negative (e.g. Chang et al., 2016; Compton et al., 1996; Tsuji et al., 2017) and neutral (e.g. Heer et al., 2010; Maccarini et al., 2018; Stevenson et al. 2002) effects reported from a wide range of frugivorous mammals. Previous research on samango monkeys found the gut passage effect significantly reduced germination of *Ficus* spp. seeds compared to the disinhibition effect but had no significant effect on *Keetia gueinzii* (climbing turkey-berry) seeds (B. Linden, unpublished data). In *S. chirindensis*, we found the gut passage effect on seed germination was neutral compared with entire fruit controls, although the gut passage effect significantly reduced germination latency. Again, results reported in the literature for *Searsia* spp. are variable (e.g. Foord et al., 1994; Mosallam, 1996).

The highly variable results reported for these species suggest there are likely to be species- and/or site-specific variables not typically measured in mammalian frugivore germination experiments that influence seed germination following gut passage. These may include chemicals in the gastrointestinal (GI) tract that may vary in respect to dietary intake of dispersers, as well as anatomical differences of the GI tract that may increase the passage time of seeds and therefore time available for chemical and mechanical scarification (Traveset et al. 2007). However, while germination latency increased in *Ficus* spp., seeds were still viable following passage through the gut, and in *S. chirindensis*, gut-passage significantly reduced germination latency.

More surprisingly, in *Ficus* spp.**,** the fertiliser effect increased germination success and reduced germination latency compared to gut passage alone, and this effect was neutral compared to entire fruit controls. We observed a similar neutral effect in *S. chirindensis*. The majority of mammalian frugivore germination studies remove seeds from faecal samples to test the gut passage effect and the literature including the fertiliser effect is scarce. In our literature search, 93% of mammalian frugivore germination studies omitted the fertiliser effect. Where the effect is included, results are again variable (Heer et al., 2010; Rojas-Martinez et al., 2015; Tutin et al., 1991; Valenta and Fedigan, 2009). Lethal effects reported in some studies (e.g. Anzures-Dadda et al., 2016) may result from seeds being removed from faecal samples and washed prior to re-covering with fresh faeces, increasing susceptibility to fungal attack (Wang and Smith 2002).

Our *in-situ* germination experiments were subject to natural variation of abiotic factors: temperature, humidity and sunlight levels and are in contrast to *ex-situ* studies conducted under controlled conditions (e.g. Anto et al., 2018; Figueiredo, 1993; Kankam and Oduro, 2012). Significant differences between the gut passage and disinhibition effects in field experiments are rarely reported, with the majority of positive influences coming from studies conducted in laboratory settings (Fuzessy et al. 2016). The significant decrease in the probability of germination we report from gut passed *Ficus* spp. seeds requires further investigation.

Ficus spp. fruits are considered keystone resources for many animal species including primates (Estrada and Coates-Estrada 1984), as their typical asynchronous year-round fruiting can be a reliable food source (Bleher et al. 2003). Figs are synconium (Galil 1977) containing hundreds of small (<2 mm) imbedded seeds (Lambert 1999). The lengthy processing time it would take to orally remove the seeds means samango monkeys are likely to swallow more than they spit out (Lambert 1999). As such, *Ficus* spp. may trade reduced quality of dispersal of gut-passed seeds for quantity, relying instead on the unpredictable heterogeneity of the environment into which samango monkeys disperse seeds (Chang et al. 2016). Similarly, *S. chirindensis* may rely on the reduced germination latency of gut-passed seeds to help them establish. Like other Cercopithecines, samango monkeys consume unripe fruit (Lawes 1991; Linden et al. 2015) acting as seed predators (Wrangham et al. 1998). As such, low germination of seeds we collected from samango monkey faeces may be because they came from unripe fruit. Alternatively, the chemical and/or mechanical scarification in the gut may damage the embryo (Samuels and Levey 2005). Embryos inside the *Ficus* spp. seeds may also have been destroyed by fig wasps (Figueiredo 1993; Righini et al. 2004).

4.2 Exclusion of ecologically relevant seed treatments

The majority of mammalian frugivore germination studies generated from our literature search exclude ecologically relevant treatments from their study design. More than 75% of studies did not use entire fruits as control groups in germination studies, instead comparing germination of gut passed seeds removed from faeces with seeds manually removed from fruit pulp. This approach has several issues. Firstly, as discussed by Samuels and Levey (2005), removal of fruit pulp is already a mechanism that can induce germination by removing chemicals within fruit pulp that act as germination inhibitors (the *disinhibition effect*). Therefore the effect of gut passage being assessed is the mechanical and/or chemical alteration (scarification) of the seed coat and not the combined effect of disinhibition *plus* gut passage, which is important as each of these mechanisms can influence germination independently (e.g. Baskin and Baskin, 2014). Secondly, in this approach there are no other treatment against which the mechanical and chemical scarification of the seed coat can be assessed, further weakening interpretation of the effect of frugivores on seed germination, extending to their role in seed dispersal.

In the present study, excluding entire fruit control groups, instead comparing germination response of seeds subject with the disinhibition effect and gut passage (*Ficus* spp. and *S. chirindensis*) or desiccation (*S. cordatum*) effect, influenced the interpretation of our analyses: we interpreted the disperser as having a negative impact on seed dispersal, as the probability of germination was significantly lower for the gut passage and desiccation effects than for the disinhibition effect. On the other hand, our analyses, which included entire fruit as the control group, indicated that samango monkeys are effective in the qualitative side of seed dispersal, as seed processing mechanisms had positive and/or neutral effects on the germination potential of seeds. Including entire fruits in the analysis meant that we isolated scarification in the gut from disinhibition and were able to include an assessment of the ecological role seed spitting plays on seed germination.

In addition to the gut passage effect, we included a separate treatment in our germination experiments to isolate the chemical and mechanical effects of gut passage. The term ‘scarification effect’ is used ambiguously in the current literature to describe the chemical and mechanical alteration of the seed coat during gut-passage (Samuels and Levey 2005; Traveset et al. 2007). However, it does not distinguish chemical treatments, which can alter and soften the seed coat, from mechanical action, which can physically damage it (Baskin and Baskin 2014). Instead, each dispersal mechanism should be assessed against a control whereby the disperser has no influence on seed fate, as well as identifying a positive or negative cumulative effect from the previous treatment (Traveset and Verdú 2002; Samuels and Levey 2005; Robertson et al. 2006). Only 9% of mammalian frugivore germination studies generated from our literature search included manual chemical and/or mechanical scarification of de-pulped seeds alongside gut-passed seeds, thereby isolating scarification processes. In the present study, we found the probability of manually scarified seeds germinating was significantly higher than gut passed *Ficus* spp. and *S. chirindensis* seeds. It may be that the methods we employed to scarify seeds were insufficient to damage the seed coat to the same extent as passage through the gut, or it may be that chemical scarification in samango monkey’s gut reduces the germination potential of seeds.

We found that in *Ficus* spp., the faecal matrix significantly increased the probability of gut-passed seeds germinating. We found a similar, albeit negligible, effect in *S. chirindensis*, and it may be that digestive acids contained within the faecal matrix continue to alter the seed coat after defaecation. Alternatively, the embryo may be able to extract nutrients from the faeces, which were no longer available once we had removed seeds from faeces. Either way, inclusion of the fertiliser effect has significant consequences on our interpretation of the influence seed handling by samango monkeys has in seed germination. Without it, especially in *Ficus* spp., the role of samango monkeys would have been significantly underestimated.

4.3 Implications for future studies

The results we obtained in *Ficus* spp. require further investigation, not least as they are in contrast to results reported in other studies. A potential limitation of our study was our analysis of the three *Ficus* spp. together because we could not distinguish each species' seeds extracted from faecal samples. Pooling these data meant that any interpretation of our findings is at the genus level and not species-specific. Whilst we acknowledge that this may have introduced bias into the germination experiments of *Ficus* spp. seeds, we took care to assign seeds and entire fruits randomly, to each treatment and each trial, to minimise such bias. We further suggest that germination experiments investigating the effect of gut passage on *Ficus* spp. seeds should share seeds from faecal samples between the gut passage and fertiliser effect treatments. The seeds we used for each of these treatments came from different faecal samples and as a result, we did not isolate the fertiliser effect using seeds from the same faecal sample and could not control for individual variation of digestive enzymes and gut-passage time. The dispersal mode of seed swallowing may be a precursor for secondary dispersers, such as dung beetles (Culot et al. 2018), and longitudinal studies incorporating secondary dispersal may elucidate to this. Our treatments for *S. cordatum* isolated the disinhibition effect and the effect of desiccation; however, we did not isolate the potential effects of individual variation in saliva chemicals on germination. We suggest that future studies should aim to use freshly de-pulped and spat out seeds in both the disinhibition and desiccation treatments.

4.4 Conclusion

Samango monkeys spend most of their time in forests compared with other South African primates and they are highly dependent on high-canopy evergreen forest (Linden et al. 2015). As both seed-spitters and seed-swallowers, samango monkeys have multiple and important roles in seed dispersal by influencing the germination probability and latency of seeds contained with the fruits they consume. Our study addressed these roles by including in our germination experiments fruit species whose seeds they disperse through both roles. Our study also demonstrated that to understand the role of frugivores in vegetation dynamics, experiments that assess the influence of seed handling on seed germination should include ecologically relevant treatments that isolate each handling mechanisms' cumulative effect, as well as a meaningful control. Germination is only one component of successful establishment of a plant in a suitable microsite, the others being the method of transport and successful arrival in that site, as well as continued delivery of niche space to maintain a plant throughout its life cycle. However, our study demonstrates that the impacts of frugivores on germination may be under- or over-estimated in ecological literature where ecologically relevant treatments and meaningful controls are excluded. There is, therefore, a distinct gap in the literature aimed at understanding the substantial role frugivores have in maintaining the resilience of ecosystems exposed to continued degradation.

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Ethical Statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All applicable institutional and/or national guidelines for the care and use of animals were followed.

Declarations of interest: none

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