

SEED DISPERSAL OF THE BRAZIL NUT TREE (*BERTHOLLETIA EXCELSA*) BY SCATTER-HOARDING RODENTS IN CENTRAL AMAZONIAN SECONDARY FOREST

KARL RICKARD PERSSON

NORWEGIAN UNIVERSITY OF LIFE SCIENCES
DEPARTMENT OF NATURAL RESOURCE MANAGEMENT
MASTER THESIS 60 CREDITS 2010



Contents

Acknowledgement	1
Abstract	3
1. Introduction	4
1.1 Background.....	4
1.2 Aims of this study.....	6
2. Study species	8
3. Methods	11
3.1 Study site	11
3.2 Forest structure variables.....	12
3.3 Experimental design	15
3.4 Data analysis.....	17
4. Results	18
4.1 Dispersal.....	18
4.2 Forest structure and seed dispersal	26
5. Discussion	28
5.1 Seed dispersers	28
5.2 Fate of seeds	29
5.3 Seasonal differences	31
5.4 Forest structure and seed dispersal	32
5.5 Conclusion and management implications	36
6. References	37

Acknowledgement

First, I would like to thank my supervisor Torbjørn Haugaasen for his support and constructive feedback throughout the process of writing this thesis, as well as giving me the opportunity to work in the depths of the Amazonian rainforest collecting data for this study. This study would not have been possible without the field expertise and invaluable help from Evandro, Evanir and Mariene de Almeida Damasceno at Lago Uauaçu. I am grateful to them and the rest of their family for their hospitality and patience with a foreigner who initially understood very little of their native language.

Furthermore, I want to thank all my friends and family who have supported me in the process of finishing my master. Especially I would like to thank my girlfriend Anette, who has been so kind to proofread early drafts of this thesis.

Abstract

The fruit from the Brazil nut tree (*Bertholletia excelsa*) is one of the most important non-timber forest products (NTFP) in the Neotropics, and relies completely upon scatter-hoarding rodents for seed dispersal. Although higher densities of Brazil nut seedlings have been documented in secondary forests compared to primary forests, no study to date has followed the fate of individual Brazil nut seeds within a secondary forest. This study investigates seed removal, predation, and caching of Brazil nuts by scatter-hoarding rodents in the wet and dry season in central Amazonian secondary forest. By following the fate of 900 thread-marked seeds I examined how seasonality influenced caching rates, dispersal distances and cache longevity. Five environmental variables were measured to investigate potential effects of forest structure on dispersal distances and removal times. Most seeds were removed by scatter-hoarding rodents within the first two weeks, and seeds were generally buried in single-seeded caches within 10 m from the seed stations. More seeds were eaten and less buried during the dry season. While season did not affect dispersal distance, it was the only variable that explained variation in removal time. None of the forest structure variables measured was found to significantly affect dispersal distance or removal time. Additionally, the results were compared with a previous study from primary forest in the same area. There appears to be differences in aspects of seed dispersal between primary and secondary forests, which may have implications for the future management of this species. This study shows that scatter-hoarding rodents are important for the natural recruitment of Brazil nuts in secondary forests, and that the foraging behaviour of these animals may explain the fact that second-growth forests are important cradles for Brazil nut regeneration. Secondary forests may therefore provide an opportunity for establishing and maintaining Brazil nut production in the long-term and secure this important extractive industry. The importance of the scatter-hoarding rodents must not be underestimated, as natural regeneration may be severely limited in the absence of these animals.

1. Introduction

1.1 Background

The majority of trees in tropical forests produce fruits that are adapted for consumption and seed dispersal by various animals. In Neotropical forests, it is estimated that 51 to 98 percent of canopy and subcanopy trees have fruits adapted to animal dispersal (Howe & Smallwood 1982). Seed dispersal is one of the key phases in the process of plant regeneration. Traveset & Rodrigues-Perez (2008) define dispersal as “the key process by which individuals move from the immediate environment of their parents to establish in an area more or less distant from them”. The animals that disperse seeds ultimately play a significant role in the ecology of forests. The interactions between plants and their subsequent dispersal agents determine the number, locations and survival of dispersed seeds and seedlings, and hence the spatial template for future plant regeneration (Wright et al. 2007). Mammals and birds are recognised as the main vertebrate groups responsible for seed dispersal in tropical regions (Koike et al. 2008), but other groups of animals also have important implications for plant regeneration (Byrne & Levey 1993; Culot et al. 2009; Terborgh et al. 1993).

Animal mediated seed dispersal is a mechanism that is potentially beneficiary for both the plants and animals involved. The most obvious advantage for the animal is food. Fruits and seeds are considered to be an important part of the diet for a wide variety of animal species. For example, at least 57 rodent species which feeds on fruits and seeds have been documented in the Brazilian Atlantic forest (Vieira et al. 2003). Plants often display morphological features in order to facilitate seed dispersal by animals (Wiesbauer et al. 2008), and it is therefore reasonable to assume that they benefit in some way by seed dispersal services provided by animals. Several hypotheses have been proposed in order to explain why plants exhibit such seed dispersal enhancing characters (Wenny 2001). One hypothesis is that it facilitates escape from high mortality caused by distance or density dependent factors in the close proximity of conspecifics. This hypothesis is supported by studies demonstrating density or distance dependent mortality of seedlings near parent trees (Sezen 2009; Terborgh et al. 1993). Seed dispersal may also increase the colonisation rate of rare or unpredictable sites (Eycott et al. 2007) or facilitate dispersal to microhabitats suitable for germination (Wenny 2001).

However, phenological studies of tropical plants have demonstrated that fruit resources for animals are not readily available at all times. Fruiting often appears to be episodic and fruit availability varies seasonally (Chapman et al. 1999; Haugaasen & Peres 2005; Prasad & Sukumar 2010). Many frugivorous and granivorous mammals and birds cope with this seasonal variation in food abundance with dietary shifts to non-fruit or keystone fruit resources, or reduced metabolic requirement (Van Schaik et al. 1993). Other animals migrate to areas of secondary growth, other types of forests or elevations in search for food (e.g. Campos et al. 2006; Loiselle & Blake 1991). An additional behaviour employed in Neotropical forests by large caviomorph rodents, agoutis (*Dactyprocta* spp.) and acouchis (*Myoprocta* spp.), is scatter-hoarding (Vander Wall 1990). Animals which scatter-hoard, bury food during times of fruit surplus in anticipation of food shortage. Studies show that the agoutis live almost exclusively on these food reserves during times of fruit scarcity (Dubost & Henry 2006; Forget 1992; Smythe 1978). Scatter-hoarders generally bury seeds in several, shallow, single-seeded caches. Scatter-hoarding may contribute to seed survival if 1) the rodent forgets the location of the cache, 2) the rodent has a super-abundance of caches and therefore do not need to return to every cache, or 3) the rodent suffers mortality and fails to return to the cache (Morrison et al. 2009). If the caching animal fails to retrieve its stored food supply, and the conditions are suitable, the seeds may germinate. Buried seeds are better protected from fungal attacks, as well as predation by other terrestrial seed predators (Maron & Simms 1997; Vander Wall 1990).

In Neotropical forests, some plant species depend completely on scatter-hoarding rodents for seed dispersal and recruitment. Scatter-hoarded seeds are often large and nutritious, and are typically produced by large trees. The Brazil nut tree (*Bertholletia excelsa*) is a typical example of such a tree. It is a large-seeded Amazonian forest emergent that produces an extremely hard globose fruit. These fruits contain seeds which remain encased after fruit-fall (Mori & Prance 1990). Under natural conditions, the fruits are gnawed open by agoutis, which also act as the main seed dispersal agent by scatter-hoarding a substantial part of the seeds (Smythe 1978). The agoutis are therefore crucial to the natural regeneration of Brazil nuts. It has been demonstrated that agoutis increase their foraging effort during times of food shortage, and that the seeds are removed quicker in the dry season compared to the wet season when fruits are more readily available (Peres et al. 1997; Smythe 1978; Tuck Haugaasen et al. 2010). Additionally, removal experiments and video recordings of agouti foraging behaviour showed that they eat relatively few seeds *in situ* (Jansen 2001). In one study, approximately

10-20% of the seeds were consumed in situ, while the rest were hoarded (Forget 1996). The ratio of seeds eaten vs. hoarded varied seasonally, with less seeds consumed immediately upon detection during times of food surplus.

It has been demonstrated that there are higher densities of Brazil nut seedlings in secondary forests compared to primary forests (Boot & Gullison 1995; Cotta et al. 2008). The Brazil nut tree is a light dependant species (Mori & Prance 1990), and thus requires a certain amount of light to grow. Second-growth areas therefore provide suitable conditions for *B. excelsa* regeneration. The studies discovered that proximity to seed source and light availability seemed to explain the higher number of seedlings (Cotta et al. 2008). It is therefore reasonable to assume that there are differences in various aspects of seed dispersal between primary and secondary forest. Secondary forest can be defined as the woody vegetation that grows back after clearance of forest for human use, such as pasture, agriculture, and clearcutting for pulp or timber (Guariguata & Ostertag 2001). Tropical secondary forest areas are regarded as important for a number of reasons, such as timber sources (Finegan 1992), sources for medicinal plants (Chazdon & Coe 1999), templates for forest rehabilitation (Lugo & Brown 1992), and providers of environmental services such as protection from erosion and atmospheric carbon fixation (Fearnside & Guimaraes 1996). Due to human activities in pristine rainforest, such as slash-and-burn agriculture, the amount of secondary forest is projected to increase significantly during the next decades (Guariguata & Ostertag 2001).

1.2 Aims of this study

As the Brazil nut is one of the most important non-timber forest products (NTFP) in the Neotropics (Clay 1997; Zuidema & Boot 2002), it is often considered a cornerstone species of the Amazonian extractive economy (Kainer et al. 2007). While several studies have investigated various aspect of seed dispersal of *Bertholletia excelsa*, no study to date has followed the fate of individual seeds in secondary forests. This study aims to examine the impact of scatter-hoarding rodents on the fate of seeds from *B. excelsa* in secondary forest. By tracking the seeds removed by these animals, I investigated how seasonal variation in food availability may influence caching behaviour, focusing on dispersal distances, removal time and cache longevity. Particularly, I tested the hypothesis that seed dispersal would be higher during the wet season. Likewise, I tested the hypothesis that more seeds would be eaten immediately upon detection during the dry season, and that dry season seeds would be

removed quicker. Additionally, I investigated potential effects of forest structure on dispersal distances and removal times, and briefly compared the results obtained in secondary forests with a previous study from primary forest in the same area (Tuck Hugaasen et al. 2010).

2. Study species

The Brazil nut tree represents a monotypic genus in the family Lecythidaceae, a pantropical tree family which includes approximately 200 Neotropical species (Mori & Prance 1990). *B. excelsa* is an exceptionally large tree species, reaching 40-50 meters in height, 1-2.7 meters in diameter at breast height (dbh) and has a crown diameter that reaches 20-35 meters (Tuck Haugaasen 2010). The species has a widespread distribution in lowland Amazonia and the Guianan Shield (Mori & Prance 1990), where it occurs throughout unflooded (terra firme) forest. Its natural distribution is reported to be within areas that receive between 1400 and 2800 mm of annual rainfall, have a mean annual temperature of 24-27°C and a mean annual relative humidity of 79-86% (Diniz & Bastos 1974). In addition, the species is mainly found on nutrient-poor, well-drained oxysol and utisol soils. It is considered to be a light-dependant species (gap-dependant; Mori & Prance 1990; Zuidema & Boot 2002), meaning it will only reach reproductive size in areas with sufficient light abundance, although seedlings in shaded understory have been reported (Peres & Baider 1997).

The fruits of *B. excelsa* are unique in the Lecythidaceae family in that the seeds remain trapped within a woody capsule after fruit fall (Mori & Prance 1990). The large seeds (on average 21 ± 3 mm \times 47 ± 6 mm in size) are encased in an extremely hard globose, woody capsule (pyxidium; 11 – 15 cm in diameter, weighing 816 ± 246 g), which drops to the ground after a maturation period of approximately 15 months (Peres & Baider 1997). The pyxidium is functionally indehiscent (Mori & Prance 1990). This means that the mature pyxidium do not break upon fruit fall. In addition, the opercular lid drops inwards and the opercular diameter is too narrow (ca 1 cm) to allow seed release, effectively trapping an average of 17.1 seeds inside each fruit (Peres & Baider 1997). The Brazil nut tree is therefore dependent on vertebrate seed predators for seed release and seed dispersal. The seeds consist of a lignified seed testa (shell) protecting a large embryo measuring 4.0 ± 0.06 cm in length, 2.0 ± 0.03 cm in width and weighing 6.7 ± 0.2 g, although fruit and seed size may experience huge variation between and within populations (Tuck Haugaasen et al. 2010). The highly palatable embryo contains 17-25% protein and 70-72% lipids and is greatly desired by seed predators (Peres 1991).

Natural dispersal and predation of seeds from the Brazil nut tree is primarily mediated by agoutis (*Dasyprocta* sp.: Forget 1990; Smythe 1978). These large-bodied diurnal caviomorph rodents weigh 3.0 – 5.9 kg, and are virtually the only animals capable of gnawing through the

hard pyxidium wall and removing mature seeds from inside the fruit. Other seed predators, including acouchis (*Myoprocta* sp.), pacas (*Agouti paca*), brown capuchin monkey (*Cebus apella*) and giant Amazonian squirrels (*Sciurus spadiceus*), are reported to be capable of overcoming the pyxidium and removing mature seeds, although this is a very rare occurrence. Other arboreal predators, such as scarlet and blue-and-yellow macaws (*Ara macao* and *A. ararauna*), red-necked woodpecker (*Campephilus rubricollis*), and smaller macaws (e.g. *A. severa*), appear to only be capable of opening immature pyxidia still hanging in tree crowns (Peres & Baider 1997). Seed dispersal services occasionally provided by these seed predators are therefore far more infrequent than those of the agouti. The Brazil nut tree thus relies almost completely on these large terrestrial rodents for their seed dispersal.

Acouchis and agoutis are the only rodents known to regularly bury large intact seeds in Neotropical forests (Dubost 1988; Forget 1990; Smythe 1978). They usually bury their food around tree trunks or other structures which through spatial memory may assist in subsequent cache retrieval. Studies on other large-seeded tree species from Panama (Forget & Milleron 1991; Smythe 1978) and French Guiana (Forget 1990) showed that agoutis can seasonally store hundreds of scatter-hoarded seeds for later retrieval. For whatever reason, the agouti may fail to retrieve some of the cached seeds, and those seeds that are not consumed within the germination period (12 – 18 months for *Bertholletia*; Müller 1981) may germinate. Studies have demonstrated that storage improve Brazil nut germination rates (Kainer et al. 1999); and that seeds may remain viable for at least 6 years (Watson 1901). However, those that are left trapped inside pyxidia are rapidly attacked by fungal pathogens once exposed to more humid conditions on the ground, and may succumb to almost 100% mortality (Peres & Baider 1997).

Throughout its natural range the Brazil nut tree is known to grow in clusters or stands, locally known as *castanhais* (Mori & Prance 1990; Peres & Baider 1997; but see Wadt et al. 2005). It has been suggested that many of the contemporary Brazil nut stands in the Amazon region were created through Amerindian interventions as a result of the establishment of agro-ecosystems. The argument is that the Brazil nut tree is a light dependant tree species, which therefore thrives on abandoned agricultural or other types of disturbed lands created by the Amerindians. However, Peres & Baider (1997) attribute the aggregated spatial distribution of Brazil nut trees to the short dispersal distances produced by agoutis. It has also been argued that this seed dispersal mechanism poses a severe limitation to the species range, because viable seeds would have great difficulty in crossing major river tributaries (Clay 1997).

However, seeds may on rare occasions, be dispersed long distances by highly mobile seed predators, such as the red-and-green macaws (*A. chloroptera*), which can easily cover several kilometres (Peres & Baidar 1997).

3. Methods

3.1 Study site

The study was conducted at Lago Uauaçu, a large, 32 kilometer long crescent-shaped black-water lake located in the lower Rio Purus region (04°14`S, 62°23`W) of central Amazonia, about 350 kilometer south-west of Manaus (Figure 1). There is a distinct seasonal variation in rainfall in this area, with July-October being the driest months (dry season), and February-June the wettest (wet season; Haugaasen & Peres 2006). The topography in this region is very flat, leading to large differences in water levels across seasons.

The 30 households inhabiting Lago Uauaçu rely primarily on collection of Brazil nuts from natural stands of Brazil nut trees, in addition to small-scale fishing and hunting for their subsistence and income. However, the study area was recently included in a large (>1 million ha) sustainable development reserve (Piagaçu-Purus), which includes several non-hunting zones. Hunting was therefore not commonly practiced in our study area and the large-mammal fauna in the region is thus largely intact.

The current work focused on ten patches of secondary forest surrounding Lago Uauaçu (Figure 1). The patches were old farm-land patches of approximately 1-2 ha, of a similar age and widely spaced (283 m - 3.3 km apart) to ensure independence. All ten areas had a large Brazil nut tree within their proximity (22.4 m - 91 m). Every patch was also surrounded by relatively undisturbed terra firme forest. A canoe with an outboard engine was used to move between the different secondary forest patches, as all areas were close to the lake margin (see Figure 1).

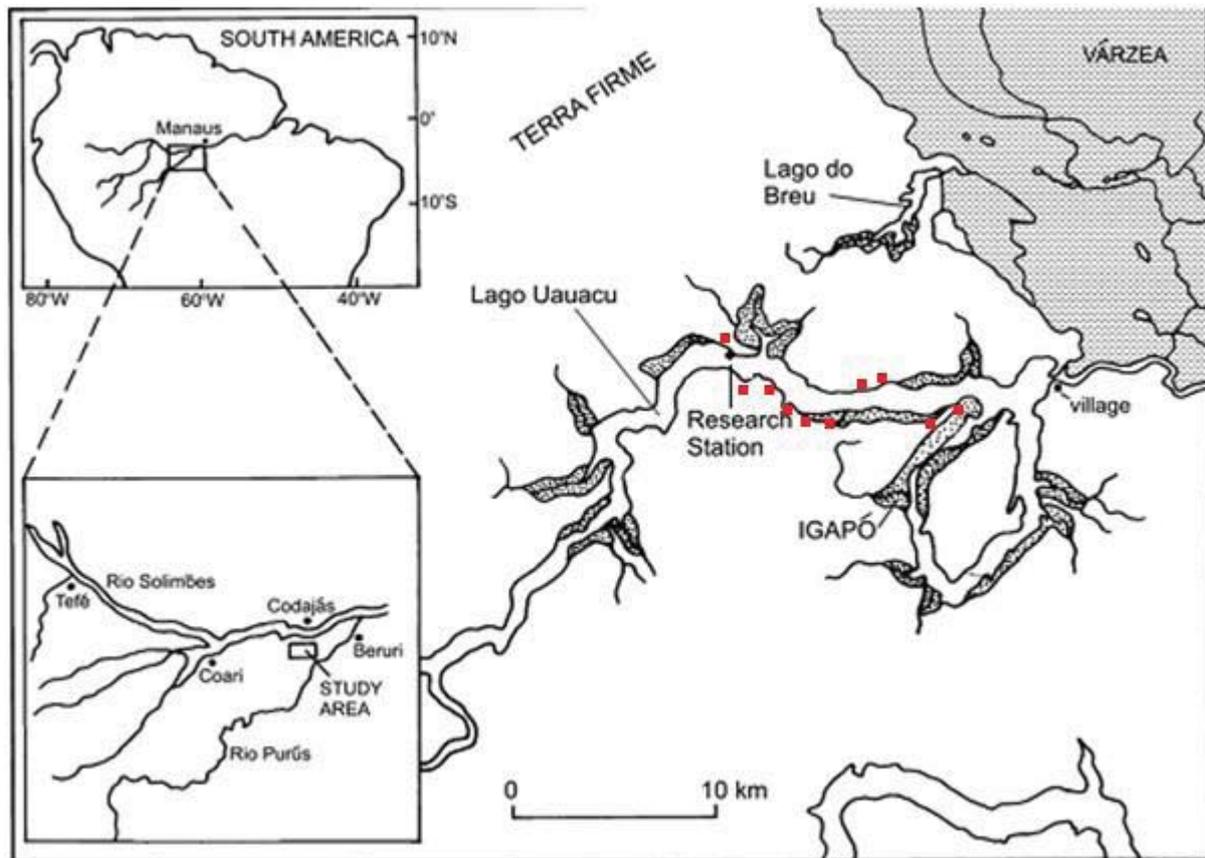


Figure 1 Map of the study area in the lower Rio Purus region, central Amazonia, Brazil. The red squares represent the ten study-patches of secondary forest.

3.2 Forest structure variables

Within each secondary forest patch, a number of forest structure variables that may potentially influence seed dispersal, were measured. These variables included canopy openness, tree density, understory vegetation density, distance from the centre of each study patch to the nearest Brazil nut tree, and the diameter at breast height (dbh) of the respective Brazil nut trees.

3.2.1 Canopy openness

Canopy openness was quantified with the use of a convex spherical densiometer. A densiometer is an apparatus that measures the amount of light penetrating the forest canopy (Figure 2a; Lemmon 1957). The densiometer is divided into 24 small grids. Following Lemmon (1957) 4 readings (facing north, east, south and west) were taken at 20 evenly

spaced locations within each patch, and the number of grids reflecting the open sky above recorded. The average value for each patch was multiplied with 1.04 and subtracted from 100 to get the percentage canopy cover.

3.2.2 Understory density

Understory density was measured using a 2.5 m tall pole, divided into 25 sections of 10 cm. Every other section was covered with orange flagging tape to distinguish each segment. A total of 20 readings were recorded in each secondary forest patch, corresponding to the number of 10 cm pole sections that were clearly visible (Haugaasen et al. 2003). The observer stood at a distance of 10 m from the pole and used binoculars to count the number of visible pole segments (Figure 2b). In order to avoid observer bias, only one person counted the segments, while another held the pole vertically upright. The number of observed segments were subtracted from the total number of segments, multiplied by 100, and divided by the total number of segments, in order to get an estimate of understory density.

3.2.3 Tree density

The number and size of trees was quantified within 25x25 m plots in all ten locations. All trees with a diameter at breast height (dbh) ≥ 10 cm were measured (Figure 2c). On occasions when a tree consisted of several smaller stems, we measured all the stems and added up the numbers.

3.2.4 Proximity and size of Brazil nut trees

The distances from the centre of each secondary forest patch to the nearest adult and reproductively active Brazil nut tree (Figure 2d) was measured with a 50 m fibreglass tape. The angle to the tree was recorded using a compass. The position of the trees was also mapped using a Garmin GPS 60 and the diameter measured using a diameter tape.



Figure 2 Photos demonstrating **a)** canopy openness in one of the study-patches, giving an impression of the light availability within the study areas, **b)** the use of a 2.5 m segmented pole, used to measure understory density, **c)** measurement of all trees with dbh ≥ 10 cm within a 25x25 m plot, and **d)** a *Bertholletia excelsa* tree the in proximity of one of the study areas, showing the impressive size of these trees. Photos: Karl R. Persson

3.3 Experimental design

Seed dispersal trials were conducted during the wet season (March), in the middle of the Brazil nut season, and then repeated in the dry season (September) when Brazil nut trees are no longer fruiting. Seeds used in the experiments were new (i.e. from the same year's seed crop) and had been cleaned and sundried until the outer shell was dry. A water immersion technique was used in order to assure the embryo's viability. This is a test widely used by local Brazil nut collectors across the Amazon, in which all seeds failing to sink are discarded (Peres & Baider 1997; pers. obs.). The seeds were then marked with a 50-cm long piece of dental floss, which was glued to the seed shell using transparent and water-resistant epoxy glue. A small piece (7 cm) of bright orange flagging tape, individually numbered using a black permanent marker, was then tied to the other end of the dental floss thread. This ensured that the flag stayed visible even after the seeds were cached by scatter-hoarding rodents. Various thread marking techniques have been used in previous experiments, and shown to be effective in tracking individual seeds following seed removal by scatter-hoarding rodents (Xiao et al. 2006).

A total of 450 seeds were placed at 30 seed stations located in the centre of 10 different secondary forest patches. Three stations were positioned within each patch in a triangular manner, with each seed station 5 m apart (Figure 3a). Seed stations were marked with a flagged pole standing upright at the center of a circular patch approximately 1.5 m in diameter, from which all litter and other debris had been carefully removed. At each station, batches of 15 marked seeds were arranged in a circular manner with their markers spread outwards (Figure 3b). The seed batch size roughly mimics the average number contained in a typical Brazil nut fruit (Figure 3c, 17.1; Peres & Baider 1997), and thus mimics the number of seeds normally found by agoutis upon opening a Brazil nut fruit.

Once seeds had been laid out, seed stations were monitored every other day by two observers until all seeds had been either eaten or removed. Where seeds were missing, intensive searches were conducted to relocate the seeds or their markers. Simultaneous searches through concentric rings radiating outwards from the seeds station were carried out to most effectively search the area, and to more effectively cover a larger area. Searches were abandoned only above 40 m away from seed stations, a procedure used in previous dispersal trials (Peres & Baider 1997; Tuck Haugaasen et al. 2010), unless all seeds or markers had already been found. Upon finding a given seed, seed fragment or marker, we determined the fate of the seed (i.e. buried, hidden, eaten, intact or only marker) and its position. The distance

between the seed and its corresponding seed station was measured using a 50 m fiberglass tape, and the displacement angle from the seed to its seed station was recorded using a compass. If the seeds were found to be intact (including those seeds buried in the ground, hidden underneath leaf litter or other debris, and those simply lying uncovered on the ground) its position was marked with a piece of flagging tape, and the fate of the seed observed every ten days thereafter. The flagging tape was attached to branches or other features of the forest at eye level immediately above the seed in order to facilitate the relocation of the seeds. However, since markers are known to provide cues for cache pilferage by diurnal rodents (Vander Wall 1990; Vander Wall & Jenkins 2003), all the markers attached to the seeds were carefully covered. If a flagged seed disappeared subsequently, intensive searches were conducted in the same manner as described above, in order to relocate and determine the fate of the seeds, although these searches were restricted to within 20 m of each primary cache location.

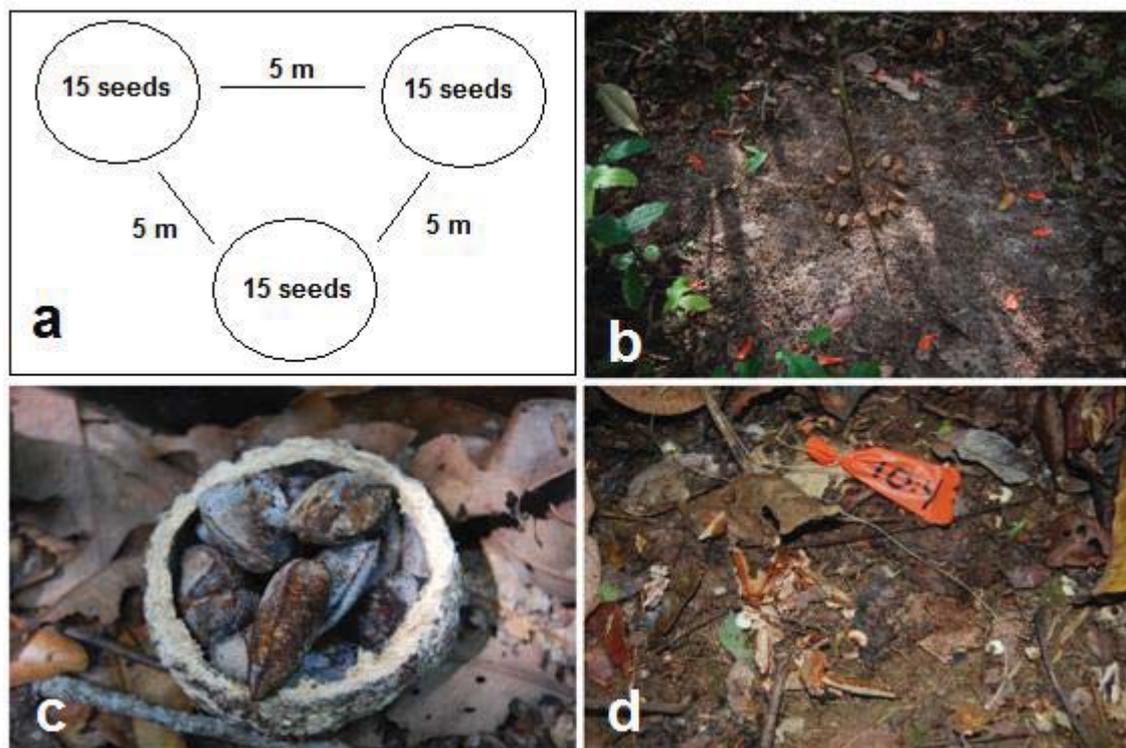


Figure 3 Illustration and photos demonstrating **a)** the three seed stations positioned in a triangular manner in each of the ten study areas, **b)** a seed station with 15 seeds that were positioned with markers pointing outwards, **c)** an opened Brazil nut fruit, containing approx. 15 seeds, and **d)** a typical recovery of a marker where the seed has been eaten. Photos: Karl R. Persson

3.4 Data analysis

Since all experimental areas were spaced by more than 250 m, they were considered to be spatially independent. However, the three seed stations within each secondary forest patch could not be considered independent and these were pooled in subsequent analyses. Due to the non-normal distribution of the data, a nonparametric Kruskal-Wallis analysis was used to assess potential differences in forest structure between the 10 secondary forest patches. Since Kruskal-Wallis tests the equality of medians among groups, the dbh of and distance to the nearest Brazil nut tree could not be included in this analysis. Two-sample t-tests was used to investigate whether there were significant differences in primary cache life-span, and the number of days until relocation of primary caches between the wet and dry season. Two sample t-tests was also applied in order to investigate significant variation in seed dispersal distances and removal time between the current study and an identical experiment carried out in primary forest (Tuck Haugaasen et al. 2010). Minitab version 15 (Minitab 2006) was used for all these analysis.

To assess potential effects of forest structure and season on dispersal distance and removal times, the data were explored using linear mixed effects regression models in the statistical programming software *R* 2 (R Development Core Team), with forest patch ID as the random variable. Top models were selected from a number of candidate models using AIC (Burnham & Anderson 2002). Mean values for environmental variables from each secondary forest patch were used in the models. All environmental variables were log-transformed for the analysis and season was entered as a categorical variable.

4. Results

4.1 Dispersal

4.1.1. *Fate of seeds*

All seed-stations, except one, were rapidly discovered by seed-eating mammals, regardless of season. Study-patch 2 deviated from the other patches in that the seed-stations remained unvisited exceptionally long in the dry season experiment. Of the 900 experimental seeds, 446 and 415 were either removed from the seed station or handled at the seed station in the wet and dry season, respectively (Table 1). The remaining seeds either succumbed to fungus infections or were presumably destroyed by other seed predators, such as leaf litter ants and primates.

The number of seeds which simply disappeared after removal from seed station was low (7.4% and 15.9% in the wet and dry season, respectively). The fate of these seeds is unknown, but they were probably moved beyond the 40 m search zone. The number of seeds which had their markers cut off was also relatively low (18.4% and 23.4% in the wet and dry season, respectively).

The vast majority of seeds were buried intact in single-seeded caches during both seasons. The proportion of seeds buried intact was much higher in the wet season (43.9%) than in the dry season (26.7%; Table 1 and Figure 4). Preferred caching sites included the base of arborescent palm and terrestrial bromeliads, in and around dead and rotting fallen tree trunks and at the base of trees (especially between buttress roots). Additionally, the proportion of seeds that were simply hidden under leaf litter or other debris was more than twice as high in the wet season than in the dry season (20.2% and 8.0%, respectively). However, a higher percentage of the seeds were eaten upon detection in the dry season (21.9 % compared to 6.7% in wet season).

Table 1 Fate of marked *Bertholletia excelsa* seeds in dispersal experiments at Lago Uauaçu, central Amazonia, Brazil. The experiments were conducted in the *B. excelsa* fruiting season (April 2009) and in the dry season (September 2009) showing the original distance the seed or marker was carried and the initial time to seed removal.

Fate of seeds	Number (%) of seeds		Distance carried (m) Mean \pm SE (range)		Removal time (d) Mean \pm SE (range)	
	Wet	Dry	Wet	Dry	Wet	Dry
	Seeds removed from stations					
Buried intact	196 (43.9)	111 (26.7)	7.1 \pm 0.5 (0.6 - 48.2)	7.0 \pm 0.5 (0.8 - 28.7)	6.8 \pm 0.4 (2 - 26)	10.2 \pm 1.4 (2 - 71)
Not buried but hidden	90 (20.2)	33 (8.0)	4.6 \pm 0.4 (0.7 - 23.3)	7.7 \pm 1.3 (1.3 - 29.6)	12.6 \pm 0.9 (2 - 36)	15.2 \pm 2.9 (2 - 71)
Eaten	20 (4.5)	44 (10.6)	3.5 \pm 0.6 (0.4 - 10.8)	8.9 \pm 0.9 (1.0 - 20.7)	13.7 \pm 2.3 (2 - 36)	14.9 \pm 3.2 (2 - 71)
Intact (neither buried nor hidden)	12 (2.7)	16 (3.9)	9.8 \pm 3.2 (1.8 - 32.2)	7.4 \pm 1.4 (1.7 - 18.0)	9.3 \pm 2.1 (2 - 20)	19.1 \pm 5.4 (2 - 71)
Markers cut off and recovered	60 (13.5)	24 (5.8)	4.9 \pm 0.5 (0.9 - 20.9)	6.2 \pm 1.1 (0.8 - 20.4)	12.4 \pm 1.2 (2 - 36)	9.1 \pm 1.7 (2 - 22)
Markers unrecovered	33 (7.4)	66 (15.9)			17.4 \pm 2.5 (2 - 38)	11.5 \pm 2.5 (2 - 71)
Seeds remaining at stations						
Buried	3 (0.7)	1 (0.2)			10.0 \pm 0.0 (10 - 10)	4.0 \pm 0.0 (4 - 4)
Eaten	10 (2.2)	47 (11.3)			15.6 \pm 3.5 (2 - 26)	8.1 \pm 1.4 (2 - 38)
Markers cut off and recovered	22 (4.9)	73 (17.6)			11.9 \pm 2.3 (2 - 36)	6.7 \pm 1.3 (2 - 60)
All seeds	446 (99.1)	415 (92.0)	6.1 \pm 0.3 (0.4 - 48.2)	7.6 \pm 0.4 (0.8 - 29.6)	10.3 \pm 0.4 (2 - 38)	11.1 \pm 0.8 (2 - 71)

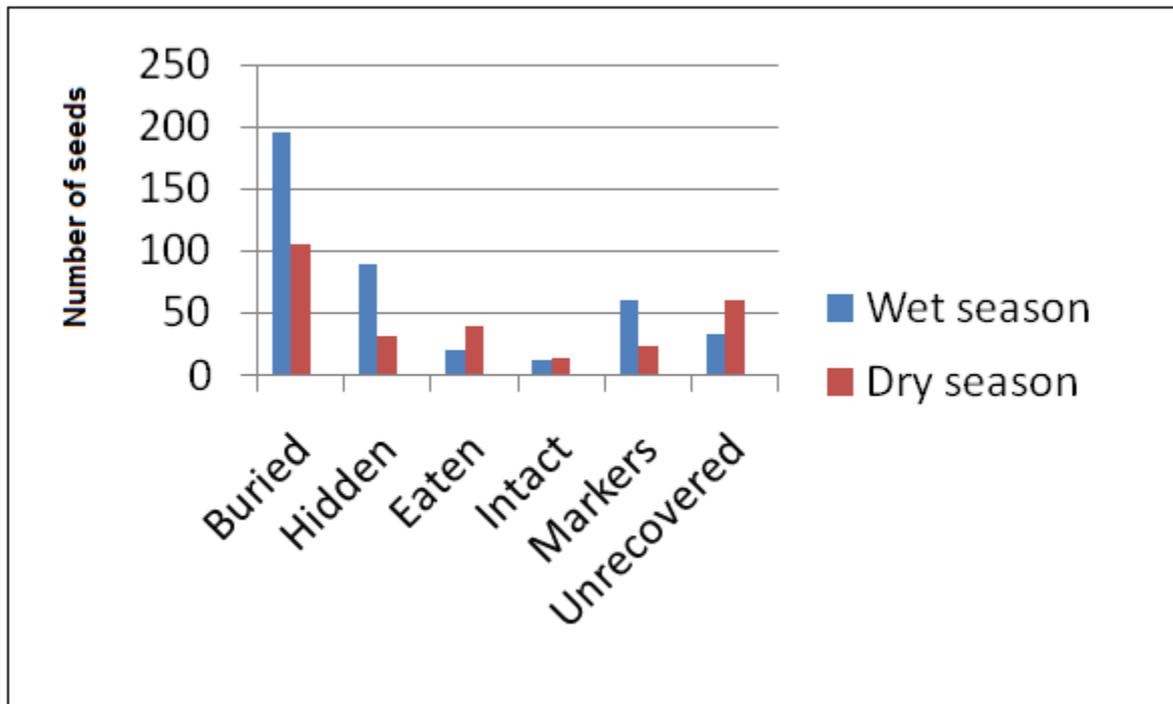


Figure 4 Fate of seeds in wet and dry season at Lago Uauaçu, central Amazonia, Brazil.

4.1.2. Removal time and dispersal distance

Most Brazil nut seeds exposed to dispersal trials were rapidly removed or eaten *in situ* (Table 1). In fact, most seeds were removed during the first week of the experiments (46.9% and 63.1% in wet and dry season, respectively; Figure 5). Nevertheless, seeds were removed from their seed stations significantly faster during the wet season ($t = 6.04$, $d.f. = 296$, $p < 0.001$).

Cached seeds were observed as far as 48.2 meters from seed stations (Table 2). However, most seeds were buried within 10 meters of their respective seed station (77.4% and 77.3% of seeds in the wet and dry seasons, respectively; Figure 6). There were no significant effect of season on dispersal distances at which seeds were buried ($t = 1.8$, $d.f. = 296$, $p = 0.07$).

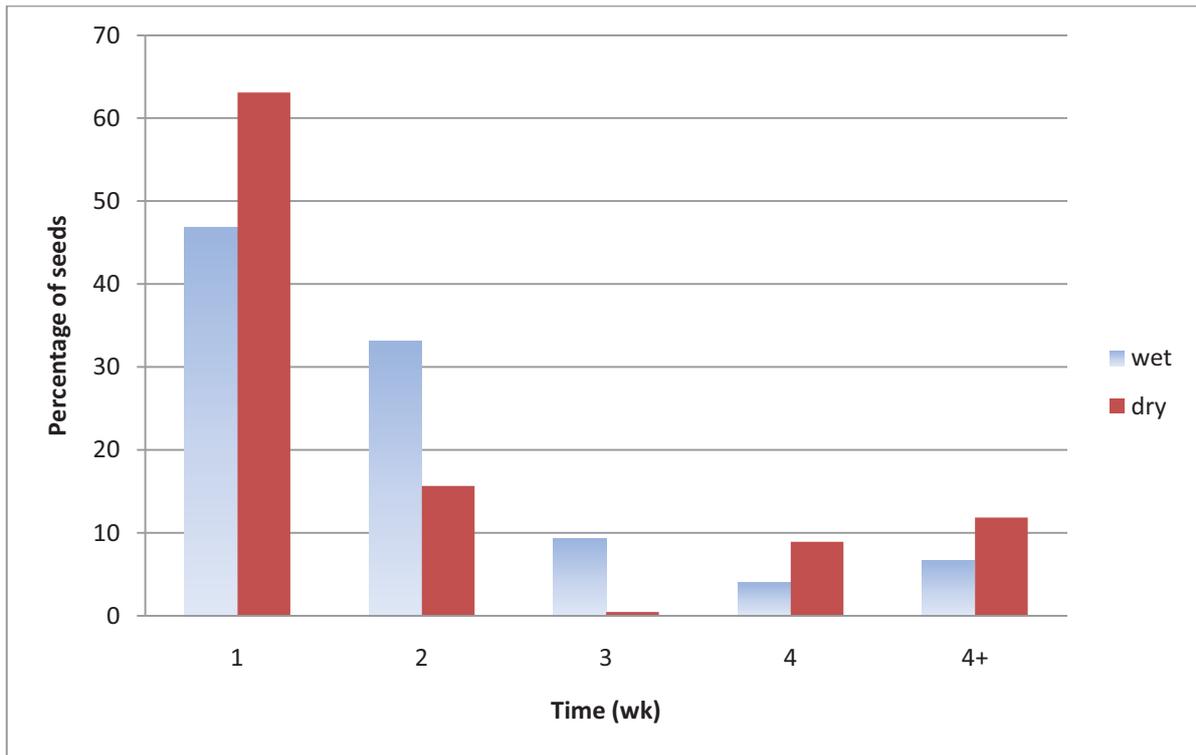


Figure 5 Removal time of thread-marked seeds during the wet and dry seasons in secondary forest areas at Lago Uauaçu, central Amazonia, Brazil.

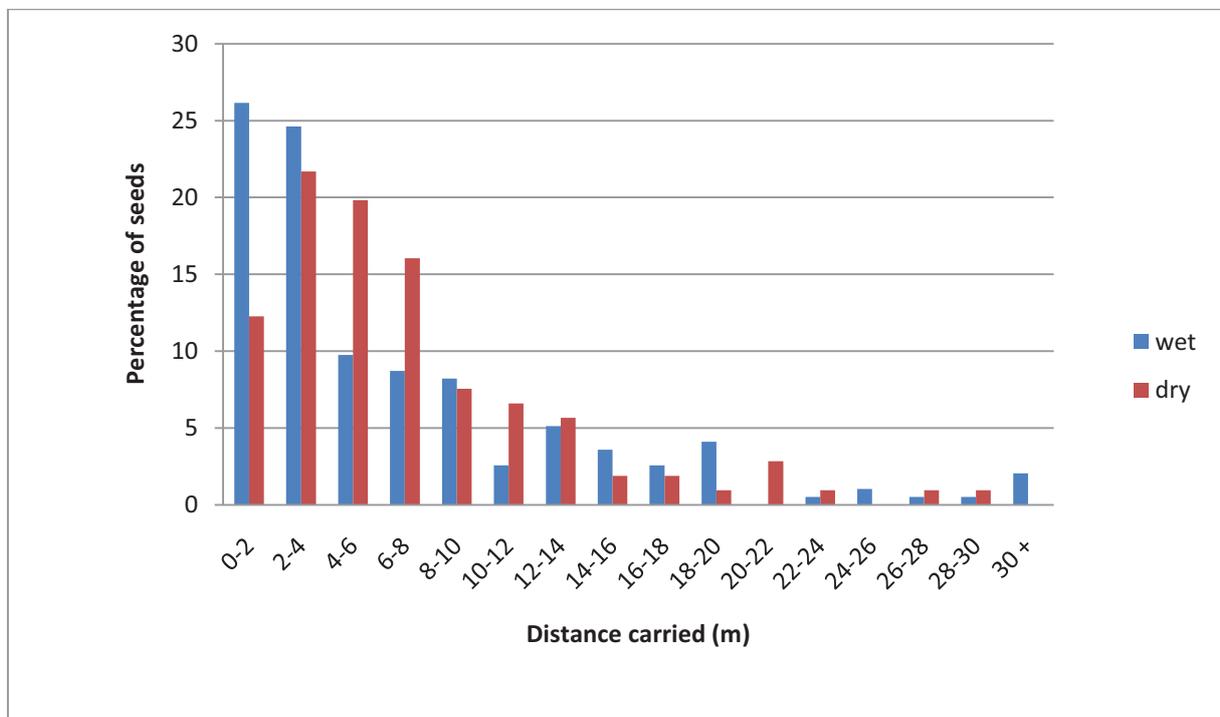


Figure 6 Removal distance of thread-marked seeds during the wet and dry season in secondary forest areas at Lago Uauaçu, central Amazonia, Brazil.

4.1.3. Fate of buried seeds

During the wet and dry season experiment, the fate of 307 primary caches was followed. Only four seeds remained buried at the end of the experiment, one year after placement of seeds at seed stations. All of these originated from the wet season experiment. None of the seeds had germinated when the caches were surveyed for the last time. Caches from the dry season had a significantly shorter life span than caches from the earlier experiment during the Brazil nut fruit-fall season ($t= 12.39$, $d.f.= 217$, $p < 0.001$). While the majority of caches in the dry season were recovered within three weeks, caches in the wet season remained buried three months, on average (Table 2).

The fate of most relocated seeds was accurately determined, as just 32.8% and 11.7% of seeds from primary caches in the wet and dry season respectively, disappeared. Most rediscovered seeds were eaten at the cache location by scatter-hoarding rodents, and a considerably higher amount of the seeds were consumed during the dry season than wet season (Table 2).

It was also discovered that 6.7% and 8.3% (in the wet and dry season, respectively) of seeds from primary caches had been relocated to secondary caches. Secondary caches were more abundant during wet season than during dry season (Table 2).

Although four seeds still remained cached, the wet season experiment was ended by the observers 362 days after the initial placement of seeds at seed stations. The dry season experiment ended after 82 days. By then, all seeds from the dry season experiment were either consumed, destroyed or had disappeared from the study-sites.

4.1.4. Reburied seeds

Secondary caches increased dispersal distances in both seasons (Table 3 and 4). In fact, reburied seeds almost doubled the average distance between seed stations and primary caches. Seeds that were relocated in the dry season were moved considerably farther, although primary caches from this season were also noticeable further from seed station. The number of days till relocation was significantly lower for seeds in the dry season ($t = 3.22$, d.f. = 10, $p = 0.009$).

No seeds were found to be reburied twice. In the wet season, six out of the 20 secondary caches were found eaten, three were destroyed by ants, three had their marker cut off and six simply disappeared. In addition, two seeds remained buried at the end of the experiment. In the dry season experiment 14 out of 15 secondary caches were eaten, and the last seed had its marker cut off.

Table 3 Fate of relocated seeds from a seed dispersal experiment established during the Brazil nut fruiting season (April) in secondary forest areas at Lago Uauaçu, central Amazonia, Brazil. The original distances and new distances are expressed as mean \pm SE (range).

	Original distance	New distance	Time (d) till relocated
Buried intact	2.6 \pm 0.63 (1.0 - 8.4)	4.7 \pm 1.0 (1.2 - 11.4)	75.6 \pm 20.8 (22.0 - 232.0)
Hidden	3.7 \pm 0.6 (1.9 - 6.5)	7.9 \pm 2.4 (2.5 - 20.4)	20.6 \pm 5.5 (12 - 42)
Intact	1.9 \pm 0.0 (1.9 - 1.9)	4.4 \pm 0.0 (4.4 - 4.4)	22 \pm 0 (22 - 22)
All seeds	2.9 \pm 0.4 (1.0 - 8.4)	5.9 \pm 1.1 (1.2 - 20.4)	52.5 \pm 13.6 (12 - 232)

Table 4 Fate of relocated seeds from a seed dispersal experiment established outside the Brazil nut fruiting season (September) in secondary forest areas at Lago Uauaçu, central Amazonia, Brazil. The original distances and new distances are expressed as mean \pm SE (range).

	Original distance	New distance	Time (d) till relocated
Buried intact	10.3 \pm 2.4 (0.8 - 28.7)	18.9 \pm 3.5 (4.9 - 45.0)	8.7 \pm 0.8 (6 - 16)
Hidden	3.3 \pm 1.3 (2 - 4.6)	22.0 \pm 17.3 (4.7 - 39.3)	11 \pm 1 (10 - 12)
Intact	7.5 \pm 0.0 (7.5 - 7.5)	11.9 \pm 0.0 (11.9 - 11.9)	10 \pm 0 (10 - 10)
All seeds	9.1 \pm 2.0 (0.8 - 28.7)	18.8 \pm 3.3 (4.7 - 45.0)	9.0 \pm 0.6 (6 - 16)

4.2 Forest structure and seed dispersal

Canopy height in the ten patches of secondary forest was between 5-20 meters, and the understory vegetation was largely dominated by species in the families Boraginaceae, Anacardiaceae, Annonaceae, Melastomataceae, Bromeliaceae and Arecaceae. There was little variation between the study-areas regarding the environmental variables measured (Table 5). However, there was a significant difference in canopy opening between patches (Kruskal-Wallis test, $H = 41.71$, d.f. = 9, $p < 0.001$). A total of 511 trees with a dbh ≥ 10 cm were measured within the ten 25x25 m plots. The number of trees measured in each patch ranged from 33 to 85 trees. Similarly, there was a significant difference in mean dbh between the ten patches (Kruskal-Wallis test, $H = 69.15$, d.f. = 9, $p < 0.001$). The understory density was dense in all areas (Table 5) and did not differ significantly between sites (Kruskal-Wallis test, $H = 11.33$, d.f. = 9, $p = 0.254$). There were large variations in distance to nearest Brazil nut tree between the ten study patches, ranging from 17.5 m to 91.0 m. Likewise, size of trees varied and dbh ranged from 71.7 cm to 194 cm.

None of these environmental variables was found to have a significant effect on removal time of *B. excelsa* seeds ($p > 0.05$). A similar trend was found for dispersal distances at which seeds were buried intact ($p > 0.05$).

Table 5 Summary of the forest structure data obtained from the ten secondary forest patches at Lago Uauaçu, central Amazonia, Brazil.

Patch	% canopy openness ± SE (range)	Mean tree size (cm dbh) ± SE (range)	No. Of trees dbh ≥ 10 cm	Understory density (%)	Distance (m) to nearest Brazil nut tree	Dbh of nearest Brazil nut tree
1	4.6 ± 0.2 (2.6 - 6.7)	12.8 ± 0.6 (10.0 - 39.0)	61	81.0 ± 2.8 (60 - 100)	34.9	90.5
2	8.4 ± 0.6 (4.4 - 14.5)	17.7 ± 1.0 (10.0 - 39.0)	44	75.8 ± 4.4 (32 - 100)	64.0	92.8
3	5.6 ± 0.6 (2.3 - 14.0)	16.9 ± 1.4 (10.3 - 59.3)	43	64.0 ± 4.8 (24 - 96)	91.0	71.7
4	5.4 ± 0.4 (1.3 - 9.1)	20.6 ± 1.7 (10.2 - 52.7)	33	80.2 ± 2.7 (60 - 100)	70.0	96.5
5	6.0 ± 0.4 (3.1 - 9.9)	15.7 ± 1.0 (10.1 - 41.0)	40	75.8 ± 4.0 (36 - 100)	23.6	87.5
6	6.9 ± 0.4 (4.7 - 11.7)	19.8 ± 1.1 (10.0 - 40.4)	52	70.6 ± 4.6 (28 - 100)	17.5	100.5
7	7.1 ± 0.6 (3.1 - 12.2)	18.7 ± 1.3 (10.0 - 48.3)	43	75.0 ± 3.9 (40 - 100)	22.4	128.0
8	7.8 ± 0.6 (3.1 - 13.0)	15.2 ± 0.5 (10.1 - 21.9)	49	78.0 ± 4.5 (44 - 100)	62.0	194.0
9	5.6 ± 0.5 (2.6 - 11.2)	24.0 ± 3.6 (10.2 - 257.0)	85	72.7 ± 2.8 (44 - 96)	87.0	131.0
10	5.8 ± 0.4 (3.1 - 11.4)	16.4 ± 1.0 (10.0 - 47.8)	61	73.0 ± 5.2 (24 - 100)	23.1	119.0
Mean	6.3 ± 0.2 (1.3 - 14.5)	18.0 ± 0.6 (10.0 - 257.0)	51 ± 4.7 (33 - 85)	75.5 ± 1.3 (24 - 100)	49.5 ± 9.0 (17.5 - 91)	111.1 ± 11.0 (71.7 - 194.0)

5. Discussion

This study followed the fate of a high number of marked seeds in ten different patches of secondary forest and is the first to examine the fate of individual Brazil nuts after caching by scatter-hoarding rodents in this forest type. The seeds were exposed on the forest floor in the proximity of an adult *Bertholletia excelsa* tree. The fact that the seeds were laid out unprotected (i.e. removed from the fruit) most likely shortened the handling times for the scatter-hoarding animals, compared to an encounter with an intact fruit (Dubost & Henry 2006). However, this approach has been used in previous Brazil nut dispersal experiment (Peres & Baider 1997; Tuck Haugaasen et al. 2010) and undoubtedly provides further understanding of how animals handle large seeds in regenerating forests in the Neotropics.

5.1 Seed dispersers

Conforming to previous studies using similar thread-marking techniques, scatter-hoarding rodents were among the first animals to detect our seeds stations (Peres et al. 1997). Since seeds were unprotected and acouchis and agoutis are sympatric in the study area, it is hard to ascertain the relative importance of each species as visitors to the seed stations. However, most seeds were likely handled by agoutis. Agoutis were observed within the study areas, and characteristic dental marks on coats of opened seeds indicate seed-handling by these animals. The large number of seeds buried in the manner of an agouti also supports this. In addition, Dubost & Henry (2006) showed that the acouchis include a greater amount of fruit pulp in their diet (60% pulp vs. 20% seeds) during times of food scarcity, whereas the agoutis mainly switch to seeds when food availability is low (76 % seeds vs. 12 % fruit pulp). It is therefore likely that agoutis removed the greater majority of seeds from the dry season experiment.

Although most seeds were handled by agoutis, there were clear indications of the presence of other seed predators. Many seeds were eaten by leaf-litter ants. This was particularly the case for seeds initially buried by scatter-hoarding rodents. The large number of seeds hidden underneath leaf-litter rather than buried indicates that smaller rodents, most likely spiny rats (*Proechimys* spp.), also visited the seed stations. At one seed station, clusters of up to 5 markers with seed fragments were found hanging from branches at heights of 1.5-2.0 m, presumably left by brown capuchin monkeys. This species, and to a lesser extent the white-fronted capuchin (*C. albifrons*), are known to be seed predators of the Brazil nut by cracking open older fruits (Peres & Baider 1997). These fruits are easier to open as they have a more

fragile pericarp, and they open them by bashing them on a suitable hard surface (Fragaszy et al. 2004).

5.2 Fate of seeds

All seeds buried throughout the experiment were buried in single-seeded caches. This seems to be a consistent feature of caviomorph rodent caches in the Neotropics, and is well documented in previous studies (Jansen et al. 2004; Peres & Baider 1997; Smythe 1978). Preferred caching sites were also similar to other studies (Forget 1990; Smythe 1978; Tuck Haugaasen et al. 2010). The majority of the thread-marked seeds were recovered (74.2% and 60.7% in the wet and dry season, respectively). These recovery percentages were lower than those of Tuck Haugaasen et al. (2010) and Peña-Claros & De Boo (2002), but higher than those of Peres & Baider (1997). The overall seed removal during the first 2-week period was 80% for wet season and 78.8% for dry season. This is lower than those results reported by Peres & Baider (1997) (87.6% in dry season) and Tuck Haugaasen et al. (2010) (90.1% and 84.5% in wet and dry season, respectively). A study by Peres et al. (1997) found consistently lower rates of seed removal in the dry season across their experimental treatments. They attributed this to seasonal differences in foraging behaviour of the scatter-hoarding rodents, where overall search effort is more intense when the probability of finding scattered seeds is greater (Peres et al. 1997).

Most of the thread marked seeds were discovered within 10 m from the seed station, during both the wet and dry season. This is in accordance with studies on seed dispersal of Brazil nuts by scatter-hoarding rodents, which show that the seeds are unlikely to be dispersed long distances. Peres & Baider (1997) explain the clustered distribution of *Bertholletia excelsa* to limitations in the scatter-hoarding rodent dispersal distances. However, Brazil nut dispersal distances of up to 100 m have been documented from a study in Madre de Dios, Peru (Peres & Baider 1997), and in the current experiment seeds were discovered as far as 48 m away from the seed station. Additionally, some seeds were re-buried, and distances were greater for secondary caches than for primary caches, regardless of season. This further increased dispersal distances by the resident scatter-hoarding rodents.

It has been suggested that the spatial differences observed in seed dispersal and seed survival are attributed to differences in the density of Brazil nut trees within the experimental neighbourhood (Tuck Haugaasen et al. 2010). Peres et al. (1997) similarly reported that the

overall removal of Brazil nuts is disproportionately higher within *Bertholletia* clusters compared to seeds in areas outside clusters, although the density of agouties did not differ significantly between areas. Conversely, a study by Jorge & Peres (2005) demonstrated that local densities of agoutis clearly co-varied with the density of Brazil nut trees. This suggests that there must be a certain amount of density-dependence in the seed foraging behaviour of the agouti, where it appears to be more intensive in areas containing high densities of reproductive *B. excelsa* trees. However, the current study did not detect any variation in dispersal activities which could be linked to the proximity of an adult Brazil nut tree. The current sampling design, where all sites had a single, large, reproductively active *Bertholletia* tree which was not part of a grove formation within 100 m, impedes the possibility of detecting density dependence in the foraging behaviour of the agouti in this case.

At the end of the experimental period, one year after the onset of the experiments, four seeds remained buried intact in their caches. All four seeds originated from the wet season experiment. This result is similar to that of Tuck Haugeaasen et al. (2010) where five seeds remained intact in their caches. The fact that seeds could remain in their caches for several months after initial caching, indicates that the agouti at least uses some of the caches as long term food reserves. This also shows that the rodent is highly adept in locating and retrieving its caches, since only four of the 307 single-seeded caches monitored during the 12 month experiments remained cached. None of these seeds had germinated at the end of the experiments. There may be several reasons for this. Firstly, the seeds may simply be located in unfavourable conditions. Secondly, 12 months do not cover the entire dormancy period of the Brazil nut, which is 12-18 months (Müller 1981). The remaining caches could therefore subsequently germinate, unless the sun-drying treatment before the onset of the experiments damaged the embryos. In any case, it is impossible to equate germination success to seedling establishment because many scatter hoarding rodents are known to eat seeds with a seedling already protruding from it (Forget 1992; Jansen et al. 2006). Conversely, seed disappearance cannot be equated to mortality. The number of seeds relocated to secondary caches is probably higher than indicated in this study given the number of unrecovered seeds following their disappearance from primary caches. A large proportion of these seeds were probably moved beyond the 20 m zone within which the observers conducted the search.

5.3 Seasonal differences

Seeds were rapidly discovered by scatter-hoarding rodents in both seasons. With the exception of one study-patch which was discovered noticeably late during the dry season, season did not affect seed removal. It has been suggested that variation in food abundance affect the caching rates and determine whether scatter-hoarding rodents act as seed dispersers or seed predators (Janzen 1971). Assuming that caching is primarily induced by food satiation, one would expect more caching during times of food surplus. The current study showed that a higher number of seeds indeed were buried in the wet season (43.9%), when alternative fruits were abundant, than in the dry season (26.7%), when fruiting trees were less prevalent in the study area (Haugaasen & Peres 2005). Additionally, it was discovered that more seeds were immediately eaten upon detection in the dry season (21.9%) than in the wet season (6.7%), which similarly supports the suggestion by Janzen (1971).

There was no significant difference in removal distances between the wet and dry season. This contrasts with the findings of Peres et al. (1997) and Tuck Haugaasen et al. (2010) who report greater removal distances in the dry season. The above authors argue that seeds are more valuable to the consumer during times of food shortages. This may lead to seeds being dispersed farther in order to make caches more spatially diffuse and thereby prevent pilferage by other seed predators (Tuck Haugaasen et al. 2010). Despite the average removal distance being similar for the dry and wet seasons in the current study, the seed dispersed farthest were taken in the wet season (48.2 m and 28.7 m in wet and dry season, respectively). Why the results presented here contradict previous studies is uncertain. However, it may indicate that agoutis employ a different foraging behaviour within second-growth areas, compared to primary forest.

It has been suggested that agoutis detect food sources quicker in the dry season, due to greater foraging effort (Smythe 1978). However, the current study found that scatter-hoarding rodents removed seeds from the seed stations significantly faster during the wet season than dry season. This was unexpected and contrasts significantly with previous studies, which found consistently shorter removal time for *B. excelsa* seeds during the dry season (e.g. Tuck Haugaasen et al. 2010). However, the fact that one patch in our study was discovered exceptionally late by scatter-hoarding rodents increased the average removal time for the dry season experiment. If the removal times from this patch are excluded, the average removal time between the wet and dry season is very similar. Yet, this does not conform to previous studies. The contrasting results presented here may indicate that food availability differs

between primary and secondary forests. Indeed, Corlett (1994) found that second-growth vegetation differs greatly in structure and plant species composition compared to old-growth forests. Secondary forest habitats often contain an abundant supply of resources, such as fruits and flowers, even during the dry season resource bottleneck observed in primary forest (Babweteera & Brown 2009; Blake & Loiselle 2001; DeWalt et al. 2003).

A total of 307 seeds were buried upon detection by scatter-hoarding rodents. Of these 23 were re-cached in secondary caches and the number of re-cached seeds was equal across seasons (11 and 12 seeds in wet and dry season, respectively). These results were unexpected. The number of secondary caches during the dry season was expected to be lower, reflecting a predicted need for scatter-hoarding rodents to consume seeds more rapidly during times of low food availability. Nevertheless, the larger number of seeds succumbing to immediate consumption during the dry season implies that seeds from *B. excelsa* experiences higher predation rates and therefore higher mortality during periods of fruit scarcity. Likewise, the time until final removal differed significantly between the seasons. It took three times longer for seeds to exit the experiment in the wet season than the dry season. This demonstrates that although an even numbers of secondary caches were found in the wet and dry season, caches have a significantly shorter life-expectancy in the dry season.

5.4 Forest structure and seed dispersal

None of the environmental variables measured within our study areas had a significant effect on dispersal distance or removal time. Few studies have investigated the actual impact of forest structure variables on the process of seed dispersal, although forest structural characteristics may influence aspects of seed dispersal (Janzen 1971). For example, the higher vegetation density in secondary compared to primary forest, may attract seed eaters looking for a place to escape predators while eating. In addition, it has been suggested that the dense vegetation may enhance successful hiding of caches from cache-pilferers (Tuck Haugaasen et al. in review). However, Peña-Claros & De Boo (2002) demonstrated that while the rates of seed removal for a number of tropical tree species decreased with an increase in forest age, this did not apply for *Bertholletia*. Brazil nut seeds were removed equally in all successional stages (2 yr, 10 yr, 20 yr, and mature forest). The authors related this finding to agouti foraging behaviour; agoutis are generally ubiquitous across forest types, occupy home ranges of 1-2 ha and forage in forests with varying degrees of canopy openness and structural

characteristics. The results presented here are thus not surprising, despite the significant differences in canopy openness and tree density found across the current study patches. In order to fully understand if, or how, forest structure characteristics influence agouti seed dispersal behaviour, it is necessary to test these results with patches of varying degrees of canopy openness, understory density and other environmental variables. This is a topic for future research.

It is, however, more reasonable to assume that environmental conditions play a role in and affect subsequent seed germination and seedling growth. For example, the canopy is often more open in secondary forest and the elevated light levels reaching the forest understory may facilitate germination and growth in more light dependent species. Indeed, mounting evidence suggests that *B. excelsa* is a long-lived pioneer species, which thrives under elevated light conditions. For example, Myers et al. (2000) showed that Brazil nut seedlings in large forest gaps were significantly taller than those in the primary forest understory, and similar results were found in a recent study in the Trombetas region of eastern Amazonia (Scoles 2010). Further evidence is the high number of seedlings found in secondary forests compared to primary forest (Cotta et al. 2008; T. Haugaasen et al. unpubl. data). The elevated Brazil nut regeneration in secondary forest may therefore be due to a combination of seed dispersal being directed towards second-growth areas as suggested above and enhanced germination success due to better light conditions.

5.4.1 Comparison with Tuck Haugaasen et al. (2010)

There were several differences in removal time and dispersal distances between the current study and Tuck Haugaasen et al. (2010); dispersal distances were longer and removal time from seed stations shorter in the dry season primary forest experiment, and final removal time significantly shorter during both seasons in primary forest (Table 6). The shorter dispersal distances in secondary forest during the dry season may reflect the intention to keep seeds within secondary forest because of the previously described increased protection from predators and pilferers in this forest type. Similarly, the significantly shorter dispersal distances and longer removal times may reflect that seeds are not as valuable in secondary forest due to the higher abundance of resources in this forest type as discussed above.

There were also several differences in seed outcomes between primary and secondary forest. The proportion of seeds buried upon detection was significantly less in the current study compared to primary forest (Table 6). Likewise, almost one third (28.6%) of all the seeds in the current experiment were eaten immediately upon detection. This is more than both Tuck Haugaasen et al. (2010) and Peres & Baider (1997). Although the difference is small, it seems to suggest that seeds have a shorter life-span in a second-growth area. This is consistent with Jorge & Howe (2009) who demonstrated that the foraging behaviour of the agouti is affected by forest disturbances, and that a higher proportion of seeds are eaten rather than hoarded within forest areas subjected to fragmentation. Similar responses have been demonstrated for other tropical tree species in areas subjected to forest degradation (Cramer et al. 2007).

A higher proportion of buried seeds were also relocated to secondary caches in the current study (Table 6). However, the proportion of seeds that disappeared after primary caching was more than twice as high in the primary forest experiment. Although the fate of these seeds is uncertain, they could subsequently have been moved to secondary caches. Seeds relocated during the wet season were moved much farther from their primary cache site in primary forest than in secondary forest. However, the removal time for these caches was noticeably shorter for seeds buried in primary forest. The fact that re-buried seeds were moved sooner, and farther in primary forest, may indicate that the probability of finding cached *B. excelsa* seeds are greater within areas of primary forests than secondary forest. It suggests that cache pilferage may be more prominent in primary forest and supports the previously discussed density dependence in foraging, where more intense foraging for nuts are carried out in areas where, or at times when, the expectancy of finding nuts are highest.

A higher percentage of hidden and intact seeds were observed in this study (Table 6). These seeds were not buried or eaten, but instead found hidden underneath leaf litter or simply lying open on the ground. Such seeds were most likely handled by spiny rats and may indicate a higher prevalence of these animals in the secondary forest areas compared to primary forests. Several previous studies have documented an increase in spiny rat abundance in secondary forests (Adler et al. 2000; Lambert & Adler 2000). As these animals do not hoard seeds, they are unlikely to contribute to Brazil nut regeneration. Rather, an increase in the density of spiny rats may lead to higher seed predation rates.

Table 6 Differences in seed dispersal between the current study from secondary forest and Tuck Hugaasen et al. (2010) from primary forest. Dispersal distance and removal time is expressed as mean (\pm SE). A two-sample t-test was used to investigate significant differences between studies. Fate of seeds is expressed as %.

	Current study		Tuck Hugaasen et al. (2010)		T		P	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Dispersal distance \pm SE	7.1 \pm 0.5	7.0 \pm 0.5	7.1 \pm 0.3	10.0 \pm 0.5	0,04	3,74	0,968	< 0.001
Removal time \pm SE	6.8 \pm 0.4	10.2 \pm 1.4	7.3 \pm 0.3	4.8 \pm 0.3	0,78	-3,75	0,435	< 0.001
Final removal time \pm SE	99.7 \pm 5.9	24.1 \pm 1.6	38.7 \pm 1.4	11.3 \pm 0.9	-10,74	-7,23	< 0.001	< 0.001
Buried (%)	44.6	26.9	74.4	38.2				
Hidden (%)	20.2	8.0	4.5	11.7				
Eaten immediately (%)	6.7	21.9	4.9	18.5				
Intact (%)	2.7	3.9	1.4	0.6				
Re-buried (%)	5.7	10.2	6.0	1.2				

The results presented here appear to demonstrate that seed survival is lower in second-growth forests compared to primary forests. This seemingly contradicts the studies showing elevated Brazil nut seedling densities in secondary forest areas. However, results from this study may be misleading. As discussed above, studies show that secondary forests are more productive and offer a higher abundance of food than primary forest, particularly during the dry season resource bottleneck (Blake and Loiselle 2001; Babweteera & Brown 2009; DeWalt et al. 2003). Even if agoutis eat more and bury less seeds upon detection in second-growth areas, they may therefore be inclined to neglect cached seeds due to the presence of alternative food sources. This study found that the time until final removal was noticeable longer than in primary forest, which also supports the idea that there may be more alternative food sources available for the scatter-hoarding rodents to consume within secondary forests. Since better light conditions are expected to enhance germination times, these seeds may be left in the ground long enough to germinate. In a case where food is not limiting, it is perhaps reasonable to assume that agoutis will discard already germinating seeds, thereby giving an alternative explanation for the high seedling numbers of *B. excelsa* in secondary forests.

5.5 Conclusion and management implications

This study confirms the hypothesis, that more seeds were eaten and less buried during the dry season. While season did not affect dispersal distance, it was the only variable that explained variation in initial and final removal time. None of the forest structure variables measured turned out to significantly affect dispersal distance or removal time. Nevertheless, there appears to be differences in aspects of seed dispersal between old-growth and a second-growth areas, which may have implications for the future management of this species.

The current study is the first to investigate dispersal of Brazil nuts in a secondary forest environment. Consideration of secondary forest areas is increasingly important as they become more abundant in the Neotropics (Guariguata & Ostertag 2001). This study shows that agoutis are important for the natural recruitment of Brazil nuts in secondary forest – perhaps particularly in secondary forest areas in close proximity to pristine, mature forests – and that agouti behaviour may explain the fact that secondary forests are important cradles for Brazil nut regeneration. Previous studies suggest that a management plan for reduced recruitment in intensively harvested areas must be considered (e.g. Peres et al. 2003; Tuck Haugaasen 2010). Secondary forests may therefore provide an opportunity for establishing Brazil nut regeneration in areas dominated by old trees, to maintain nut production in the long-term and secure this important extractive industry. However, based on results presented here, the importance of the agouti must not be underestimated. Natural regeneration may be severely limited in the absence of these animals. Although hunting did not occur in the current study area, hunting of agoutis generally accompanies the seasonal harvest of Brazil nuts across Amazonia (Rumiz & Maglianesi 2001). Several studies show that quantitative dispersal services are limited where intense hunting occurs. For example, Holbrook & Loiselle (2009) discovered that a proportionally larger number of seeds were removed from fruiting trees at non-hunted sites (89.4%) than at hunted sites (66.8%). A study by Babweteera and Brown (2009) demonstrated that in frugivore impoverished forests, 70-90% of juvenile seedlings established beneath adult conspecifics. In less disturbed forest areas with normal frugivore community, juveniles were established up to 80 m from adult conspecifics. The continued harvest of this non-timber forest product may therefore also depend on adequate measures to protect seed dispersers (Peres et al. 2003).

6. References

- Adler, G. H., Becerra, M. T., Prado, F. & Travi, B. L. (2000). Ecology of spiny rats, *Proechimys canicollis*, in northern Colombia. *Mammalia*, 64: 145-153.
- Babweteera, F. & Brown, N. (2009). Can remnant frugivore species effectively disperse tree seeds in secondary tropical rain forests? *Biodiversity and Conservation*, 18: 1611-1627.
- Blake, J. G. & Loiselle, B. A. (2001). Bird assemblages in second-growth and old-growth forests, Costa Rica: Perspectives from mist nets and point counts. *Auk*, 118: 304-326.
- Boot, R.G.A. & Gullison, R.E. (1995). Approaches to developing sustainable extraction systems for tropical forest products. *Ecological Applications*, 5: 896-903
- Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach*. Second ed. New York, USA: Springer-Verlag.
- Byrne, M. M. & Levey, D. J. (1993). Removal of seeds from frugivore defecations by ants in a Costa-Rican rain forest. *Vegetation*, 108: 363-374.
- Campos, W. G., Schoereder, J. H. & DeSouza, O. F. (2006). Seasonality in neotropical populations of *Plutella xylostella* (Lepidoptera): resource availability and migration. *Population Ecology*, 48: 151-158.
- Chapman, C. A., Wrangham, R. W., Chapman, L. J., Kennard, D. K. & Zanne, A. E. (1999). Fruit and flower phenology at two sites in Kibale National Park, Uganda. *Journal of Tropical Ecology*, 15: 189-211.
- Chazdon, R. L. & Coe, F. G. (1999). Ethnobotany of woody species in second-growth, old-growth, and selectively logged forests of northeastern Costa Rica. *Conservation Biology*, 13: 1312-1322.
- Clay, W. C. (1997). Brazil nuts: the use of a keystone species for conservation and development. In Freese, C. H. (ed.) *Harvesting Wild species: implications for biodiversity conservation*, pp. 246-282: The Johns Hopkins University Press.
- Corlett, R. T. (1994). What is secondary forest. *Journal of Tropical Ecology*, 10: 445-447.
- Cotta, J. N., Kainer, K. A., Wadt, L. H. O. & Staudhammer, C. L. (2008). Shifting cultivation effects on Brazil nut (*Bertholletia excelsa*) regeneration. *Forest Ecology and Management*, 256: 28-35.
- Cramer, J. M., Mesquita, R. C. G., Bentos, T. V., Moser, B. & Williamson, G. B. (2007). Forest fragmentation reduces seed dispersal of *Duckeodendron cestroides*, a central amazon endemic. *Biotropica*, 39: 709-718.
- Culot, L., Huynen, M. C., Gerard, P. & Heymann, E. W. (2009). Short-term post-dispersal fate of seeds defecated by two small primate species (*Saguinus mystax* and *Saguinus fuscicollis*) in the Amazonian forest of Peru. *Journal of Tropical Ecology*, 25: 229-238.
- DeWalt, S. J., Maliakal, S. K. & Denslow, J. S. (2003). Changes in vegetation structure and composition along a tropical forest chronosequence: implications for wildlife. *Forest Ecology and Management*, 182: 139-151.
- Diniz, T. D. A. S. & Bastos, T. X. (1974). Contribuição ao conhecimento do clima típico da castanha do Brasil. *Boletim Técnico IPEAN*, 64: 59-71.
- Dubost, G. (1988). Ecology and social-life of the red acouchy (*Myoprocta exilis*) - comparison with the orange red-rumped agouti (*Dasyprocta leporina*). *Journal of Zoology*, 214: 107-123.
- Dubost, G. & Henry, O. (2006). Comparison of diets of the acouchy, agouti and paca, the three largest terrestrial rodents of French Guianan forests. *Journal of Tropical Ecology*, 22: 641-651.

- Eycott, A. E., Watkinson, A. R., Hemami, M. R. & Dolman, P. M. (2007). The dispersal of vascular plants in a forest mosaic by a guild of mammalian herbivores. *Oecologia*, 154: 107-118.
- Fearnside, P. M. & Guimaraes, W. M. (1996). Carbon uptake by secondary forests in Brazilian Amazonia. *Forest Ecology and Management*, 80: 35-46.
- Finegan, B. (1992). The management potential of Neotropical secondary lowland rain-forest. *Forest Ecology and Management*, 47: 295-321.
- Forget, P. M. (1990). Seed-dispersal of *Vouacapoua americana* (Caesalpiniaceae) by caviomorph rodents in French-Guiana. *Journal of Tropical Ecology*, 6: 459-468.
- Forget, P. M. & Milleron, T. (1991). Evidence for secondary seed dispersal by rodents in Panama. *Oecologia*, 87: 596-599.
- Forget, P. M. (1992). Seed removal and seed fate in *Gustavia superba* (Lecythythaceae). *Biotropica*, 24: 408-414.
- Forget, P. M. (1996). Removal of seeds of *Carapa procera* (Meliaceae) by rodents and their fate in rainforest in French Guiana. *Journal of Tropical Ecology*, 12: 751-761.
- Fragaszy, D., Izar, P., Visalberghi, E., Ottoni, E. B. & De Oliveira, M. G. (2004). Wild capuchin monkeys (*Cebus libidinosus*) use anvils and stone pounding tools. *American Journal of Primatology* (4): 359-366.
- Guariguata, M. R. & Ostertag, R. (2001). Neotropical secondary forest succession: changes in structural and functional characteristics. *Forest Ecology and Management*, 148: 185-206.
- Haugaasen, T., Barlow, J. & Peres, C. A. (2003). Surface wildfires in central Amazonia: short-term impact on forest structure and carbon loss. *Forest Ecology and Management*, 179: 321-331.
- Haugaasen, T. & Peres, C. A. (2005). Tree phenology in adjacent Amazonian flooded and unflooded forests. *Biotropica*, 37: 620-630.
- Haugaasen, T. & Peres, C. A. (2006). Floristic, edaphic and structural characteristics of us flooded and unflooded forests in the lower Rio Purus region of central Amazonia, Brazil. *Acta Amazonica*, 36: 25-35.
- Holbrook, K. M. & Loiselle, B. A. (2009). Dispersal in a Neotropical tree, *Virola flexuosa* (Myristicaceae): Does hunting of large vertebrates limit seed removal? *Ecology*, 90: 1449-1455.
- Howe, H. F. & Smallwood, J. (1982). Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, 13: 201-228.
- Jansen, P. A., Bongers, F. & Hemerik, L. (2004). Seed mass and mast seeding enhance dispersal by a neotropical scatter-hoarding rodent. *Ecological Monographs*, 74: 569-589.
- Jansen, P. A., Bongers, F. & Prins, H. H. T. (2006). Tropical rodents change rapidly germinating seeds into long-term food supplies. *Oikos*, 113: 449-458.
- Jansen, P. A., & Forget, P.M. (2001). Scatterhoarding rodents and tree regeneration. In Bongers, F., Charles-Dominique, P., Forget, P.M. & Thery, M. (ed.) *Nouragues: Dynamics and plant-animal interactions in a neotropical rainforest* pp. 278-288: Kluwer Academic Publishers.
- Janzen, D. H. (1971). Seed predation by animals. *Annual Review of Ecology and Systematics*, 2: 465-492.
- Jorge, M. L. S. P. & Howe, H. F. (2009). Can forest fragmentation disrupt a conditional mutualism? A case from central Amazon. *Oecologia (Berlin)*, 161: 709-718.
- Jorge, M. S. P. & Peres, C. A. (2005). Population density and home range size of red-rumped agoutis (*Dasyprocta leporina*) within and outside a natural Brazil nut stand in southeastern Amazonia. *Biotropica*, 37: 317-321.

- Kainer, K. A., Duryea, M. L., Malavasi, M. D., da Silva, E. R. & Harrison, J. (1999). Moist storage of Brazil nut seeds for improved germination and nursery management. *Forest Ecology and Management*, 116: 207-217.
- Kainer, K. A., Wadt, L. H. O. & Staudhammer, C. L. (2007). Explaining variation in Brazil nut fruit production. *Forest Ecology and Management*, 250: 244-255.
- Koike, S., Morimoto, H., Goto, Y., Kozakai, C. & Yamazaki, K. (2008). Frugivory of carnivores and seed dispersal of fleshy fruits in cool-temperate deciduous forests. *Journal of Forest Research*, 13: 215-222.
- Lambert, T. D. & Adler, G. H. (2000). Microhabitat use by a tropical forest rodent, *Proechimys semispinosus*, in central Panama. *Journal of Mammalogy*, 81: 70-76.
- Lemmon, P. E. (1957). A new instrument for measuring forest understory density. *Journal of Forestry*, 55.
- Loiselle, B. A. & Blake, J. G. (1991). Temporal variation in birds and fruits along an elevational gradient in Costa-Rica. *Ecology*, 72: 180-193.
- Lugo, A. E. & Brown, S. (1992). Tropical forests as sinks of atmospheric carbon. *Forest Ecology and Management*, 54: 239-255.
- Maron, J. L. & Simms, E. L. (1997). Effect of seed predation on seed bank size and seedling recruitment of bush lupine (*Lupinus arboreus*). *Oecologia*, 111: 76-83.
- Minitab. (2006). *Minitab 15.1.0.0*, Minitac inc. (ed.).
- Mori, S. A. & Prance, G. T. (1990). Taxonomy ecology and economic botany of the Brazil nut (*Bertholletia excelsa*) Humb. and Bonpl. Lecythidaceae. In *Advances in Economic Botany, Prance, G. T. And M. J. Balick*, pp. 130-150.
- Morrison, S. F., Pelchat, G., Donahue, A. & Hik, D. S. (2009). Influence of food hoarding behavior on the over-winter survival of pikas in strongly seasonal environments. *Oecologia*, 159: 107-116.
- Myers, G. P., Newton, A. C. & Melgarejo, O. (2000). The influence of canopy gap size on natural regeneration of Brazil nut (*Bertholletia excelsa*) in Bolivia. *Forest Ecology and Management*, 127: 119-128.
- Müller, C. H. (1981). Castanha-do-Brasil: estudos agronomicos. *documentos*, 1: 1-25.
- Pena-Claros, M. & De Boo, H. (2002). The effect of forest successional stage on seed removal of tropical rain forest tree species. *Journal of Tropical Ecology*, 18: 261-274.
- Peres, C. A. (1991). Seed predation of *Cariniana micrantha* (Lecythidaceae) by Brown capuchin monkeys in central Amazonia. *Biotropica*, 23: 262-270.
- Peres, C. A. & Baider, C. (1997). Seed dispersal, spatial distribution and population structure of Brazilnut trees (*Bertholletia excelsa*) in southeastern Amazonia. *Journal of Tropical Ecology*, 13: 595-616.
- Peres, C. A., Schiesari, L. C. & DiasLeme, C. L. (1997). Vertebrate predation of Brazil-nuts (*Bertholletia excelsa*, Lecythidaceae), an agouti-dispersed Amazonian seed crop: A test of the escape hypothesis. *Journal of Tropical Ecology*, 13: 69-79.
- Prasad, S. & Sukumar, R. (2010). Context-dependency of a complex fruit-frugivore mutualism: temporal variation in crop size and neighborhood effects. *Oikos*, 119: 514-523.
- R Development Core Team. (2008). *R: a language and environment for statistical computing*. Computing, R. F. f. S. (ed.). Vienna, Austria. ISBN 3-900051-07-0
- Rumiz, D. I. & Maglianesi, M. A. (2001). Hunting impacts associated with Brazil nut harvesting in the Bolivian Amazon. *Vida Silvestre Neotropical*, 10: 19-29.
- Scoles, R. (2010). *Ecologia e extrativismo da castanheira (Bertholletia excelsa, Lecythidaceae) em duas regiões da Amazônia Brasileira*. Phd-thesis. Manaus, Instituto Nacional de Pesquisas da Amazonia - INPA

- Sezen, U. U., Chazdon, R.L. & Holsinger, K.E. (2009). Proximity is not a proxy for parentage in an animal-dispersed Neotropical canopy palm. *Proceedings of the Royal Society B-Biological Sciences*, 276: 7.
- Smythe, N. (1978). The natural history of the central american agouti (*Dasyprocta punctata*). *Smithsonian Contributions to Zoology*, 257: 1-52.
- Terborgh, J., Losos, E., Riley, M. P. & Riley, M. B. (1993a). Predation by vertebrates and invertebrates of the seeds of 5 canopy tree species of an Amazonian forest. *Vegetatio*, 108: 375-386.
- Traveset, A., Rodrigues-Perez, J. (2008). Seed Dispersal. In Elsevier, B. V. (ed.) *General Ecology*, pp. 3188-3194.
- Tuck Haugaasen, J. M., Haugaasen, T., Peres, C. A., Gribel, R. & Wegge, P. (2010). Seed dispersal of the Brazil nut tree (*Bertholletia excelsa*) by scatter-hoarding rodents in a central Amazonian forest. *Journal of Tropical Ecology*, 26: 251-262.
- Tuck Haugaasen, J. M., Haugaasen, T., Peres, C.A., Gribel, R. & Wegge, P. (In review). A new method to evaluate natural seed dispersal of the Brazil nut tree. *Biotropica*.
- Van Schaik, C. P., Terborgh, J. W. & Wright, S. J. (1993). The phenology of tropical forests - adaptive significance and consequences for primary consumers. *Annual Review of Ecology and Systematics*, 24: 353-377.
- Vander Wall, S. B. (1990). *Food hoarding in animals*: The University of Chicago Press. 445 pp.
- Vander Wall, S. B. & Jenkins, S. H. (2003). Reciprocal pilferage and the evolution of food-hoarding behavior. *Behavioral Ecology*, 14: 656-667.
- Vieira, E. M., Pizo, M. A. & Izar, P. (2003). Fruit and seed exploitation by small rodents of the Brazilian Atlantic forest. *Mammalia*, 67: 533-539.
- Wadt, L. H. O., Kainer, K. A. & Gomes-Silva, D. A. P. (2005). Population structure and nut yield of a *Bertholletia excelsa* stand in Southwestern Amazonia. *Forest Ecology and Management*, 211: 371-384.
- Watson, W. (1901). Germination of seeds of *Bertholletia excelsa*. *Annals of Botany*, 15: 99-102.
- Wenny, D. G. (2001). Advantages of seed dispersal: A re-evaluation of directed dispersal. *Evolutionary Ecology Research*, 3: 51-74.
- Wiesbauer, M. B., Giehl, E. L. H. & Jarenkow, J. A. (2008). Morphological patterns of diaspores from animal-dispersed tree and treelet species at Parque Estadual de Itapua, Rio Grande do Sul State, Brazil. *Acta Botanica Brasilica*, 22: 425-435.
- Wright, S. J., Stoner, K. E., Beckman, N., Corlett, R. T., Dirzo, R., Muller-Landau, H. C., Nunez-Iturri, G., Peres, C. A. & Wang, B. C. (2007). The plight of large animals in tropical forests and the consequences for plant regeneration. *Biotropica*, 39: 289-291.
- Xiao, Z. S., Jansen, P. A. & Zhang, Z. B. (2006). Using seed-tagging methods for assessing post-dispersal seed fate in rodent-dispersed trees. *Forest Ecology and Management*, 223: 18-23.
- Zuidema, P. A. & Boot, R. G. A. (2002). Demography of the Brazil nut tree (*Bertholletia excelsa*) in the Bolivian Amazon: impact of seed extraction on recruitment and population dynamics. *Journal of Tropical Ecology*, 18: 1-31.