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Defaunation Increases the Survivorship of the Palm *Astrocaryum gratum* in a Submontane Tropical Forest

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Abstract

Overhunting of mammals may negatively affect plant populations though indirect impacts on mammal-plant interactions such as herbivory. In this paper, we examined how hunting of terrestrial mammals impacts the survival of seedlings and juveniles of the palm *Astrocaryum gratum*. To determine *A. gratum* seedling survivorship patterns, an experiment with seedlings in 25 × 250-m plots exclosures and different levels of mammal species presence was conducted: all mammals, only *Tayassu peccari* potentially a major seedling predator and no animals excluded. More than 50,600 non-adults were measured for 27 months. We replicated these treatments in three forest categories: 1) no hunting, with an intact mammal community, 2) a lightly hunted region and 3) a heavily hunted area. Seedling survivorship under natural non-experimental conditions was highest in the heavily hunted (S(T) = 0.72), lower in moderate and lowest in unhunted forests. Experiments revealed that *T. peccari* was the main agent of palm seedling mortality and the most important factor determining seedling and juvenile survivorship, for example they caused the 84.61% deaths of the seedlings in unhunted forests. Thus, *T. peccari* feeding habits can influence forest dynamics and forest structure. *T. peccari* was also susceptible to hunting pressure and as the main seed and seedling predator in the system, its extinction should affect the survivorship and distribution of *A. gratum* in forests.

Keywords

Defaunation, Empty Forest, Exclosure, Half Empty Forest, Seedling Survivorship, White-Lipped Peccary

1. Introduction

The overhunting of animals can impact plant species abundance and diversity through linked ecological processes in tropical forests (Aliaga-Rossel, 2011 [1], Dirzo *et al.*, 2014 [2]). Overhunting has been described as leaving forests that are structurally intact but missing key animal species (the so-called empty forest hypothesis, Redford, 1992 [3]). The process of emptying forests, also known as defaunation, describes how the loss of terrestrial vertebrates may alter plant communities through the loss or reduced consequences of plant-animal interactions (Asquith *et al.*, 1997 [4]; Dirzo and Miranda, 1991 [5]; Dirzo *et al.*, 2014 [2]) and decreased both seed dispersal and predation are important determinants of seedling community compositional change in the functional trait composition as a consequence of defaunation in tropical forests.

Dirzo and Miranda, 1999 [5] first noted that the absence of mammal species, especially large ungulates, increased seedling recruitment. Subsequent studies reported that the absence of ungulates and rodents is correlated with increased seedling densities (DeMattia *et al.*, 2006 [6]; Terborgh and Wright, 1994 [7]; Terborgh *et al.*, 2001 [8]). In contrast, Asquith *et al.*, 1997 [4] found that reducing mammal populations increased seedling mortality. These differential responses of plant communities to the reduction and local extirpation of vertebrate populations motivated the present study. However, the influence of any one vertebrate species, even one credited as a potential keystone, remains understudied.

White-lipped peccary (WLP; *Tayassu pecari*) and the Chonta palm *Astrocaryum gratum* are ecologically important species (Aliaga-Rossel, 2011 [1]). Chonta palm is a widespread Neotropical lowland forest species that grows up to 15 m tall with characteristic spines on trunk and leaves. Adult trees produce large edible fruit throughout the year (Aliaga-Rossel, 2011 [9]) and the production of both fruits and seeds brings together an important guild of frugivore animals in Bolivia (Aliaga-Rossel and Moraes, 2014 [10]). In other species of the same genus, seeds and seedlings experience mortality from insects, desiccation, and disease, but principally from mammalian herbivores including WLP (Antonik, 2005 [11]; Beck and Terborgh, 2002 [12]; Silman *et al.*, 2003 [13]). White-lipped peccaries may form large herds of over 300 individuals that range over extended areas feeding mainly on palm fruits, seeds and seedlings year-round (Aliaga-Rossel and Painter, 2020 [14]; Fragoso, 2005 [15]); they are adapted to harvest hard seeds such as palm endocarps of 46 species affecting their distribution (Aliaga-Rossel and Moraes, 2014 [10]; Beck, 2006 [16]). The WLP is also the preferred prey of many indigenous and rural peoples (Aliaga-Rossel, 2011 [1]; Fragoso *et al.*, 2016 [17]); with a frequency of 43 (33.08%), 33.5 kg and 13.92 of importance as a species, it has a high rate of defaunation in semi-deciduous forests of lowlands, which depends not only on its conservation, but also on the variation of its population density (Giacomini and Galetti, 2013 [18]).

Here, we use WLP and Chonta palm as model to understand the effect of removing or reducing populations of a potential keystone vertebrate consumer of

seedlings versus all vertebrate species. First, using a natural experimental approach, we compare seedling survivorship in three types of forests with high, low and no hunting pressure, testing the conflicting predictions of most defaunation studies that vertebrate species can increase or decrease seedling mortality. We first test the hypotheses under conditions of complete and partial extirpation or normal levels of vertebrate species using an observational approach and a natural experiment. We also tested the hypotheses experimentally by controlling access to planted seedlings in plots (exclosures) in examples of the three forest types. In the natural experiment, we predicted that adult and seedling densities of *A. gratum* would be lowest in unhunted forests and highest in areas where all vertebrate herbivores (e.g. agouti *Dasyprocta punctata*, peccary *Tayassu peccary*, tapir *Tapirus terrestris*) have been reduced or extirpated. To tease apart the effect of all vertebrates present versus only the WLP, we predicted that experimental treatments would follow the patterns observed naturally in the three forest types: 1) allowing access to treatments by all mammals (Control) would result in the lowest seedling survivorship and recruitment levels. 2) Exclosures simulating total defaunation would show the highest palm survivorship. 3) We expected no difference in seedling survivorship between peccary-exclusion treatments in all forest types and the control situation of all vertebrates excluded. We also predicted that all effects would remain constant in the three forest types.

2. Material and Methods

2.1. Study Area

The study was conducted in two areas of lowland forest in Bolivia, the Madidi National Park, and Natural Area of Integrated Management (13˚20' S - 14˚00'S, 68˚10' W - 69˚10'W), and Pilón Lajas Biosphere Reserve and Indigenous Territory (14˚25' S - 15˚27'S, 66˚55' W - 67˚40'W). These adjacent areas share comparable landscapes and altitudes (altitude range = 200 - 6000 masl) and support similar ecosystems and species (Aliaga-Rossel, 2011 [1]). The mean annual temperature is 26˚C and the annual precipitation of over 2000 mm is highly seasonal with well-defined dry (May-October) and wet (December-April) seasons. The vegetation in the region is a Piedmont submontane evergreen forest (Navarro and Maldonado, 2004 [19]) that mixes with moist lowland forest with both Amazonian and Andean plant affinities.

Three forest types with similar vegetation experiencing different levels of hunting pressure and therefore different mammal density were selected for study (Aliaga-Rossel, 2011 [1]; Perez, 2008 [20]; Aliaga-Rossel and Fragoso 2015 [21]); 1) an unhunted area that supported high densities of WLPs (10.7 individuals km-2; unpublished radio tracking data Wildlife Conservation Society WCS-Bolivia), 2) a moderately hunted forest that maintained low WLP densities, and 3) a heavily hunted forest where WLP no longer existed (Perez, 2008 [20]).

The unhunted area was located inside Madidi National Park, where terrestrial mammal communities remain intact (Perez, 2008 [20]; WCS-Bolivia unpubl.

data). The moderately hunted forest, consisted of sites located 10 - 15 km from the Asunción del Quiquibey indigenous community center, an area where hunting is regulated by the community. The heavily hunted forest was located in the Pilón Lajas - Asunción del Quiquibey indigenous community, within a 10-km radius of the village center where other studies (Iwanura *et al.*, 2014 [22]) have shown that fauna is intensively hunted. Although we did not directly estimate hunting pressure or animal populations, we assumed that these parameters correlated with distance from village based on the work of other researchers (evidence presented by Iwamura *et al.*, 2014 [22]).

Following Antonik, 2005 [11] we used a study design that included natural observational0 and experimental approaches. For the natural observational, we randomly placed four 25 × 250-m plots, each separated by at least 4 km, in each of the three forest categories. A 2 × 250-m transect was established along the centre of each plot in which all individuals of *A. gratum* were identified, measured, and tagged with green flagging. In addition, the height of each seedling was measured monthly for 27 months, from November 2008 to January 2011. A local expert tracker and hunter accompanied all visits and assisted with the identification of animal sign and the causes of seedling and juvenile mortality.

For survivorship analysis, we divided *A. gratum* into five growth categories: *Seedling* includes plants up to 50 cm in height, and individuals with emerging plumules or with two bifid euphylls. This size range equals seedlings up to about two years old (Aliaga-Rossel, 2011 [1], Aliaga-Rossel, 2011 [9]). *Seedling 1* - individuals 50 - 150 cm tall, with three to five pinnate leaves, with fully developed pinnae or leaflets along each side of the rachis. *Juvenile* - taller individual 150 - 250 cm tall, with several thicker leaves and sharp spines along the rachis. Saplings - plants taller than 250 cm, with a developing trunk, and many spines along leaves larger than 15 cm. *Adult*—An individual, with a well-defined trunk covered with spines and signs of reproductive activity—inflorescence and/or fruit.

2.2. Density

The density of *Astrocaryum* was the number of individual plants present in the determined area. The number of adult *A. gratum* capable of producing flowers and fruits was also noted along the 250 × 2-m transect. All sub-adult *A. gratum* within each transect were also recorded and checked monthly, with their condition recorded as alive or dead.

2.3. Survivorship

If dead, the cause of mortality was determined based on physical evidence and animal sign and classified as: 1) Insect attack: usually caused by different caterpillar species or leaf cutter ants. Species' sign consisted of furrows left on the leaves or small holes on the leaf surface; 2) White-lipped peccary: seedlings uprooted and root base bitten off, unmistakable hoof tracks and/or characteristic soil removal and rooting; 3) Other vertebrate: mainly deer as determined by the

presence of tracks, or sign of herbivory on leaves; 4) Fungus and other microbial pathogens: either a hole in the leaf epidermis, or fungus growing through stomata. Spores of some fungi attack leaves, creating dead spots or killing the whole leaf; 5) Unknown: the agent of mortality could not be determined, for example, indeterminate causes of desiccation (seedling standing but completely dried).

The non-parametric Kaplan-Meier survival estimator S [T] (Kaplan and Maier, 1958 [23]) was used to calculate survival estimates with 95% confidence intervals for seedlings, juveniles, and saplings (separately and combined). The Kaplan-Meier statistic allows for the addition of new individuals into the calculation at any point in time, as well as allowing the inclusion of plants of unknown fates (e.g. plants not found; crushed by trees, or status unknown). Age classes are sampled randomly and the survival times of different individuals are independent (Aliaga-Rossel, 2011 [1]). The survival function (S [T]) was estimated monthly. To measure the correlation of temperature and precipitation with mortality (Pearson's correlation coefficient), we compared monthly data for these parameters (rainfall and temperature data obtained from the National Service of Hydrography and Meteorology-SENAHMI).

2.4. Experiment

To determine the effect of vertebrate seedling predators on the survivorship and recruitment of *A. gratum*. We used a randomized block design to quantify the effects of seedling consumption by vertebrates. Treatments consisted of experimental plots where: 1) all vertebrates were excluded (total fenced-exclosure), 2) only WLP were excluded (peccary fenced-exclosure), or 3) no species were excluded (no fencing around plot). A block sample consisted of each of the three treatments spaced 1 - 5 m apart. Three blocks (nine treatments), each separated by 50 - 75 m formed a plot. A total of 12 blocks where located in each forest type.

The all-vertebrate (total) exclosure consisted of a 1×1-m soil area enclosed by a galvanized wire mesh fence (50 cm high, <1 cm^2 mesh size). The wire mesh was fixed to vertical wooden poles at the corners and staked to the soil to prevent mammals from pushing underneath. The top of the structure was covered with the same wire material to complete enclosure. All contact points between mesh and stakes were secured tightly with wire. A test run of the exclosure at the beginning of the study, indicated that leaves accumulating on top of the mesh might reduce sunlight and affect seedling growth. Thus, leaves and debris were removed each month from the top of all total exclosures.

The peccary exclosure consisted of the same galvanized wire fencing but mesh size was 2 cm^2. Fencing began at 15 to 20 cm above the ground and reached a 50 cm height. Wooden poles running parallel to the ground at a 15 cm height served to secure the wire mesh. This design prevented peccaries (white-lipped peccaries and potentially collared peccaries) from rooting into the sides of the treatment but allowed smaller vertebrates such as rodents to pass beneath and through the mesh. There was no covering mesh on this exclosure type, allowing

larger mammals such as deer, tapir, and birds access over the fencing. Peccary head and neck morphology did not enable them to reach into exclosures, similar to Antonik, 2005 [11].

The open or control treatment consisted of an unfenced area marked with 5 - 10 cm long wooden poles placed at the four usual corners as the exclosures. This treatment allowed access for all animals. Soil was cleaned around the treatment to facilitate the identification of the tracks of any terrestrial vertebrate visiting the site.

Each experimental treatment (total, WLP and open) contained 16 seedlings, planted in four rows of four with at least 20 cm between rows. Seedlings planted in the treatments were collected opportunistically from beneath different adult trees from distant unstudied areas. Only seedlings in good condition, with an emerged plumule and endocarp still attached were collected for experiments. The proportion of open canopy above each sample was measured using a spherical crown densiometer to ensure similar light conditions for each treatment.

Treatments were set out in November to December 2008 and monitored monthly until January 2011 (25 mo). Survivorship of seedlings was recorded at each visit and causes of mortality noted. Notably, the exclosures were designed to restrict vertebrate predation, and all other sources of seedling mortality, such as fungal attack, were assumed to be constant among plot type treatments.

A factorial ANOVA was conducted to determine if statistical differences in seedling survivorship were present among treatments and forest types with the plots and treatments also included, and to determine the effects of the exclosures in the forest a simple ANOVA was used. The survival rate (σ) of seedlings was derived for each forest category (unhunted, moderately hunted and hunted) and treatment for those that survived to each recording event (s) divided by the number of live plants identified at the start of the study (N) Thus, $\sigma = s/N$. The probability for moving from seedlings growth stage to the next was obtained by considering the number of plants reaching the next size class after 1 y: $\gamma = r/s$.

3. Results

3.1. Density

Mean adult palm densities differed amongst the three forest types, with the unhunted forest supporting mean $\pm$ SD 19.6 $\pm$ 12.50 indiv·ha^{-1} (n = 49), the moderately hunted with 21.6 (n = 54, SD = 4.65) and the hunted one with 46 indivi·ha^{-1} (n = 115, SD = 19.20). Average densities for seedlings, seedling 1, juveniles and saplings were all significantly different amongst the three types of forest, with the hunted and moderately hunted forest supporting higher seedling densities relative to the unhunted sites. The greatest difference was between forests with heavy hunting pressure relative to the others. There was little difference between forests with no-hunting and moderate hunting. A positive relationship occurred between adult and seedling density in all forests (PTE R^2 = 0.6, p = 0.043). Areas with high adult density had higher seedling densities.

3.2. Survivorship

More than 50,600 non-adult *A. gratum* were recorded and monitored in three types of forests. An average of 513 individuals (SD = 60) occurred in unhunted, 685.2 (SD = 48.98) in moderately hunted and 1455 in hunted forest at the end of the study (Figure 1).

Seedling survivorship in all size classes was significantly different amongst the three forest classes ($F_{2,9}$ = 16.4; p < 0.001), with the lowest occurring in unhunted (S(T) = 0.39, 95% C.I. ±0.03), followed by moderately hunted (S(T) = 0.66, 95% C.I. ±0.03) and lastly the heavily hunted forest (S(T) = 0.72, 95% C.I. ±0.02) (Figure 2). Ninety four percent of total deaths in the unhunted forest occurred during one event, when a herd of WLP passed through and killed these seedlings.

The estimator used independently for seedlings indicates a significant difference in the survivorship rates of the three types of forest ($F_{2,9}$ = 22.34; p < 0.0001); with the lowest seedling survivorship in the unhunted (S(t) = 0.255, 95% C.I. ±0.02); followed by the moderately hunted (S(t) = 0.58, 95% C.I. ±0.034) and then the hunted forest (S(t) = 0.672, 95% C.I. ±0.03 (Figure 2). For the seedling 1 group survivorship was also significantly different among forest types ($F_{2,9}$ = 16.29; p < 0.0001), with the lowest seen in the unhunted forest, followed by the moderately and the hunted ones (Figure 2); juveniles also presented a difference in survivorship amongst the forest types (F_1 7.06; p < 0.001), with once again the lowest in the unhunted, higher for the moderately hunted and highest in the hunted one (Figure 2). In contrast saplings did not present differences in survivorship between the forest types (F_2 = 0.288; p = 0.75), it being high in all three type of forests; S(t) = 0.94, 95% C.I. ±0.11 for the unhunted; S(t) = 0.93, 95% C.I. ±0.09 moderately hunted; and S(t) = 0.958, 95% C.I. ±0.1 for the hunted forest (Figure 2). Temperature and precipitation had a non-significant correlation with *A. gratum* mortality (r = 0.2, p = 0.251).
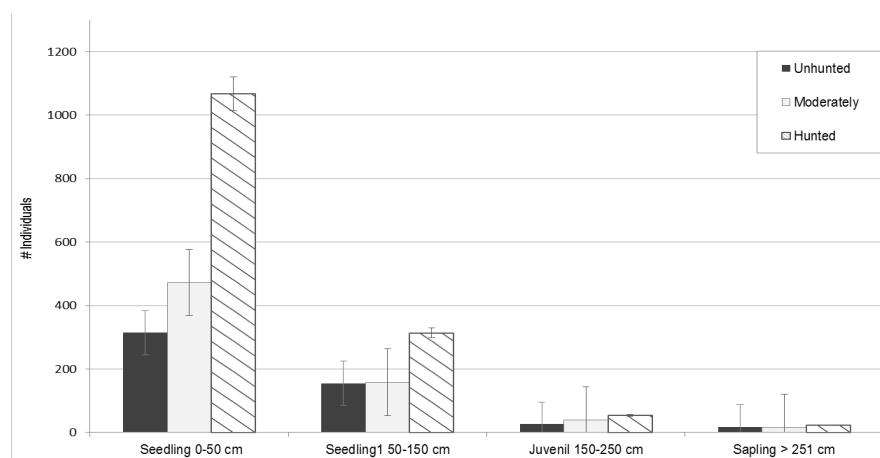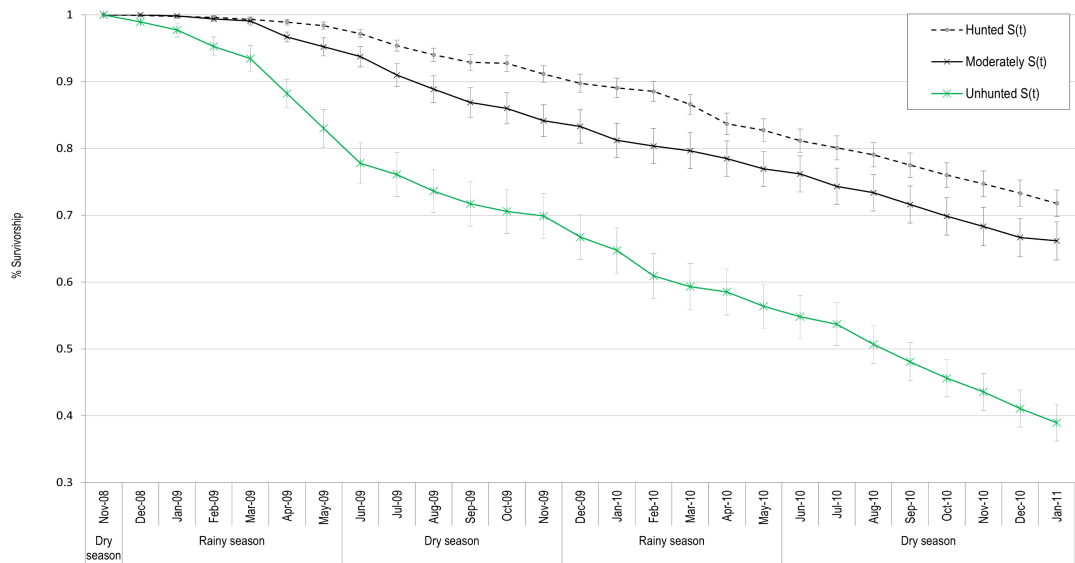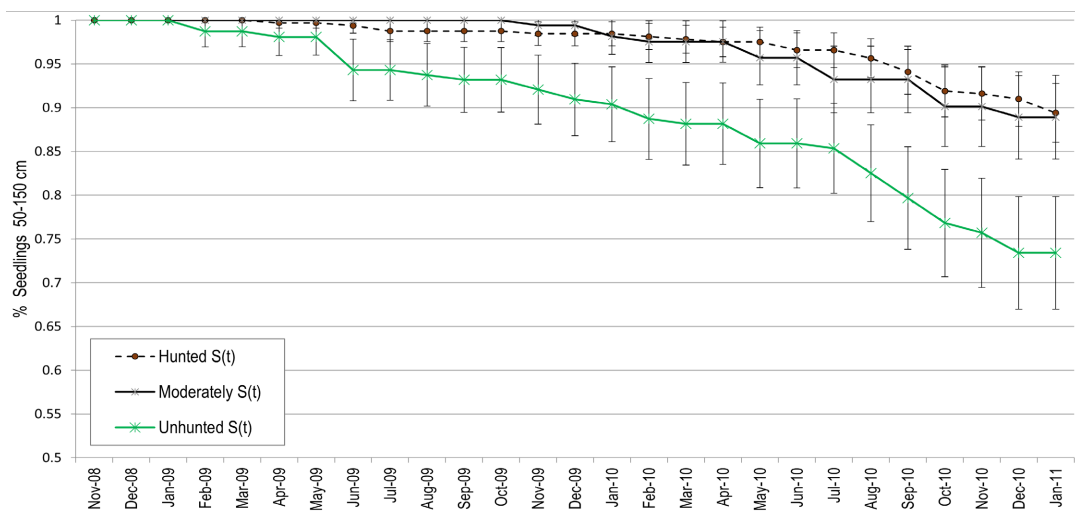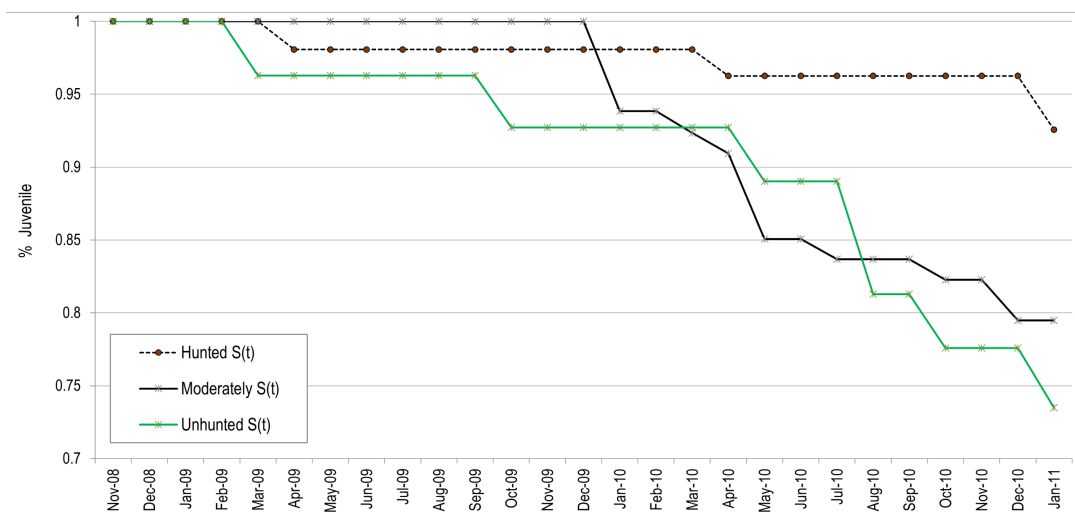

Figure 1. Number of Chonta palm (*Astrocaryum gratum*) non-adults surveyed from November 2008 to January 2011. Seedling (0 - 50 cm), seedling 1 (50 - 150 cm), juveniles (150 - 250) and saplings (>300 cm). In three types of forests; unhunted, moderately hunted and hunted forest.
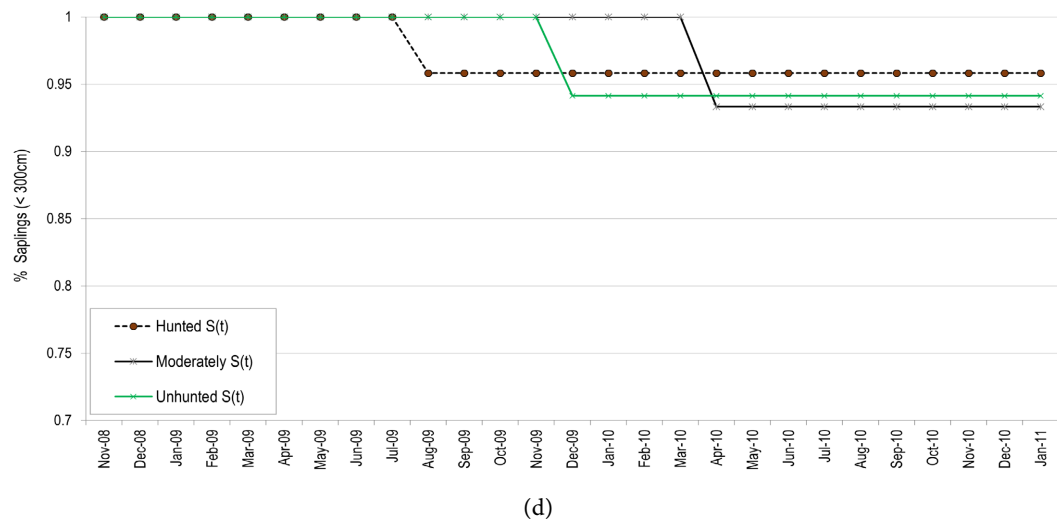
(a)

(b)

(c)

(d)

Figure 2. Chonta palm (*A. gratum*) survivorship (from November 2008 to January 2011), in three type of forests; unhunted, moderately hunted and hunted; a) Seedlings (5 - 50 cm) one month- 2 years; b) youngling (50 - 150 cm); c) juvenile (150 - 300 cm); d) sapling (<300 cm, some with developed trunk but not reproductive). Figure (a), Axis Y starts at 0.3 and figures (b), (c), (d) starts at 0.7. (a) Seedlings 0 - 50 cm. Not hunted: $S(t) = 0.255\%$ (95% CI = 0.023); moderately hunted $S(t) = 0.58\%$ (95% CI = 0.034) and hunted forest $S(t) = 0.67\%$ (95% CI = 0.028). (b) Seedling 1 (50 - 150 cm). Unhunted: $S(t) = 0.734\%$ (95% CI = 0.064); moderately hunted $S(t) = 0.88\%$ (95% CI = 0.048) and hunted forest $S(t) = 0.89\%$ (95% CI = 0.033). (c) Juvenile (150 - 300 cm). Unhunted: $S(t) = 0.735\%$ (95% CI = 0.064); moderately hunted $S(t) = 0.79\%$ (95% CI = 0.092) and hunted forest $S(t) = 0.92\%$ (95% CI = 0.033). (d) Sapling (<300 cm). Unhunted: $S(t) = 0.94\%$ (95% CI = 0.016); moderately hunted $S(t) = 0.93\%$ (95% CI = 0.093) and hunted forest $S(t) = 0.96\%$ (95% CI = 0.077).

3.3. Mammal Seedling Predators on the Survivorship and Recruitment of *Astrocaryum gratum*

From January 2009 to January 2011, 576 seedlings of *A. gratum* were followed in the three experimental treatments. We observed different degrees of seedling survivorship amongst treatments with a significant interaction between exclosure type and forest, as well as significant main effects of both forest and exclosure type (Table 1). A further exploration of this interaction using factorial ANOVAs revealed significant difference between seedling survivorship in the three forests and experimental treatments (see Table 1). As predicted, survival was much higher in the total exclosures (Figure 3) in unhunted (75%), moderately hunted (79%) and in hunted forest (85.4%). This difference was first noted at the 4-month mark and was maintained until the end of the two-year study (52.6%, 53.12% and 58.8%, respectively) (Figure 3). The total exclosures worked successfully, with no vertebrate or mammal activity ever registered inside (Total excl: Forest $F_{2,9} = 0.14$; p = 0.871). In all forest types seedling survival in the total and peccary exclosures was similar (peccary excl: Forest $F_{2,9} = 0.86$; p = 0.457).
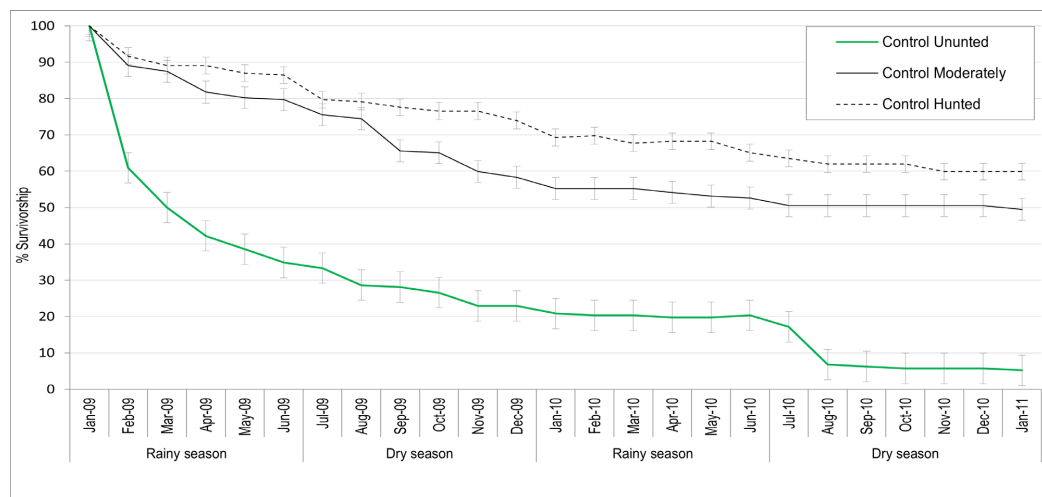
In contrast, seedling survival in the control plots differed significantly amongst the three forest types (control exclosure: Forest $F_{2,9} = 164.56$; p < 0.0005), with the highest mortality recorded during the first three months of the study. In the
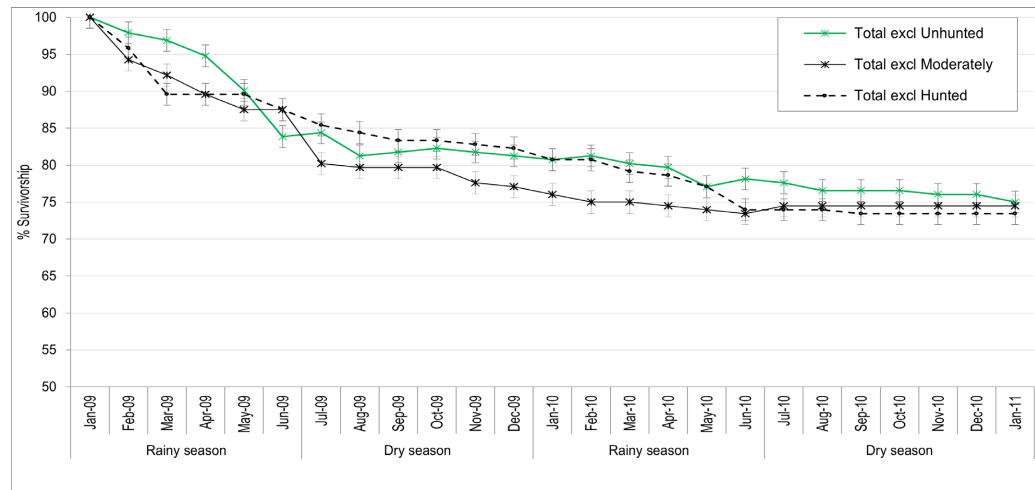
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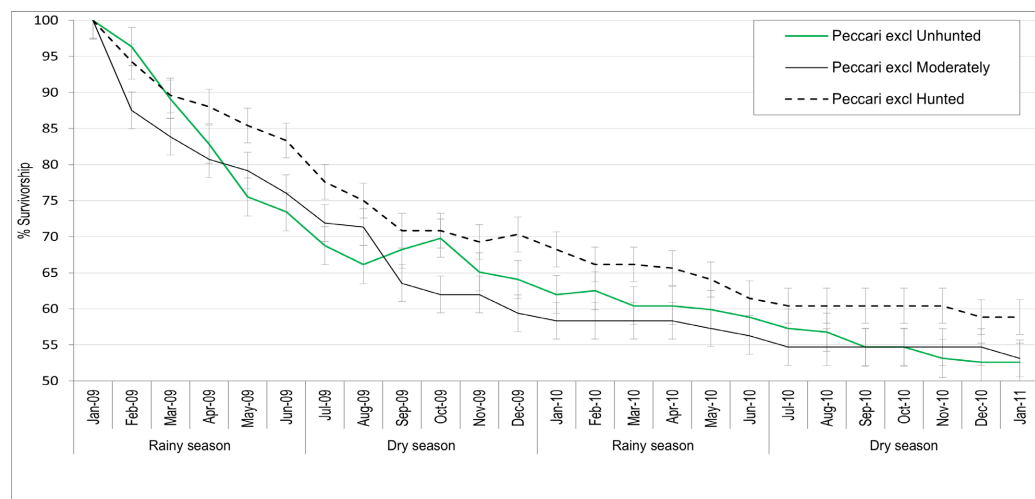
(b)

(c)

Figure 3. Per cent survivorship of *Astrocaryum gratum* seedlings, within three experimental plot treatments (a) Total exclosure; (b) peccary exclosure; (c) control (no exclosure), in three type of forests with various hunting pressure: Unhunted, moderately hunted and hunted. Figures (a), (b) starts at 0.5.

Table 1. Factorial analysis of variance for experimental treatment of seedlings (exclosure treatment), using Adjusted SS for Tests.able type styles.

| Source	DF	Seq SS	Adj SS	Adj MS	F	P
Forest	2	195.019	195.019	97.509	54.72	0.000
Plot (Forest)	9	25.278	25.278	2.809	0.68	0.715
Block (Forest Patch)	24	98.444	98.444	4.102	1.52	0.092
Exclosure	2	602.074	602.074	301.037	111.76	0.000
Forest* Exclosure	4	554.62	554.62	138.66	9.78	0.000
Error	66	177.778	177.778	2.694		
Total	107	1429.407				

first month for the controls, the unhunted forest presented a survivorship of 60.93%, the moderately hunted forest 89.06%, and the heavily hunted forest 91.66%. During the second month, seedling survivorship fell by half, dropping to 87.5% and 89.1% for the moderately and heavily hunted forest, respectively. After a year, survivorship fell to 20.8% in the unhunted, 55.21% in the moderately hunted and 69.3% for the hunted forest. After two years survivorship dropped 5.02% in the unhunted, 49.4% in the moderately and 59.89% for the heavily hunted forest (Figure 3).

We were unable to identify the main cause of mortality in total exclosures for all forest types (81.25% for unhunted forest, 87.75% in the moderately hunted and 99.23% for the heavily hunted forest). The next most important agent of mortality was fungus and insect infection (less than 20%) (Figure 4).

In only one case did peccaries impact seedlings in a peccary exclosure, when three (3.3% of the total) were dug up by white-lipped peccaries in the unhunted forest. Sign remnants indicated that rodents and other mammals did enter these exclosures. We registered only two events of mammal herbivory on seedlings inside these, both in the unhunted forest. In both instances a deer (*Mazama sp.*) foraged over the fence and consumed a few leaves but the seedling survived. In month six, a single seedling death was caused by an insect eating the apical meristem. A higher number of attacks by insects were detected in the moderately and hunted forest compared to the unhunted forest (Figure 4).

In the control plots, the highest seedling mortality (Table 2) occurred in the unhunted forest, where WLP were killed 84.61% of the seedlings (154 killed out of 192 planted). There was no evidence of seedling predation by peccaries in the control plots in forests with heavy hunting pressure. This pattern also occurred in the moderately hunted forest. Seedling mortality caused by other vertebrates was low in the control plots in the unhunted (1.09%), moderately hunted (10.52%) and hunted forest (2.6%).
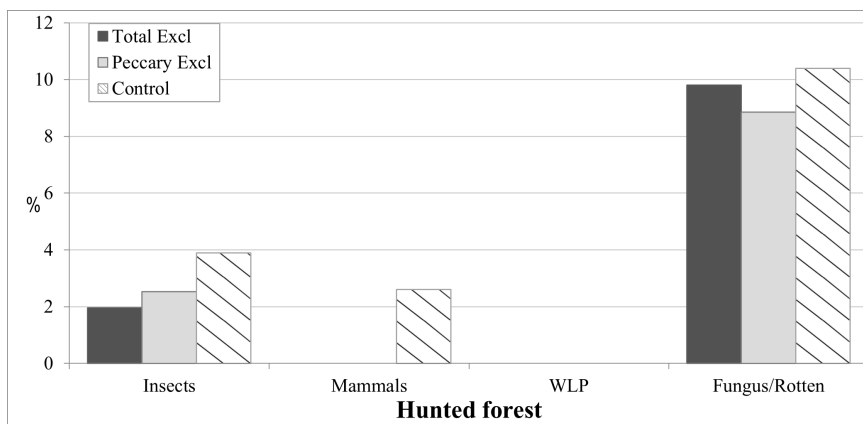
A few seedlings were killed by falling trees and branches in two exclosures and, but most experiencing such events survived until the end of the experiment (Table 2). We observed high fungal infection rates on some seedling's leaves, but
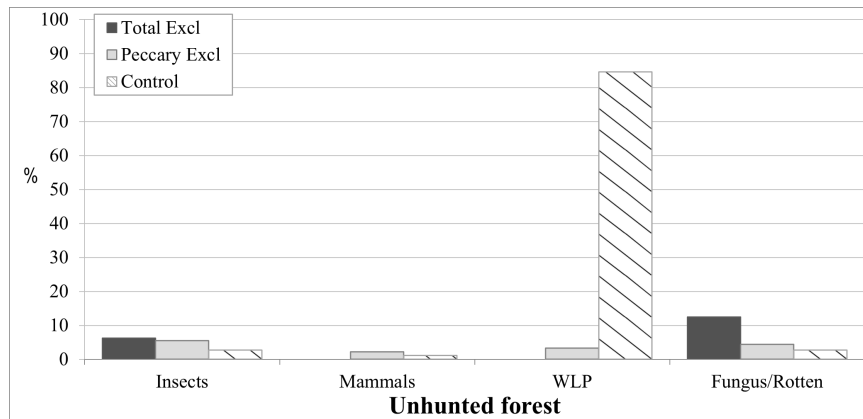
(a)
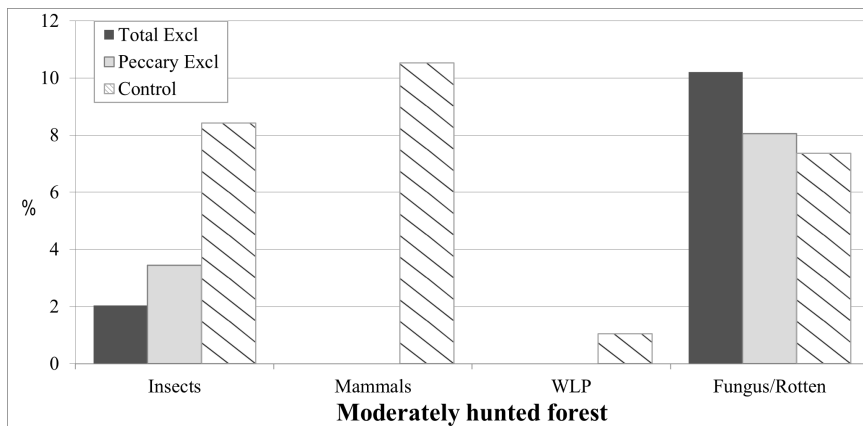
(b)

(c)

Figure 4. Causes of seedling mortality-insects, all animals, white-lipped peccaries, fungus/rotten-within the three treatments: total exclosure, peccary exclosure; control (no exclosure) in three types of forests (a) Unhunted forest, (b) Moderately hunted and (c) Hunted. Unknown causes, were not included in the graph, they represented more than 50% of deaths. Figure (b), (c) max to 12%.

this rarely translated into death. There was some insect attack on several seedlings, but the first death caused by insects (herbivory by caterpillars) was registered only in the heavily hunted forest (in the peccary exclosure) in the third

Table 2. Number of *A. gratum* seedlings killed by agent under three experimental treatments in forests experiencing three levels of hunting pressure: Unhunted, moderately and hunted forests. Each treatment started with 16 seedlings. Results are expressed as percentage mortality by cause. This percentage is based from the initial 192 seedlings.

	Exclosure	Insect	Mammals	Peccary	Fungus	Unknown
Unhunted	All	6.25 (3)	0	0	12.50 (6)	81.25 (39)
	Peccary	5.50 (5)	2.19 (2)	3.29 (3)	4.39 (4)	84.61 (77)
	Control	2.74 (5)	1.09 (2)	84.6 (154)	2.74 (5)	8.79 (16)
Moderately	All	2.04 (1)	0	0	10.2 (5)	87.75 (43)
	Peccary	3.44 (3)	0	0	8.05 (7)	88.5 (77)
	Control	8.4 (8)	10.5 (10)	1.05 (1)	7.37 (7)	72.63 (69)
Hunted	All	1.96 (1)	0	0	9.80 (5)	88.24 (45)
	Peccary	2.50 (2)	0	0	8.86 (7)	88.61 (70)
	Control	3.89 (3)	2.60 (2)	0	10.39 (8)	83.11 (64)

survey month. Unknown causes accounted for 8.0%, 72.6% and 83.1% of mortality (including desiccation) in unhunted, moderately and the heavily hunted forest respectively.

4. Discussion

As *Astrocaryum gratum* was evaluated in having aggregated patterns in seedlings and juvenile stages in natural stands (Choo, *et al.*, 2017 [24]), our natural experiment indicates that *A. gratum* seedlings, juveniles and sapling densities differed among unhunted, moderately hunted and heavily hunted forests, with abundance being greatest in areas with the highest defaunation. For those seedlings tracked over time on transects, survivorship was lower in the unhunted site relative to the hunted forest. Analyzing the different seedling life stages independently, revealed that newborn and young *A. gratum* seedlings had higher mortality rates, rather that juveniles and saplings, with survivorship lowest in unhunted forest areas. Most known deaths resulted from WLP, and rodents eating the yet attached endocarp. As the seedlings aged leaves became thicker, cell walls developed higher lignin concentrations, and spines in the rachis became tougher. All this makes the plants less attractive to vertebrate consumers (Henderson, 2002 [25]), eliminating vertebrate induced mortality and allowing densities to equalize. This may explain why in the non experimental data at the oldest life stages (saplings) survivorship was similar amongst the three forest types. Our observations are like what was seen for *A. gratum* seedlings (originally mentioned to be *A. murumuru*) that survive their first year at least at one other site in the Amazon region (35%; Cintra, 1997 [26]).

White-lipped peccaries were responsible for seeds, and most of the Seedling mortality in unhunted forest sites (Aliaga-Rossel and Fragoso, 2017 [21]). In the hunted and moderately hunted forests, white-lipped peccaries were absent or

rare, and seedling survivorship was significantly higher. However, the reason for many seedling deaths remained unknown. Some of this mortality trends could result from flooding during the rainy season or extreme desiccation during the dry season (Engelbrecht *et al.*, 2006 [27]). Other vertebrates such as armadillos did not cause a relevant effect on these seedlings (Aliaga-Rossel *et al.*, 2021 [28]). In addition, pathogens such as fungus were difficult to detect with certainty as causing mortality, because at all life stages, at least one leaf of any plant was infected by fungus. In these instances, sometimes an entire leaf died but plants responded by growing another leaf.

We did not found evidence of deaths caused by vertebrates in the juvenile age class. For this class, most deaths were due to tree falls or causes we could not identify. Saplings had the highest survival rate in all three forest types (survivorship higher than 90%), possibly because (as mentioned above) herbivore species prefer seedlings and softer plants (Krebs, 2001 [29]).

In the moderately hunted forest, small groups of 5 to 10 WLP remained. In this forest, we only registered one seedling death, when WLP rooted in a control plot. In comparison, the unhunted forest supported a high density of WLP (and other mammals), and groups of up to 300 WLP disturbed and turned over litter and soil layers within *A. gratum* areas. Here, they killed 65 percent of seedlings within 5 months of their sprouting, mainly uprooted seedlings to eat attached endocarps. Other vertebrates or agents did not generate significant mortality. In contrast, the moderately hunted forest supported very few WLPs and as predicted few seedlings (only one) were killed in control plots. No WLPs were observed in the hunted forest and no seedlings were killed by the species there. Deaths were attributed to unknown causes, such as pathogens, larvae, and rodents. These results support the WLP-plant dynamics observed with other plant species elsewhere, where defaunation results in higher seedling densities (Fragoso, 2005 [15]; Silma *et al.*, 2003 [13]; Terborgh and Wright, 1994 [7]; Aliaga-Rossel and Fragoso, 2017 [21]).

Our experimental and observational results thus indicate that WLP are key agents of seedling death and potentially can create significant changes in tropical forest structure as suggested by others (Antonik, 2005 [11]; Choo *et al.*, 2017 [24]; Silman *et al.*, 2003 [13]; Fragoso, 2005 [15]). Whereas peccaries, rather than soil pathogens, were also the primary contributors of distance-effects for *A. gratum* population Choo *et al.*, 2017 [24]). Critically, the influence of the WLP on *Astrocaryum* seedlings, juveniles and saplings survivorship was much greater than that of rodents and other vertebrates. For example, we registered lower seedling mortality from tapirs (*Tapirus terrestris*), agouti (*Dasyprocta punctata*) and collared peccary *(Pecary tajacu*).

Seedling mortality in the open control treatment was higher than at the other exclosure types and followed the pattern observed naturally on the transects. Seedling mortality during the first four months in the controls was similar amongst unhunted and mildly hunted forests (75% and 79%, respectively). This similarity can be attributed to agoutis uprooting seedlings to consume the at-

tached endosperms in the latter area compensating for the lack of death due to WLP. Total exclosures recreated a situation of a forest with only invertebrate predators; this treatment maintained similar numbers of seedlings across the three forest types. As white -lipped peccaries where the main agent of seedling mortality where they are present in normal densities, their exclusion from these plots, probably explain why seedlings here experienced nearly complete survival in all forest classes.

Research on the effects on seedling mortality caused by vertebrates or invertebrates has shown varying results in different studies (e.g., Beck *et al.*, 2013 [30], Terborgh *et al.*, 2001 [8]; Wright and Duber, 2001 [31]). However, we observed that complete or partial defaunation leads to higher seedling densities. Uniquely, our study indicates that the low density of *A. gratum* seedlings and juveniles in unhunted forest was the result of extensive seedling consumption by WLP. Consequently, the natural disappearance or extirpation by hunting of this species should lead to reduce seed, seedling, and juvenile mortality rates, potentially leading to significant cascading effects on the population dynamics of the palm and its dependent wildlife. It may be that most seedling recruitment only occurs when populations of white-lipped peccaries undergo population declines, as described by Fragoso, 2005 [15]. Other mammals (such as agoutis or tapirs) had little impact on *A. gratum* seedling populations. Consequently, the loss of these animals would have less impact than the loss of the WLP. However, we also observed that sapling densities seemed unaffected by higher seedling mortality rates, suggesting that compensatory effects may occur at older life stages, potentially nullifying the importance of high death rates at earlier life stages.

White-lipped peccary populations may fluctuate greatly over decades perhaps because of outbreaks of disease (Fragoso, 2005 [15]), or due to overhunting by human (Silman *et al.*, 2003 [13]; Wright and Duber, 2001 [31]). Across a forested landscape the varying dynamics of WLP populations may yield a mosaic of varying palm populations that in turn would influence populations of smaller seed predators and dispersers and eventually peccary populations which in turn would influence palms, and so on (Aliaga-Rossel, 2011 [1]; Fragoso, 2015 [15]). This was also the case for the palm *A. huicungo* in Peru, where seed and seedling densities almost doubled once WLP disappeared for over a decade from an area (Silman *et al.*, 2003 [13]), seedling densities decreased when WLPs returned to the area.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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