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How can seed removal rates of zoochoric tree species be assessed quickly and accurately?

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ABSTRACT

The quantification of seed dispersal and predation processes has been gaining increased importance in the assessment of forest responses to anthropogenic disturbance, but also in developing an understanding of forest dynamics facing particular reproductive strategies. Seed removal rate is a reliable estimator of animal activities relating to these processes and can be quickly and easily estimated using a rapid assessment method (RAM) described by Lermyte and Forget (2009) and Boissier et al. (2014). This method consists in selecting trees reaching a given fruit crop in plots of interest and estimating, under each tree, the proportion of removed seeds in a single quadrat among the places having the highest crops; the proportion of removed seeds is obtained by enumeration of fruit scraps and intact fruits and estimation of their seed contents. The objective of this work is to evaluate the reliability of this method and to propose alternative estimation protocols (APs) in order to obtain an index of animal interaction with seeds.

To do so, we estimated produced and removed seed numbers in up to 30 random 1 sq.m. quadrats under a total of 19 trees of *Afzelia bipindensis*, *Dialium pachyphyllum/zenkeri* and *Xylopia staudtii*. Secondly, we investigated the influence of tree size and fruit production on seed removal rate using a generalized linear mixed model. Thirdly, we used a generalized linear mixed model and a bootstrap procedure to test if RAM and APs are biased. Then, we compared their accuracy throughout their mean squared error, also obtained with a bootstrap approach.

Despite its interesting accuracy, we showed that the RAM is positively biased. Removal rate was obviously influenced by canopy size and fruit production whereas the quadrats with higher fruit production have higher seed removal rates. Thus, trees with representative sizes and crops of the studied plots have to be sampled. Secondly, as an AP, random selection of several quadrats was found to be the best method. Based on these results, we recommend using the mean of three random quadrats per tree to estimate seed removal rate. It is an unbiased estimator, more accurate and more time efficient than the RAM. However, attention should be paid to select a proper quadrat size, in line with seed and fruit numbers, since the accuracy of the methods depends on these quantities. Such a choice could be made using a mean squared error criterion obtained from a preliminary intensive sampling of some specimens of the focal species.

1. Introduction

Ecological processes reflect the health status of ecosystems. As pointed out by Boissier et al. (2014), there is a need of rapid protocols to assess those processes at large scale because most of the ecosystems are under growing threats (logging, hunting, wood collection, fragmentation, forest agriculture, fire), particularly in the tropics. Moreover, these threats are interlinked in their occurrences and consequences (Malhi et al., 2014). Disentangling their respective effects on ecosystem processes requires large sampling efforts across landscapes using effective methods. Zoochory by vertebrates is a remarkable process of interaction between animals and plants. According to several reviews (Howe and Smallwood, 1982; Willson et al., 1989; Jordano, 2000), it is one of the main mechanisms of seed dispersal in tropical forests (occurring in 70–100% of the ligneous plants) and could be also common in other ecosystems, such as nemoral temperate forests (between 9 and 100%), Mediterranean scrubs and forests (sometimes as high as 60%) or warm temperate forests of the southern hemisphere (up

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to 59%). In primary dispersal, animals remove seeds from the canopy, while in secondary dispersal, other animal species move seeds already fallen on the ground towards a new position. Primary dispersal is mostly carried out by frugivores which do not eat the seeds, but swallow them or spit them out possibly after temporary storage in gizzard or in cheek pouches. Secondary dispersal is more complex and concerns seeds already fallen on the ground, still in the fruits or not. It mainly relies on seedeaters in a process called 'scatterhoarding' (Brewer and Rejmánek, 1999; Feer and Forget, 2002, Aliyu et al., 2014), on animals attracted by elaiosomes, very often ants (Lengyel et al., 2010) but also other animal species, for instance slugs (Calvino-Cancela and Rubido-Bará, 2012; Türke et al., 2012) and on dung beetles which accidentally catch seeds with the excrements they collect (Culot et al., 2011).

Attempts to quantify zoochory and seed predation rely on different methods, such as direct observation of animals, fruit collectors, genetic parentage analysis, seed tagging or direct count of fallen fruit (Forget and Wenny, 2005; Wang et al., 2007; Steele et al., 2011; Suselbeek et al., 2013). However, such methods are time-consuming and limit the number of trees and sites which can be studied (Boissier et al., 2014).

Thus, it is valuable to dispose of a rapid method to evaluate seed removal activity in order to yield information about the interaction intensity between animals and seeds. Such a method would allow to simultaneously study large numbers of sites or possibly of species, not only to disentangle the respective contribution of various human pressures, but also to understand the consequences of particular reproductive strategies such as mast-fruiting (Norden et al., 2007) or to follow functionality recovery in forest restoration projects. In this spirit, Lermyte and Forget (2009), followed by Boissier et al. (2014), proposed a rapid assessment method of seed removal rate (RAM) applicable to individual tree with the aim to characterize plots by examining tree samples. They computed a seed removal rate as the ratio between removed and produced seeds which integrates the results of primary dispersal, secondary dispersal and predation rate at the source. We will use the term 'seed removal rate' further through this work to refer to this quantity. The RAM consists of estimating the seed removal rate using a single quadrat (of e.g. 1 sq.m. but larger or smaller according to fruit crop, see Lermyte and Forget (2009) and Boissier et al. (2014)) where the fruit density seems maximal (i.e. without prior estimation of this quantity); thus it is somehow based on a random choice of quadrats but in places where fruit abundance are higher. One requirement of the method is to study species having fruits leaving husk exocarps, fruit peduncles or other fruit remains. Indeed, the fruit remains allow estimating the number of consumed fruits and consequently also the number of removed seeds, knowing the number of seeds per fruit. The number of produced seeds over the quadrat is derived from the sum of intact fruits plus the number of consumed fruits, possibly also counting the loose seeds. If one is interested in comparing sites or species, RAM per tree is averaged over all sampled trees from the same site or over the same species, discarding trees with too low fruit productions. The authors only used trees with fruit numbers in any quadrat higher than ten. They estimated the accuracy of their method by sampling several quadrats with the highest fruit densities and comparing their results with varying sampling effort per tree and quadrat. They also demonstrated the efficiency by comparing sites with various hunting pressures. Although this method is only applicable to certain species, its simplicity and time efficiency are particularly appealing for field work.

However, the RAM protocol raised two questions. First, drawing the quadrat at random among the places where the density is higher could skew the estimation of seed removal rate for a given tree. Indeed, it relies on the hypothesis that seed or fruit removal in the canopy and on the soil are independent of fruit density or that all seeds or fruits have the same probability to be removed. Most often seeds are removed with the fruits but it is not uncommon in tropical species that arillated seeds are picked up individually from dehiscing fruits. This occurs for instance in Myristicaceae, Leguminosae or Annonaceae members. The ability of animals to move up to the fine branches for feeding strongly varies among animal species (e.g., McClearn, 1992, Rosenberger, 1992; Flörchinger et al., 2010). Some species could also take advantage of particular places in the canopy; for instance, in Gabon, hornbills feed higher up in the canopy than primates (Poulsen et al., 2002). Thus patterns of fruit and fruit scrap deposition beneath the canopy could vary with disperser guild in the canopy. Terrestrial species take advantage of fallen fruits but also of fruits dropped by the activity of birds (Hernández, 2008). On the soil, the fruit consumption and seed predation could also vary with fruit density according to the behaviour of the animal species (e.g., Hulme, 1997; Blendinger and Díaz-Vélez, 2010; Guitián and Munilla, 2010). Clumps of fruits would be more appealing than single fruits because the reward for a given effort would be higher (Jones and Comita, 2010). The second question concerning the RAM protocol is that rejecting trees with low fruit production could skew site estimations. Indeed, trees with ample fruit production could attract proportionally more frugivores/seedeaters (Beckman and Muller-Landau, 2007; Janmaat et al., 2013; Suarez, 2014), but larger fruit production could also reduce the proportion of consumed fruits (Briani and Guimarães, 2007) as a result of frugivore satiation.

With regards to the interest of disposing of a reliable rapid assessment method of ecological interactions, this paper had three goals. First, we provided insights on how trees could be selected to estimate seed removal rate for a given site or eventually for a given species by investigating the relationship between tree size, fruit production and seed removal rate. Secondly, we assessed if the RAM provides an unbiased estimation of the true seed removal rate (denoted $E(R)$, for expectation of the removal rate), for individual trees, by studying its statistical properties. Thirdly, since the RAM proved to be biased, we studied also the statistical properties of ratios obtained with alternative protocols (APs) in which we varied the quadrat sampling rule and the number of quadrats and we compared APs' accuracy.

To this end, we evaluated produced and removed seeds under the canopy of three zoochorous large-seeded afrotropical species (*Dialium pachyphyllum* Harms/*D. zenkeri* Harms and *Xylopia staudtii* Engl. & Diels, *Afzelia bipindensis* Harms), using random quadrats, to estimate produced and removed seed numbers. This extensive sampling work allowed us to obtain a precise estimation of the true seed removal rate of the considered trees and is used as a benchmark measure.

Using the obtained data, we first examine how to conduct tree selection to obtain good seed removal rate estimates of forest plots or of tree species owing to the possibility that tree dimensions and fruit production influence its seed removal rate. In this perspective, we used generalized linear mixed models (GLMM) to investigate the existence of a relationship between the seed removal rate and two characteristics of the tree, namely the size and a proxy of the fruit production. Secondly, we investigate at tree level the relationship between the estimated seed removal rate and the fruit density on the ground. Using a bootstrap method, we were able to estimate the bias of RAM, and to assess if it was significantly different from zero. We also compared the accuracy of the RAM and APs, measured by their mean squared error (MSE) with respect to the estimated seed removal rate obtained from our extensive sampling strategy. Once again, these quantities were obtained with a bootstrap technique.

2. Materials and methods

2.1. Study sites

Data were collected in dense evergreen humid forests of Africa. The first site was located in Eastern Central Gabon, around the 'Concession Forestière sous Aménagement Durable' of the 'Precious Wood Gabon Society - Compagnie Equatoriale des Bois' in Lastourville (12.50–14.00°E, 0.50–1.00°S). According to information from local people, this site would be well-stocked with game. The three other sites, with intensive hunting activities, were situated in the western

Democratic Republic of Congo, near the WWF Malebo research station (16.41–16.56°E, 2.45–2.66°S). There, we sampled trees in three forest patches about 10 km apart (Mbanzi, Mbominzoli and Nkala), separated by man maintained savannas.

2.2. *Tree species and data collection*

Fruits, mericarps, their scraps and eventually individual seeds were counted in up to 30 quadrats with surface 1 sq.m. strictly under the crown of each focal tree crown. The quadrats were randomly selected using the field rule that they must contain at least one fruit. For the statistical analysis, we decided to keep only the quadrats containing at least one mature fruit, intact or more or less consumed. For each tree, we recorded crown size (distance from the base of the trunk to the end of the projection of the crown in the four cardinal directions) and diameter at breast height (dbh).

The first sampled species was the complex consisting of *Dialium pachyphyllum* and *D. zenkeri.* The fruits are indehiscent drupaceous one-seeded legumes and are mainly produced between March and May (Meunier et al., 2015). Stalks are not consumed, leaving a trace of frugivore activity. The seeds are primary dispersed by large primate species who swallow them. Fruits of *Dialium* species are of major importance in the diet of bonobos in DR Congo (Beaune et al., 2013; Trolliet et al., 2016), of young gorillas in Gabon (Flohic et al., 2015) and of chimpanzees in Republic of Congo (Krief et al., 2004). Sampling was undertaken in RD Congo. We randomly selected 6 trees, two in the Mbanzi forest and four in the Nkala forest. Under each tree, we sampled 30 quadrats during fructification peak. Due to the fact that some quadrats did not contain any mature fruits, they were excluded from the statistical analysis, leading to final sample sizes of 29, 30, 30, 20, 29 and 30 quadrats.

The second sampled species was *Xylopia staudtii*. Fruits of this species are composed of three to five carpels becoming free at maturity. Fructification peak occurs between May and September (Poulsen et al., 2002). Mericarps split like follicles, bearing one to five orange arillated seeds (on average three seeds per mericarp). Seeds of this species are primary dispersed by birds, especially by hornbills, and by primates (Clark et al., 2001; Poulsen et al., 2002); rodents act as seed predators or secondary dispersers (Gautier-Hion et al., 1985, Rosin and Poulsen, 2016). Eight trees were sampled in the Mbominzoli forest during fructification peak of the species. Mericarps were sorted as intact or consumed. Old mericarps from fructification of the previous year were not counted. They could be recognized because they were invaded by understory roots, and their seeds did not resist to finger pressure. The numbers of seeds were directly counted in intact open fruits. Taking into account loose seeds, the numbers of removed seeds were deduced from the numbers of empty cells in the open fruits and the numbers of eaten carpels multiplied by 3, the mean number of seeds per carpel. Some quadrats were discarded because of slight discrepancies between produced and removed seed numbers, leading to final sample sizes of 28, 29, 30, 30, 30, 28, 27 and 27 quadrats.

The third species, sampled in the Gabon site, was *Afzelia bipindensis*. Fruits of this species consist of large kidney-shaped legumes which open when ripe (Wilks and Issembé, 2000) and contained between one and ten seeds. At maturity, the seeds are shiny black and partly surrounded by an orange-red aril. Flowering occurs almost throughout the year (Louppe and Gérard, 2011). The primary dispersers consume the aril. This species is probably primary dispersed by primates (Gartlan and Struhsaker, 1972). Seed numbers were obtained as for *X. staudtii* but no carpel was found eaten. Due to time constraints and the fact that the results were rather constant, the sampling objective was to cover about 5% of the area under the canopy (sample sizes: 11, 12, 14, 20 and 8 quadrats). Sampling was done under trees with copious flowering.

2.3. *Statistical analysis*

The seed removal rate for a given quadrat is defined as the ratio between the estimation of the number of removed seeds and the estimation of the number of seeds produced by the tree over the quadrat (referred below as 'seed density'). It is denoted by R_i, for i = 1, …, n (n being the number of quadrat for a given tree). The best possible estimation of the true seed removal rate, or ratio, for a specific tree was computed as the mean over the n quadrat ratios and is denoted $\widehat{E(R)}$. Indeed, due to the numbers of quadrats per tree, this quantity could be close to what would have been obtained by a full enumeration procedure.

2.3.1. *Estimation of seed removal rate of forest plots*

We tested whether $E(R)$ varies as a function of tree characteristics using GLMM. Indeed, it is established that plant reproduction and notably fruit production increase with plant size (even if no generalization has been made on the shape of this relationship) but also that the availability of resources is also an important factor controlling fruit production (Naito et al., 2008). We used $\widehat{E(R)}$ of each tree as the dependent variable and, as independent variables, diameter at breast height, crown size as well as the product between the mean fruit density of the tree and its crown size. This last variable is a proxy of the fruit production (it is only a rough estimate of fruit production because only places with fruits were sampled at one moment). We allowed for a random intercept according to locality, also accounting for species (Mbanzi or Nkala for *D. pachyphyllum/zenkeri*, Mbominzoli for *X. staudtii,* Gabon for *A. bipindensis*). We only tested for a random intercept, owing to the small sample size. Since we were dealing with a dependent variable varying between 0 and 1, we assumed a binomial distribution for the response variable, with a logit link function, following Bolker et al. (2008). We compared the full model with the null model containing only the random effects, using a likelihood ratio test to assess the significance of the fixed effects. Analysis was conducted in R with the glmmPQL function of the MASS package (Venables and Ripley, 2002).

2.3.2. *Bias of RAM and APs*

First we investigated the relationship between R_i and the fruit density per quadrat. For this purpose, we used a GLMM similar to the one described above, with R_i as dependent variable and fruit density as independent variable. We allowed for a random intercept according to individual trees and a random slope according to the species. Secondly, we assessed if the differences between $\widehat{E(R)}$ and the RAM or the AP estimations are significantly different from zero using a bootstrap technique (Efron, 1979; Efron and Tibshirani, 1993). As APs, we tested the ratio of one random quadrat (ran1), the mean ratio of 2–4 random quadrats (ran2 to ran4), the ratio of the quadrat with median fruit density (med), the mean ratio of the quadrat with median fruit density plus the two or the four values on either side of this median (med + 2, med + 4), the mean ratio of the quadrats having the 2 to the 4 highest fruit densities (max2 to max4). For each initial sample i.e., observed ratios in the quadrats under a single tree, we generated 2000 resamples of size n, with replacements. Thus, we obtained estimated distributions of differences and corresponding confidence interval bounds with the percentile bootstrap method (Efron and Tibshirani, 1993). We used 90% confidence intervals to avoid a large type-II error, i.e., a large probability of not detecting an existing bias (especially since we have to deal with small sample sizes). When a confidence interval did not include zero, we concluded that the estimator was biased. Notice that here RAM is based on the maximum observed fruit density and some may argue that bootstrap fails in this situation as the observed maximum will be resampled only 63.2% of the time. However, in practice, RAM is based on the quadrat where the fruit density seems maximal. Consequently, taking a random quadrat among those with a high fruit density 36.8% of the time introduces a realistic additional randomness in the analysis. At worst, due to the possible relationship between fruit density and expected removal, the test might simply lack of power (and underestimate the MSE of the RAM, see next subsection). These features (which favour the RAM) may not change our conclusions.

2.3.3. *Accuracy of RAM and APs*

To evaluate the accuracy of the estimations provided by RAM and APs, we computed their mean squared errors (MSE), using the same resampling method as described above. The interest in using the MSE comes from the fact that it takes into account both the bias and the variability of the estimates, and summarizes them in a single measure. To do so, we computed the squared differences between $\widehat{E(R)}$ and the RAM or the considered AP, computed on the bootstrap resamples. The final MSE for a given tree and a particular method was obtained by taking the average of these differences over 2000 bootstrap resamples. Additionally, we derived a MSE per species (by taking the average of the MSE over all trees of the same species). We also reported the standard error of the MSE per species. These measures of RAM and AP accuracies allowed classifying the different estimation techniques.

3. Results

3.1. *Estimation of seed removal rate of forest plots*

We found a significant effect of tree characteristics on $\widehat{E(R)}$. Canopy surface had a significant positive effect while the proxy of fruit production had a negative effect (Table 1, Fig. 1). We tested the robustness of this relationship by removing the *A. bipindensis* observations which varied less than the other ones. We still obtained a significant slope coefficient for canopy surface (coefficient = 0.0118, p-value = 0.0310), but not for the proxy of fruit production.

3.2. *Bias of RAM and APs*

For the three studied species, numbers of removed seeds generally followed seed density and accordingly fruit density, but the intensity of the relationship was stronger for *A. bipindensis* than for *X. staudtii* and *D. pachyphyllum/zenkeri* (examples in Fig. 2). For *A. bipindensis*, two trees had all their quadrats with R_i (i.e., the observed seed removal rate in a given quadrat) equal to one, two others had all their quadrats but one with R_i equal to one, and a single tree had quadrats with more scattered R_i values, i.e. some lower values for lower fruit densities (Fig. 3). Nevertheless, R_i was higher than 0.9 for all quadrats. For *X. staudtii* and *D. pachyphyllum/zenkeri,* some trees had most of their R_i values ranging between 0.8 and 1.0, but R_i was generally more variable for lower seed and fruit densities as observed by Boissier et al. (2014).

Table 1
Generalized linear mixed model (family = binomial, link function = logit) of expected seed removal rate as a function of tree diameter at breast height (dbh), of canopy surface, of proxy of fruit production (fixed effects) and of locality (intercept random effect), and likelihood ratio test.

Model	Number of groups: 4 Number of observations: 19			
Random effect	Standard deviation			
Locality (intercept)	0.000017			
Residuals	0.2925			
Fixed effects	Coefficient value	Degree of freedom	t-statistic	p-value
Intercept	0.1277	12	0.2002	0.8446
dbh	−0.0106	12	−0.6961	0.4997
Canopy surface	0.0164	12	4.4969	0.0007
Proxy of fruit production	−0.0007	12	−2.4465	0.0308
Likelihood ratio test				
Chi-squared	Degree of freedom	p-value		
13.6626	1	0.0002		

GLMM analysis revealed a significant relationship between R_i and fruit density (Table 2, Fig. 3). Confidence intervals to test for bias (Fig. 4) did not include zero for a significant proportion of the RAM results. Both results strongly suggested that the removal rate is dependent of fruit density and that the RAM is positively biased. The results of the confidence intervals of APs based on the quadrats with the highest fruit densities were quite similar to those obtained with the RAM, while the results for APs based on quadrats with a median fruit density always included zero (Fig. 4). APs based on randomly selected quadrats were not biased by construction (since the sample mean of a random selection is an unbiased estimator of its unconditional expectation, see Mood et al. (1974)).

3.3. *Accuracy of RAM and APs*

The MSEs combine bias (squared) and variance and allow comparing the accuracy of the different protocols (Fig. 5). For *D. pachyphyllum/zenkeri* and *X. staudtii,* the APs based on a single quadrat at random or with median fruit density were the least suitable estimators. The RAM yielded better estimations than a single quadrat, but not for *A. bipindensis*. Besides, MSE obviously decreased with the number of quadrats taken into account. APs based on several random quadrats yielded slightly better results than APs based on quadrats around median fruit density. This was probably due to the apparently Poisson-like nature of the fruit density distribution. In this case, the quadrats with median fruit density had fruit and seed densities below average, which increased the variability of the ratios. We also observed that three or more random quadrats provided a better estimation than the RAM.

4. Discussion

Regarding the influence of tree size and fruit production on seed removal rate, our results confirmed the intuition that larger trees or larger fruit crops could influence seed dispersal. Thus, crown diameter positively interferes with zoochoric seed removal. Large trees are particularly important because they produce large numbers of fruits, crop increasing with tree size. Fruit crop size hypothesis suggests that plants with larger fruit production attract larger numbers of dispersers (Ortiz-Pulido and Rico-Gray, 2000), probably because by feeding mainly on those trees with high fruit production, animals minimize their probability to visit trees without fruit. Trees with a small fruit production tend to be neglected, the offered reward being not sufficient (Howe and Smallwood, 1982; Foster, 1990; Stevenson et al., 2005; Moreira et al., 2017), and larger trees might have a larger proportion of dispersed seeds (Leighton and Leighton, 1982; Chapman et al., 1992; Duncan and Chapman, 1999; Toh et al., 1999; Wenny, 2000). On the contrary, larger fruit production could negatively influence seed removal rate. Species having their seeds dispersed by large vertebrates are more deeply affected by hunting when crop is large because of satiation of the remaining animals (Forget and Jansen, 2007; Trolliet et al., 2017). Thus, when estimating seed removal rate*,* it is important to also estimate crown size and crop size using tree metrics like diameter size, canopy volume and index of fruit production and to use these factors as cofactors in statistical analyses with seed removal rate as dependent variable.

Concerning the estimation of the seed removal rate, our results showed that RAM and APs based on the quadrats with the highest fruit densities are biased while those based on the quadrats with a median fruit density or on randomly selected quadrats are not biased. These

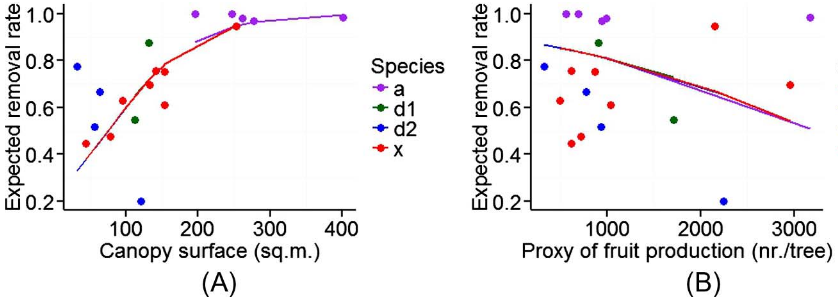

Fig. 1. Distribution of expected removal rate for each tree of the three species (d1: *Dialium pachyphyllum/zenkeri* in Mbanzi, d2: *Dialium pachyphyllum/zenkeri* in Nkala, x: *Xylopia staudtii* in Mbominzoli, a: *Afzelia bipindensis* in Gabon), as a function of (A) canopy surface and (B) proxy of fruit production and prediction lines of GLMM for each considered explanatory variable (see Table 2).

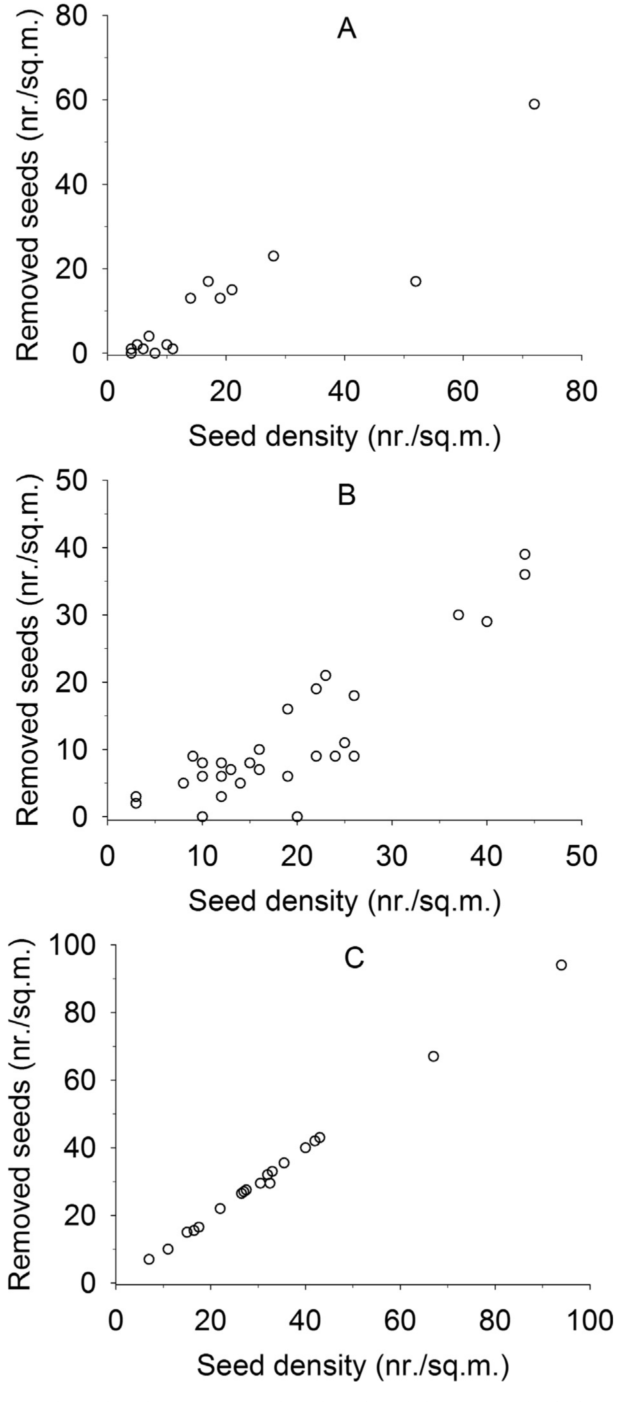

Fig. 2. Relationships between removed seeds and seed density in individual 1 sq.m. quadrats for three species (A: *Dialium pachyphyllum/zenkeri* 3, B: *Xylopia staudtii* 2, C: *Afzelia bipindensis* 4).

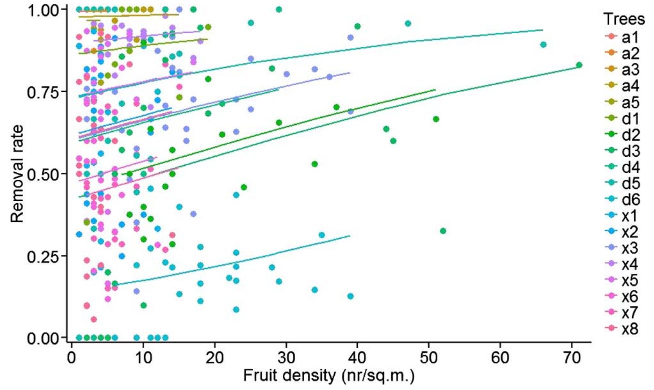

Fig. 3. Seed removal rate in the 1 sq.m. quadrats as a function of fruit density for the three species (d1 to d6: *Dialium pachyphyllum/zenkeri*, x1 to x8: *Xylopia staudtii,* a1 to a5: *Afzelia bipindensis*) with prediction lines of GLMM (see Table 2).

Table 2
Generalized linear mixed model (family = binomial, link function = logit) of quadrat seed removal rate as a function of fruit density (fixed effect), species (slope random effect) and individual trees (intercept random effect), and likelihood ratio test.

Model	Number of groups: 19 Number of observations: 459			
Random effects	Standard deviation			
Tree (intercept)	3.9132			
Dialium (slope)	2.7717			
Xylopia (slope)	3.1958			
Residuals	0.4699			
Fixed effects	Coefficient value	Degree of freedom	t-statistic	p-value
Intercept	0.5976	439	2.4118	0.0163
Fruit density	0.0261	439	3.7102	0.0002
Likelihood ratio test				
Chi-squared	Degree of freedom	p-value		
7.1581	1	0.0075		

conclusions have been drawn from the study of the confidence intervals for the differences between the expected seed removal rate (estimated from our intensive field work) and the RAM or APs. Moreover, APs based on several random quadrats apparently yielded the best estimates of seed removal rate in term of mean squared error. Nevertheless, RAM showed the potential to be used to minimize the variability of the estimation of the seed removal rate, which can be particularly large when proportions are computed with small numbers at the denominator (i.e., the so-called ‘small frequency bias’).

Hence, a trade-off between having a large bias or a high variance should be found. The study of the mean squared error showed that, among the estimation methods based on a single quadrat, RAM effectively yielded good results for *D. pachyphyllum/zenkeri* and *X. staudtii*. For these two species, our results are explained by the fact that the

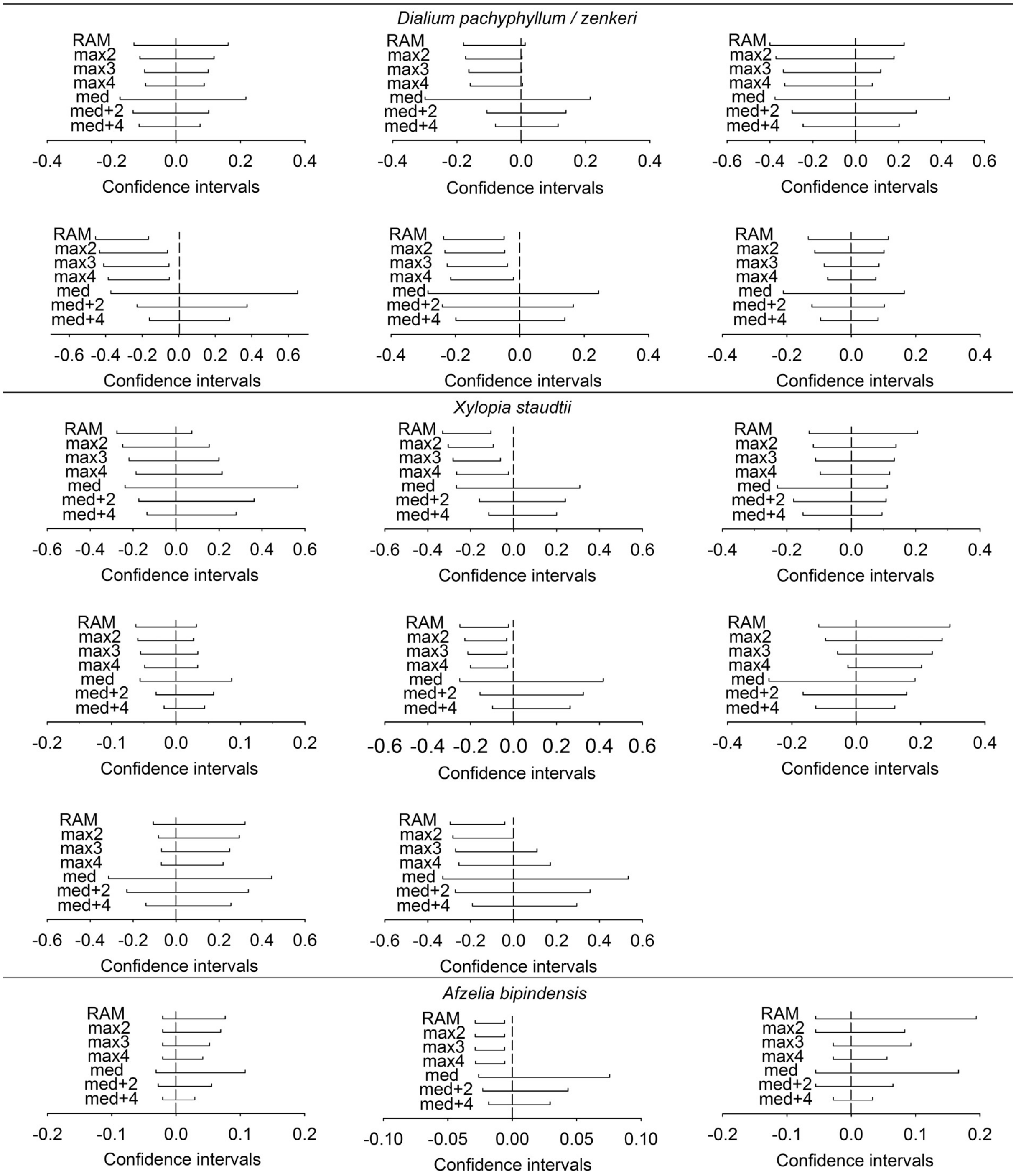

Fig. 4. Ninety percent confidence intervals of RAM and alternative sampling protocols* (RAM: rapid assessment method; max2 to max4: the mean ratios of the quadrats having the 2–4 highest fruit densities; med: ratio of the quadrat with median fruit density; med + 2 and med + 4: the mean ratios of the quadrat with median fruit density plus the two or the four values on either side of this median) of the individual trees.

reduction in variability can offset the positive contribution of the bias to the mean squared error. Besides, RAM did not perform better than APs based on a single quadrat for *A. bipindensis*. Here, the seed removal rate expectation was close to one and the variability among quadrats was close to zero because the seed removal has reached its maximum. In this configuration, the gain in variability obtained with RAM was not sufficient to offset the effect of the bias and to achieve a lower mean squared error. The obvious observation that the MSE decreased with the number of quadrats used to estimate the seed removal rate suggests the use of an AP based on several quadrats. In particular, randomly selecting three quadrats proved to be a very good method since it leads to a reduction of the mean squared error close to 50%, compared to RAM. It might be argued that these results are obtained at the cost of a longer collecting time in the field. However, due to the Poisson-like distribution of the fruit density in the quadrats, the sum of the counts to be done in three quadrats selected at random is very likely to be smaller than the count to be performed in a single quadrat with one of the highest density of fruits. However, taking more than 3 quadrats would probably reduce this advantage.

We did not test the influence of quadrat size on the mean squared error. We can suppose that the effect should be marginal provided that some mean fruit density threshold is reached, but careful attention

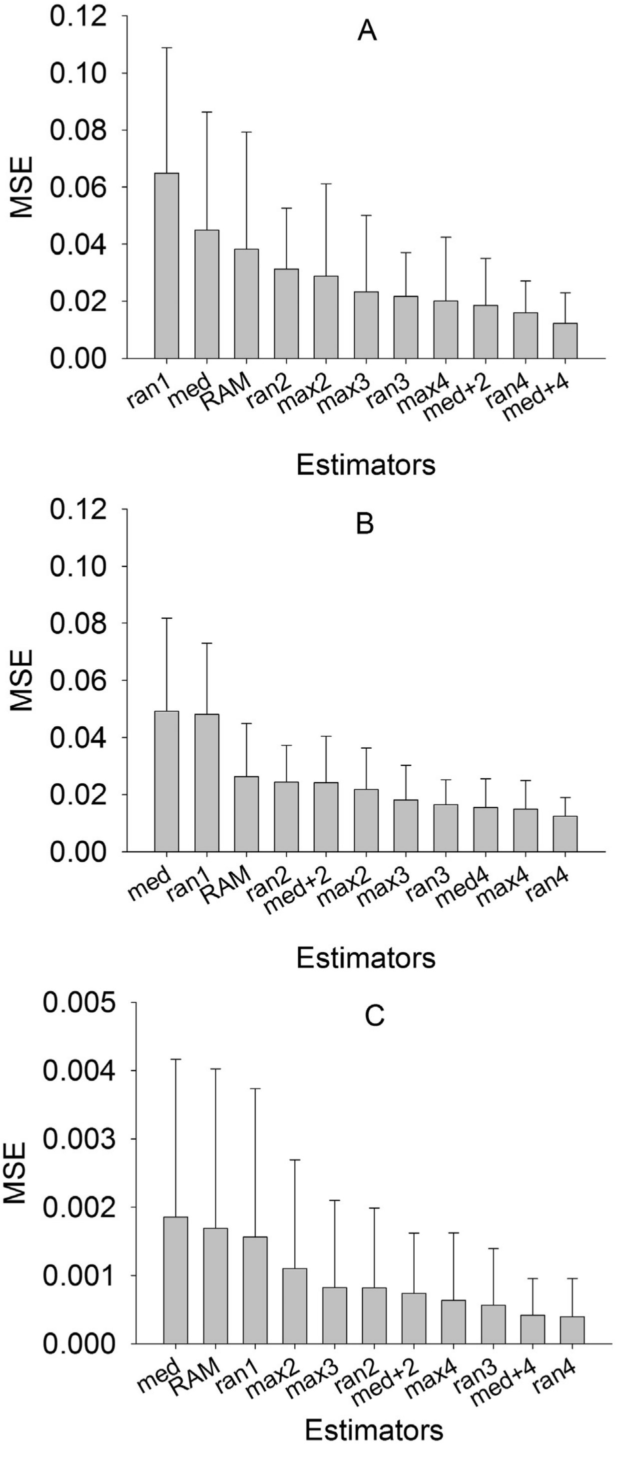

Fig. 5. Mean squared errors and standard deviations per species (A: *Dialium pachyphyllum/zenkeri*, B: *Xylopia staudtii,* C: *Afzelia bipindensis*) according to sampling protocol (RAM: rapid assessment method; ran1: one random quadrat; ran2 to ran4: the mean ratios of 2–4 random quadrats; max2 to max4: the mean ratios of the quadrats having the 2–4 highest fruit densities; med: the ratio of the quadrat with median fruit density; med + 2 and med + 4: the mean ratios of the quadrat with median fruit density plus the two or the four values on either side of this median).

should be paid to this factor. Indeed, too few fruits in the sampled quadrats would expose us to a high variability of the estimated seed removal rate, even with APs based on a random selection. On the contrary, too many fruits in the quadrats would lower the efficiency. Here a surface of 1 sq.m. was selected following Boissier et al. (2014) and this surface seemed appropriate with the selected species to reach the objectives of the study. Nevertheless, to reproduce our results, it is necessary to aim at least at obtaining a mean number of fruits per quadrat in the range of this study. The mean number of fruits varied sensibly: between two and ten for *A. bipindensis*, between five and eighteen for *D. pachyphyllum/zenkeri* and between four and twenty-one mericarps for *X. staudtii,* with an overall mean of six. A target between five and ten fruits seems reasonable but it might be better to test this suggestion, particularly for fruits smaller than those encountered in the present study and if these tend to be more numerous. Overall, densities are ranging between a few dozens of fruits per sq.m. for the larger fruits and up to several hundred for the smaller ones like in the Sapotaceae family (O. Boissier pers. obs., Boissier, 2012).

We noted that fruit and scrap numbers could be obtained by weighing, which could facilitate the enumeration. We advise to intensively sample some specimens of the selected species and then to estimate the mean squared error of the selected AP, adapting the size of the quadrats to fruit size and crop (see R code in Supporting information for details on the bootstrap procedure that has been used). The resulting value could be compared with those of our study, ranging between 0 and 0.044 (mean = 0.014) with the expected seed removal rate varying between 1 and 0.2 (mean = 0.73). In addition, the specimens sampled for determining quadrat size may cover the range of the expected seed removal rate because the mean squared error at tree level increases when the expected seed removal rate decreases.

To compare sites for activity of dispersers as proposed by the authors of RAM, it is crucial to have an index of animal interactions with seeds. Still, a method based on enumeration of seeds from intact fruits and from fruit scraps on the ground has several limitations. The first one is that selected tree species must bear fruits leaving scraps after their seeds have been removed, in order to estimate the original number of seeds (Boissier et al., 2014). Nevertheless, a large number of species produces fleshy fruits, but also dry dehiscent fruits such as capsules, follicles or legumes, leaving scraps. The second limitation is that seed removal rate gives only a measure of overall seed removal without clear distinction of seed dispersal and seed predation. To have a better idea of the processes involved in each study site, it is possible to combine seed removal rate estimation with additional observations. The recording of traces of frugivores and seed predators on fruits scraps, as suggested by Boissier et al. (2014), focal observations of the trees and the use of camera-traps, which are currently more affordable (Trolliet et al., 2014), could allow identification of the species involved in the processes and the distinction of predator relative contribution in seed removal. Also, careful observation of the ripening stage of the fruits on the ground could provide further valuable information. The dispersal of seeds from unripe fruits might follow other ways than seeds of mature fruits since they are less edible and less attractive to frugivores (Izhaki, 2002; Schaefer et al., 2003). Last, the seeds from unripe fruit could germinate, but following contrasted patterns (e.g. Lottf et al., 1999; Barnett et al., 2012; Niederhauser and Matlack, 2015).

5. Conclusion

In this study, we showed that RAM is an efficient, but unfortunately biased estimator of the seed removal rate (in 47% of the studied cases), probably because seed removal rate inside or below the canopy could increase with fruit density. Thus, its use might be restricted to the comparison of sites and considering a single species. An unbiased, more accurate (50% decrease of the mean squared error compared to RAM) and probably more time efficient estimation of the seed removal rate was obtained by using the average seed removal rate of three random quadrats. Regarding the size of the quadrats, we recommend aiming at a mean fruit number per quadrat between five and ten to minimize the small frequency bias and to keep counts moderate. However, the operator should estimate the mean squared error at least on one focal tree to compare it with the values of the present study, particularly for smaller fruits.

We also showed that seed removal rate could depend on tree size (crown diameter) and fruit production. This result implies that, if stands are to be compared, the sampled trees might be representative. Besides, attention could be paid to the ripening stage of the fruits, to the traces of teeth and beaks on the fruit scraps as well as to the identification of the dispersers and predators. Indeed, this information would allow to separately assess the seed removal rate for the unripe and the ripe fruits and to infer the relative contributions of primary and secondary dispersers and predators.

Data accessibility

- Field data can be found at https://figshare.com/s/83cda3a927c1ad954d96
- R script is uploaded as online supporting information.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.07.042.

References

Aliyu, B., Adamu, H., Moltchanova, E., Forget, P.-M., Chapman, H., 2014. The interplay of habitat and seed type on scatterhoarding behavior in a fragmented Afromontane forest landscape. Biotropica 46, 264–267.

Barnett, A.A., Boyle, S.A., Pinto, L.P., Lourenc, W.C., Almeida, T., Silva, W.S., Ronchi-Teles, B., Bezerra, B.M., Ross, C., MacLarnon, A., Spironello, W.R., 2012. Primary seed dispersal by three Neotropical seed-predating primates (*Cacajao melanocephalus ouakary*, *Chiropotes chiropotes* and *Chiropotes albinasus*). J. Trop. Ecol. 28, 543–555.

Beaune, D., Bretagnolle, F., Bollache, L., Hohmann, G., Surbeck, M., Bourson, C., Fruth, B., 2013. The Bonobo-*Dialium* Positive Interactions: Seed Dispersal Mutualism. Am. J. Primatol. 75, 394–403.

Beckman, N.G., Muller-Landau, H.C., 2007. Differential effects of hunting on pre-dispersal seed predation and primary and secondary seed removal of two neotropical tree species. Biotropica 39, 328–339.

Blendinger, P.G., Díaz-Vélez, M.C., 2010. Experimental field test of spatial variation in rodent predation of nuts relative to distance and seed density. Oecologia 163, 415–423.

Boissier, O., 2012. Impact des pressions anthropiques sur les communautés de frugivores et la dispersion des graines en forêt guyanaise (Thèse de doctorat). Museum National d'Histoire Naturelle, Paris.

Boissier, O., Bouiges, A., Mendoza, I., Feer, F., Forget, P.-M., 2014. Rapid assessment of seed semoval and frugivore activity as a aool for monitoring the health status of tropical forests. Biotropica 46, 633–641.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S., 2008. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135.

Brewer, S.W., Rejmánek, M., 1999. Small rodents as significant dispersers of tree seeds in a Neotropical forest. J. Veg. Sci. 10, 165–174.

Briani, D.C., Guimarães Jr, P.R., 2007. Seed predation and fruit damage of *Solanum lycocarpum* (Solanaceae) by rodents in the cerrado of central Brazil. Acta Oecol. 31, 8–12.

Calvino-Cancela, M., Rubido-Bará, M., 2012. Effects of seed passage through slugs on germination. Plant Ecol. 213, 663–673.

Chapman, C.A., Chapman, L.J., Wangham, R., Hunt, K., Gebo, D., Gardner, L., 1992. Estimators of fruit abundance of tropical trees. Biotropica 24, 527–531.

Clark, C.J., Poulsen, J.R., Parker, V.T., 2001. The role of arboreal seed dispersal groups on the seed rain of a lowland tropical forest. Biotropica 33, 606–620.

Culot, L., Mann, D.J., Muñoz Lazo, F.J.J., Huynen, M., Heymann, E.W., 2011. Tamarins and dung deetles: an efficient diplochorous dispersal system in the Peruvian Amazonia. Biotropica 43, 84–92.

Duncan, R.S., Chapman, C.A., 1999. Seed dispersal and potential forest succession in abandoned agriculture in tropical Africa. Ecol. Appl. 9, 998–1008.

Efron, B., 1979. Bootstrap methods: another look at the Jackknife. Ann. Stat. 7, 1–26.

Efron, B., Tibshirani, J.T., 1993. An Introduction to the Bootstrap. Chapman & Hall CRC, Boca Raton.

Feer, F., Forget, P.-M., 2002. Spatio-temporal variations in post-dispersal seed fate. Biotropica 34, 555–566.

Flohic, G.L., Motsch, P., DeNys, H., Childs, S., Courage, A., King, T., 2015. Behavioural ecology and group cohesion of juvenile western lowland gorillas (*Gorilla g. gorilla*) during rehabilitation in the Batéké Plateaux National Park, Gabon. PLoS ONE 10, e0119609.

Flörchinger, M., Braun, J., Böhning-Gaese, K., Schaefer, H.M., 2010. Fruit size, crop mass, and plant height explain differential fruit choice of primates and birds. Oecologia 164, 151–161.

Forget, P.M., Jansen, P.A., 2007. Hunting increases dispersal limitation in the tree *Carapa procera*, a nontimber forest product. Conserv. Biol. 21, 106–113.

Forget, P.-M., Wenny, D., 2005. How to elucidate seed fate? A review of methods used to study seed removal and secondary seed dispersal. In: Forget, P.-.M., Lambert, J.E., Hulme, P.E., Vander Wall, S.B. (Eds.), Seed Fate: Predation, Dispersal, and Seedling Establishment. CABI, Wallingford, pp. 379–394.

Foster, M.S., 1990. Factors influencing bird foraging preferences among conspecific fruit trees. Condor 92, 844–854.

Gartlan, J.S., Struhsaker, T., 1972. Polyspecific associations and niche separation of rain-forest anthropoids in Cameroon, West Afr. J. Zool. 168, 221–266.

Gautier-Hion, A., Duplantier, J.-M., Quris, R., Feer, F., Sourd, C., Decoux, J.-P., Dubost, G., Emmons, L., Erard, C., Hecketsweiler, P., Moungazi, A., Roussilhon, C., Thiollay, J.-M., 1985. Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. Oecologia 65, 324–337.

Guitián, J., Munilla, I., 2010. Responses of mammal dispersers to fruit availability: Rowan (*Sorbus aucuparia*) and carnivores in mountain habitats of northern Spain. Acta Oecol. 36, 242–247.

Hernández, Á., 2008. Cherry removal by seed-dispersing mammals: Mutualism through commensal association with frugivorous birds. Pol. J. Ecol. 56, 127–138.

Howe, H.F., Smallwood, J., 1982. Ecology of seed dispersal. Annu. Rev. Ecol. Syst. 131, 201–228.

Hulme, P.E., 1997. Post-dispersal seed predation and the establishment of vertebrate dispersed plants in Mediterranean scrublands. Oecologia 111, 91–98.

Izhaki, I., 2002. Emodin - a secondary metabolite with multiple ecological functions in higher plants. New Phytol. 155, 205–217.

Janmaat, K.R.L., Ban, S.D., Boesch, C., 2013. Chimpanzees use long-term spatial memory to monitor large fruit trees and remember feeding experiences across seasons. Anim. Behav. 86, 1183–1205.

Jones, F.A., Comita, L.S., 2010. Density-dependent pre-dispersal seed predation and fruit set in a tropical tree. Oikos 119, 1841–1847.

Jordano, P., 2000. Fruits and frugivory. In: Fenner, M. (Ed.), Seeds: the Ecology of Plant Regeneration in Plant Communities. CABI, Wallingford, pp. 125–166.

Krief, S., Jamart, A., Hladik, C.M., 2004. On the possible adaptive value of coprophagy in free-ranging chimpanzees. Primates 45, 141–145.

Leighton, M., Leighton, D.R., 1982. The relationship of size of feeding aggregate to size of food patch: howler monkeys (*Alouatta palliata*) feeding in *Trichilia cipo* fruit trees on Barro Colorado Island. Biotropica 14, 81–96.

Lengyel, S., Gove, A.D., Latimer, A.M., Majer, J.D., Dunn, R.R., 2010. Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: a global survey. Perspect. Plant Ecol. 12, 43–55.

Lermyte, C., Forget, P.-M., 2009. Rapid assessment of dispersal failure and seedling recruitment of large-seeded non- timber forest products trees in a tropical rainforest. Trop. Conserv. Sci. 2, 404–424.

Lottf, Y.V.D., Thatcher, R.L., Rossomv, L., Reimiardt, C.F., 1999. The influence of baboon predation and time in water on germination and early establishment of *Opuntia stricta* (Australian pest pear) in the Kruger National Park. Koedoe 42, 43–50.

Louppe, D., Gérard, J., 2011. Afzelia bipindensis Harms. In: Lemmens, R.H.M.J., Louppe, D., Oteng-Amoako, A.A. (Eds.), Prota 7(2): Timbers/Bois d'œuvre 2. [CD-Rom]. PROTA, Wageningen, Netherlands.

Malhi, Y., Gardner, T.A., Goldsmith, G.R., Silman, M.R., Zelazowski, P., 2014. Tropical forests in the anthropocene. Annu. Rev. Environ. Resour. 39, 125–159.

McClearn, D., 1992. Locomotion, posture, and feeding behavior of kinkajous, coatis, and raccoons. J. Mammal. 73, 245–261.

Meunier, Q., Moumbogou, C., Doucet, J.L., 2015. Les arbres utiles du Gabon. Les Presses Agronomiques de Gembloux, Gembloux.

Mood, A.M., Graybill, F.A., Boes, D.C., 1974. Introduction to the Theory of Statistics. McGraw-Hill, New-York.

Moreira, J.I., Riba-Hernández, P., Lobo, J.A., 2017;al., in press. Toucans (*Ramphastos ambiguus*) facilitate resilience against seed dispersal limitation to a large-seeded tree (*Virola surinamensis*) in a human-modified landscape. Biotropica 49 (4), 502–510.

Naito, Y., Kanzaki, M., Numata, S., Obayashi, K., Konuma, A., Nishimura, S., Ohta, S., Tsumura, Y., Okuda, T., Lee, S.L., Muhammad, N., 2008. Size-related flowering and fecundity in the tropical canopy tree species, *Shorea acuminata* (Dipterocarpaceae) during two consecutive general flowerings. J. Plant Res. 121, 33–42.

Niederhauser, E.C., Matlack, G.R., 2015. All frugivores are not equal exploitation competition determines seed survival and germination in a fleshy-fruited forest herb. Plant Ecol. 216, 1203–1211.

Norden, N., Chave, J., Belbenoit, P., Caubère, A., Châtelet, P., Forget, P.-M., Thébaud, C., 2007. Mast fruiting is a frequent strategy in woody species of Eastern South America. Plos One 2, e1079.

Ortiz-Pulido, R., Rico-Gray, V., 2000. The effect of spatio-temporal variation in understanding the fruit crop size hypothesis. Oikos 91, 523–527.

Poulsen, J.R., Clark, C.J., Connor, E.F., Smith, T.B., 2002. Differential resource use by primates and hornbills Implications for seed dispersal. Ecology 83, 228–240.

Rosenberger, A.L., 1992. Evolution of feeding niches in New World monkeys. Am. J. Phys. Anthropol. 88, 525–562.

Rosin, C., Poulsen, J.R., 2016. Telemetric tracking of scatterhoarding and seed fate in a Central African forest. Biotropica 49, 170–176.

Schaefer, H.M., Schmidt, V., Winkler, H., 2003. Testing the defence trade-off hypothesis How contents of nutrients and secondary compounds affect fruit removal. Oikos 102, 318–328.

Steele, M.A., Bugdal, M., Yuan, A., Bartlow, A., Buzalewski, J., Lichti, N., Swihart, R., 2011. Cache placement, pilfering, and a recovery advantage in a seed-dispersing rodent could predation of scatter hoarders contribute to seedling establishment? Acta Oecol. 37, 554–560.

Stevenson, P.R., Link, A., Ramírez, B.H., 2005. Frugivory and seed fate in *Bursera inversa* (Burseraceae) at Tinigua Park, Colombia Implications for primate conservation. Biotropica 37, 431–438.

Suarez, S.A., 2014. Ecological factors predictive of wild spider monkey (*Ateles belzebuth*) foraging decisions in Yasuní. Ecuador. Am. J. Primatol. 76, 1185–1195.

Suselbeek, L., Jansen, P.A., Prins, H.H.T., Steele, M.A., 2013. Tracking rodent-dispersed large seeds with Passive Integrated Transponder (PIT) tags. Methods Ecol. Evol. 4, 513–519.

Toh, I., Gillespie, M., Lamb, D., 1999. The role of isolated trees in facilitating tree seedling recruitment at a degraded sub-tropical rainforest site. Restor. Ecol. 7, 288–297.

Trolliet, F., Forget, P.-M., Huynen, M.-C., Hambuckers, A., 2017. Forest cover, hunting pressure, and fruit availability influence seed dispersal in a forest-savanna mosaic in the Congo Basin. Biotropica 49, 337–345.

Trolliet, F., Serckx, A., Forget, P.M., Beudels-Jamar, R., Huynen, M.C., Hambuckers, A., 2016. Ecosystem services provided by a large endangered primate in a forest-savanna mosaic landscape. Biol. Conserv. 203, 55–66.

Trolliet, F., Vermeulen, C., Huynen, M.-C., Hambuckers, A., 2014. Use of camera traps for wildlife studies a review. BASE 18, 446–454.

Türke, M., Andreas, K., Gossner, M.M., Kowalski, E., Lange, M., Boch, S., Socher, S.A., Müller, J., Prati, D., Fischer, M., Meyhöfer, R., Weisser, W.W., 2012. Are gastropods, rather than ants, important dispersers of seeds of myrmecochorous forest herbs? Am. Nat. 179, 124–131.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Springer Science + Bussiness Media, New York.

Wang, B.C., Sork, V.L., Leong, M.T., Smith, T.B., 2007. Hunting of mammals reduces seed removal and dispersal of the afrotropical tree *Antrocaryon klaineanum* (Anacardiaceae). Biotropica 39, 340–347.

Wenny, D.G., 2000. Seed dispersal, seed predation, and seedling recruitment of a Neotropical montane tree. Ecol. Monogr. 70, 331–351.

Wilks, C., Issembé, Y., 2000. Les arbres de la Guinée Equatoriale Région continentale. CUREF, Bâta (Guinée Equatoriale).

Willson, M.F., Irvine, A.K., Walsh, N.G., 1989. Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographic comparisons. Biotropica 21, 133–147.