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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