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Species identity influences secondary removal of seeds of Neotropical pioneer tree species

Selina A. Ruzi · Daniel P. Roche · Paul-Camilo Zalamea · Abigail C. Robison · James W. Dalling

Abstract Primary dispersal agents move seeds from the maternal plant to the soil surface where they are often moved again by secondary dispersal agents. However, the extent to which different species in the same location experience secondary dispersal is often unknown despite the importance of this mechanism for determining recruitment opportunities and consequently community structure. Here we examine the secondary removal rates of 12 Neotropical pioneer species placed either on or 2 cm below the soil surface at five locations in lowland tropical forest on Barro Colorado Island, Panama. We investigated whether species identity, primary dispersal mode (animal or wind), dormancy type, seed mass, and capacity to persist in the seed bank were correlated with removal rate. We also investigated whether season (dry or wet) influences removal from the soil surface. In general, both superficial and buried seeds were highly mobile. We found an effect of primary dispersal mode and dormancy type on removal rates both on (12 species) and beneath the soil surface (six species). However, this pattern was largely driven by species identity. Season had no influence on seed removal rates from the soil surface. The dispersal of small-seeded pioneer species is highly species dependent, indicating that generalizations made using broader categories, such as primary dispersal mode or dormancy type, do not accurately describe the observed patterns hindering our understanding of community assembly within even a single functional group of plants.

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Introduction

Plants face a wide variety of obstacles to the recruitment of new individuals into the population. Plants are limited not only by the number of seeds that they can produce ('source limitation'), but also whether those seeds escape pre-dispersal predation, survive dispersal, and reach a suitable microsite for germination, while surviving to emergence and establishment (Nathan and Muller-Landau 2000). In tropical forests, some pioneer tree species produce sufficient numbers of small seeds to overcome source limitation (Dalling et al. 2002), yet only a small fraction of those seeds reach microsites suitable for onward growth. This indicates that they are either limited in their ability to effectively disperse seeds ('dispersal limitation') or that constraints imposed by reducing source limitation negatively affect the range of sites favorable for seedling establishment (Dalling and Hubbell 2002).

Dispersal away from the parent plant or conspecifics is important for seedling recruitment in plant communities, both to increase the probability of encountering suitable microsites and to avoid predators and pathogens that act in a density-dependent manner (Janzen 1970; Connell 1971; Comita and Hubbell 2009; Mangan et al. 2010; Bagchi et al. 2014). In most forests, treefall gaps provide the microsites required for germination and establishment of pioneer trees (Swaine and Whitmore 1988; Hubbell et al. 1999). However, treefall gaps are uncommon, they exist for short periods of time, and their spatial location and time of formation are fairly unpredictable (Young and Hubbell 1991; Schnitzer et al. 2000). As a consequence, pioneer tree species are under selection to disperse seeds widely, and/or for their seeds to persist for decades in the seed bank (Dalling and Brown 2009).

Primary dispersal, the initial movement of seeds away from the parent tree (Nathan and Muller-Landau 2000), can be accomplished through a variety of means, including animal, wind, gravity, and ballistic dispersal (Seidler and Plotkin 2006). After primary dispersal has occurred, seeds may experience additional movement events (see Vander Wall et al. 2005) resulting in secondary dispersal or predation. The activity of these secondary removal agents ultimately influences how many seeds are available for germination and can be important at structuring plant communities (Chambers and MacMahon 1994). Potential benefits of secondary dispersal include protection from predation, reduction of competition with conspecifics, and movement to microsites beneficial for germination (Vander Wall and Longland 2004). Overall, whether secondary dispersal activity is beneficial or not to seeds is context dependent and relies on many different factors (Chambers and MacMahon 1994). For example, secondary dispersal often comes with a price: many secondary dispersers also consume some of the seeds they remove (Vander Wall and Longland 2004). However, the benefits accrued by the seeds that survive may outweigh the loss of seeds due to predation (Chambers and MacMahon 1994).

Incorporation of seeds into the soil seed bank (defined as the viable seeds present both on the soil surface and in the soil profile; Long et al. 2015) is a critical part of dispersal activities. The seed bank is often referred to as a safe site for seeds; however, the seed bank is dynamic with many factors including seed, species, and environmental characteristics influencing whether seeds persist or exit the seed bank, either through decay or germination (reviewed in Long et al. 2015). Seed characteristics, such as dormancy type, can influence when seeds are able to respond to the environmental cues they require for germination, while seed size, seed coat, and the presence of appendages or exudates can influence the vulnerability of seeds to predators and pathogens (Long et al. 2015). For example, some physically dormant seeds may avoid predation while in the seed bank because their impermeable seed coat reduces the diffusion of olfactory cues used by rodents to detect them (Paulsen et al. 2013). Spatial and temporal variation in environmental conditions associated with climate seasonality and microsite heterogeneity can also influence whether a seed is likely to persist (Long et al. 2015), and these conditions may be altered through additional dispersal events into new microsites. While secondary dispersal of seeds into the soil profile is frequently reported, it is unknown whether seeds experience the same potential for further movement both at the soil surface and in the topsoil.

Table 1 Soil and flora characteristics of sampling areas (plots) within Barro Colorado Island

Plot/location name	Forest age (years)	Soil type/soil form/parent material
25 Ha	80–120	Marron/brown fine loam/Andesite
Armour	80–140	AVA/red light clay/Andesite
Drayton	400+	Fairchild/red light clay/Bohio
Pearson	400+	Standley/brown fine loam/Bohio
Zetek	400+	Marron/brown fine loam/Andesite

Forest age is approximated from classifications mapped in Mascaro et al. (2011) that were generated based on land-use data obtained from Enders (1935). Information on soil type, soil form, and parent material is from Baillie et al. (2006)

In this study, we examine whether secondary removal rates of seeds vary among pioneer species found in lowland forest of central Panama. For 12 tree species, we tested whether the individual species identity, season, primary dispersal mode (animal or wind), and dormancy type (physical, physiological, or quiescent) influenced the rate of seed removal from the forest floor. We also examined if two seed traits, seed mass and seed persistence in the soil seed bank (defined here as the proportion of viable seeds that survive 18 months of burial enclosed in mesh bags), were correlated with removal rates. For six species, we further tested whether seed removal occurred once seeds were incorporated into the top 2 cm of soil. We tracked the fate of 3000 seeds to test the following alternative, but not mutually exclusive, hypotheses: (1) primary dispersal mode would serve as a strong predictor of secondary dispersal rates, predicting that animal-dispersed seeds would experience higher above-ground removal rates than wind-dispersed seeds (Fornara and Dalling 2005a); (2) seeds with physical dormancy, and that are therefore impermeable to water, would also have lower removal rates, reflecting reduced availability of olfactory cues for seed predators (Paulsen et al. 2013); and (3) the capacity for long-term survival in the soil seed bank is contingent on avoiding seed predators, resulting in a negative correlation between seed removal rate and seed persistence in the soil in the absence of predators.

Methods

Study site and species

The study was carried out in 2013 on Barro Colorado Island (BCI) (9°10′N, 79°51′W) in the Republic of Panama. BCI supports seasonal semi-deciduous forest at an average elevation of 70 m above sea level with an annual rainfall of 2600 mm (Windsor 1990). The forest experiences a pronounced dry season starting late December or early January and continuing until late April or early May (Windsor 1990). Seed removal experiments were located at five sites at least 350 m apart, and at least 20 m from conspecific trees, within either old-growth or secondary forest and representing a range of soil types (Zalamea et al. 2015; Table 1).

We selected 12 pioneer species found on BCI that vary in seed size, primary dispersal mode, dormancy type, and seed persistence capacity for experiments during the dry and wet seasons (Table 2). The wet season experiments also included artificial seeds (30.5 ± 0.038 mg, mean ± SE, mass silica beads). Ripe fruits were collected directly from the parent tree or from beneath the crown and then cleaned to remove seeds from fruit pulp (animal-dispersed species except *Zanthoxylum ekmanii* (Urb.) Alain) or kapok-like fibers (*Cochlospermum vitifolium* Willd. and *Ochroma pyramidale* Urb.). None of the species involved in this experiment have elaiosomes to attract ants.

Seed removal experiments

The experiments were divided into two parts: an above-ground removal experiment that investigated seed removal from the soil surface and a below-ground removal experiment that investigated the removal of seeds buried 2 cm beneath the soil surface. The above-ground experiment was conducted once in the dry season and once in the wet season of 2013, and the below-ground experiment was conducted once in the wet season of 2013.

The above-ground removal experiment used similar methods to Fornara and Dalling (2005a).

Table 2 Pioneer tree species used throughout the study

Species (species code)	Family	Species distribution	Fruiting period	Primary dispersal mode	Dormancy type	Seed mass (mg)	Seed persistence (18 months)	Used in above-ground	Used in below-ground
Apeiba membranacea Aubl. (Ape)	Tiliaceae	Mexico–Brazil[a]	February–March[a]	Animal	Physical[b]	13.58 ± 1.23	0.75[c]	x	x
Cecropia longipes Pitt. (Cec)	Urticaceae	Panama–Colombia[d]	July–August[e]	Animal	Quiescent[c]	0.9 ± 0.07	0.85[c]	x	x
Cochlospermum vitifolium Willd. (Coc)	Bixaceae	Mexico–Brazil[a]	February–April[a]	Wind	Physical[c]	24.4 ± 2.27	0.24[c]	x	
Ficus insipida Willd. (Fic)	Moraceae	Mexico–Brazil[f]	Variable[g]	Animal	Quiescent[c]	1.56 ± 0.12	0.46[c]	x	
Guazuma ulmifolia Lam. (Guz)	Sterculiaceae	Mexico–Colombia[h]	March–April[h]	Animal	Physical[b]	3.38 ± 0.28	0.86[c]	x	
Hieronyma alchorneoides Allemao (Hie)	Euphorbiaceae	–	January, June, July, December[i]	Animal	Physiological[b]	6.55 ± 0.33	0.98[c]	x	
Jacaranda copaia (Aubl.) D. Don. (Jac)	Bignoniaceae	Belize–Brazil[j]	July–September[k]	Wind	Quiescent[c]	1.16 ± 0.18	0.33[c]	x	x
Luehea seemannii Triana and Planch. (Lue)	Tiliaceae	Mexico–Venezuela[a]	February–April[a]	Wind	Physical[b]	1.85 ± 0.3	0.88[c]	x	
Ochroma pyramidale Urb. (Och)	Malvaceae	Mexico–Brazil[a]	March–May[a]	Wind	Physical[b]	5.73 ± 0.29	0.88[c]	x	x
Trema micrantha (L.) Blume "black" (TrBl)	Cannabaceae	–	August–October[i]	Animal	Physiological[b]	3.2 ± 0.21	0.97[c]	x	x
Trema micrantha (L.) Blume "brown" (TrBr)	Cannabaceae	–	August–October[k]	Animal	Quiescent[c]	1.71 ± 0.1	1.00[c]	x	
Zanthoxylum ekmanii (Urb.) Alain (Zan)	Rutaceae	–	February–March[i]	Animal	Physiological[c]	16.2 ± 0.81	1.00[c]	x	x

All seeds were used for the above-ground experiment, while a subset was chosen for the below-ground experiment

[a] Zalamea et al. (2015)

[b] Sautu et al. (2007)

[c] P.-C. Zalamea unpubl. data

[d] Zalamea et al. (2012)

[e] Zalamea et al. (2011)

[f] Phillips (1990)

[g] Milton et al. (1982)

[h] Janzen (1975)

[i] Zimmerman et al. (2007)

[j] Scotti-Saintagne et al. (2013)

[k] Dalling et al. (1997)

Table 3 Models testing for the effects of season (above-ground only), dispersal mode, and dormancy type both with and without species identity included as a random effect for above- and below-ground experiments

Experiment	Model	DF	AIC	BIC	LogLik	p value
Above-ground	Proportion ~ season + dispersal + dormancy + (1|plot/trial)	8	236	264	−110	–
Above-ground	Proportion ~ season + dispersal + dormancy + (1|plot/trial) + (1|species)	9	202	233	−92	<0.001
Below-ground	Proportion ~ dispersal + dormancy + (1|plot/trial)	7	42.8	57.4	−14.4	–
Below-ground	Proportion ~ dispersal + dormancy + (1|plot/trial) + (1|species)	8	43.7	60.5	−13.9	0.312

p values are from comparing models without and with species identity as a random effect for the above- or below-ground experiments separately

Experiments were located along a 12-m transect established at the edge of five rectangular (9 × 15 m) plots where seeds of the same species were buried inside mesh exclosures to determine seed persistence capacity (Zalamea et al. 2015). Leaf litter was partially removed, and 9-cm-diameter Petri dish lids were inverted and placed on the ground 1 m apart. Ten seeds of one species were placed in each dish, with the position of each species assigned at random. No vertebrate exclosures were used, as most seed removal observed had been previously attributed to invertebrates (Fornara and Dalling 2005a). A 1.0 m wide × 1.0 m long × 0.5 m tall transparent plastic shelter was centered over each dish to protect seeds from being washed away by rain or failing debris.

Observations of seed removal were initiated at 10:00. The number of seeds present was recorded at hourly intervals for each species until 16:00. The seeds were left out overnight. Observations continued 24 h after the first time point at 10:00 and again until 16:00 of the second day. One final observation was made at 09:00 on the third day. Overall, the seeds were left out for 47 h.

Two trials were performed at each of the five plots. Each trial consisted of one 47-h period of seed removal for all 12 species along one side of each experimental plot chosen at random. A second trial was performed at least 1 week after the first trial along a different randomly selected plot edge. Dry season sampling was conducted from mid-March to mid-April 2013, and wet season sampling from mid-July to mid-August 2013.

The below-ground removal experiment used six of the 12 species in the above-ground study (Table 2). Two trials were randomly assigned to sides of the same five plots as in the above-ground experiment.

Holes approximately 2 cm deep and two and a half centimeters wide were dug into the soil along the plot 1 m apart. Ten seeds of one species were mixed with sieved, forest soil (autoclaved at 121 °C for 2 h) and then buried in each hole. Plastic shelters were not used for below-ground trials. As a control, we buried ten silica beads in a seventh hole to estimate 'background' rates of seed loss including loss by rainfall.

Each of the two trials at a plot was buried simultaneously, though plots were buried on separate days (Online Resource 1). Seeds were left buried for 4 weeks from mid-July to late August 2013. During collection, an area larger than the original burial holes (approximately 5 cm deep and 8 cm wide) was dug up to ensure collection of all seeds remaining. The soil was sieved, and any remaining seeds were recovered and counted.

Statistical analysis

We used general linear mixed-effects models (LMMs) to analyze the proportion of seeds removed to seeds placed among species for both the above- and below-ground experiments with the assumption that missing seeds had been actively removed. We also used LMMs to test for the fixed effects of season (above-ground experiment only), primary dispersal mode, and dormancy type. These models were calculated with and without species as a random effect to determine the added value of including species (model selection values reported in Table 3). Above-ground and below-ground data were analyzed separately. We also tested whether controls (silica beads) had a lower removal rate than seeds for both the wet season above-ground data and below-ground data. All LMMs for both experiments consisted of the proportion of the total
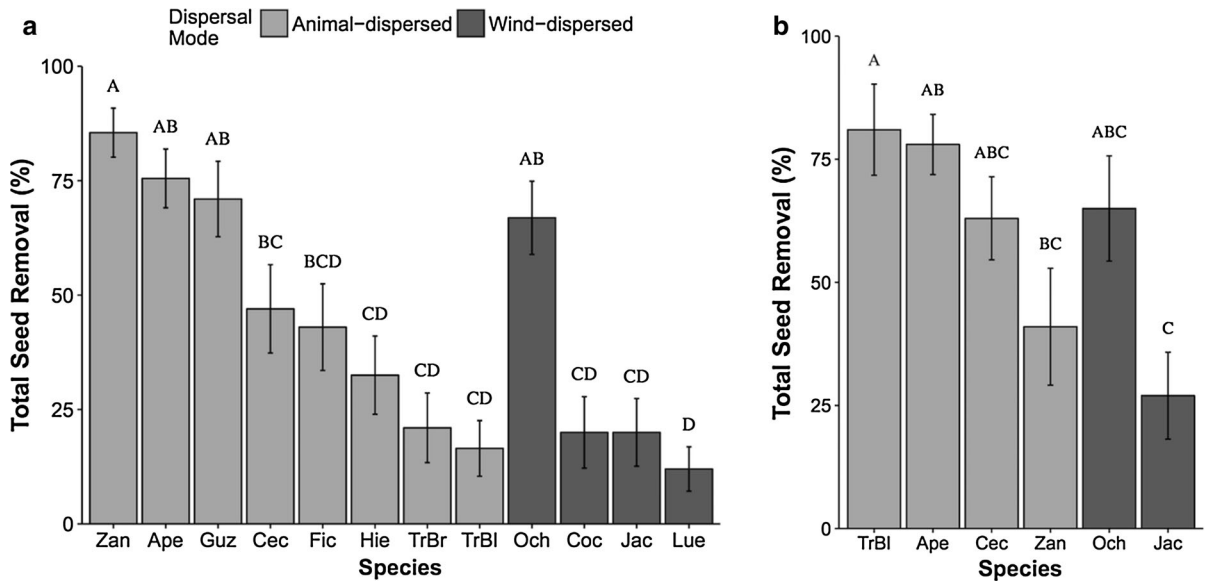
Fig. 1 Average seed removal by species for above-ground ($n = 200$ seeds) (**a**) and below-ground ($n = 100$ seeds) (**b**) experiments. *Error bars* correspond to SE. *Letters* denote significant differences as determined by Tukey's HSD means separation tests. *Light gray* and *dark gray bars* represent species categorized as being primarily animal- or wind-dispersed, respectively. See Table 2 for species codes

number of seeds removed as the response variable and location within the forest (plot) and trial as the nested random effects. Tukey's post hoc means separation tests were conducted for significant fixed effect factors in the above-ground experiment and below-ground experiment. Separate analyses of variance (ANOVAs) were used to determine if dispersal mode or dormancy type influenced seed mass. Spearman's rank correlation tests were conducted to determine if species highly removed in the above-ground experiment were also highly removed in the below-ground experiment, as well as whether seed mass was correlated with seed removal. Pearson correlation tests were also used to determine the relationship between the proportions of buried seeds that survive in the soil for 18 months (P.-C. Zalamea, unpubl. data) and removal. We conducted variance partitioning (Borcard et al. 1992) to analyze the relative importance of season (above-ground experiment only), species identity, primary dispersal mode, dormancy type, and location within the forest (plot) on seed removal. For these analyses, above- and below-ground data were treated separately and linear models were used to obtain R^2 values. All analyses were completed using R version 3.3.0 (R Developmental Core Team 2016) using the *nlme* (version 3.1-127, Pinheiro et al. 2015), and modifying the varPart

function from *ModEvA* (version 1.3.2, Barbosa et al. 2016) to work with four or five factors.

Results

For the above-ground experiment, we found that seeds were more frequently removed than silica beads ($F = 12.92$, df = 1, 119, $p < 0.001$). Combining all the species and plots together during the wet season, on average $46 \pm 3.8\%$ ($n = 1200$ seeds) (mean $\pm$ SE) of the seeds were removed from the dishes, while $2 \pm 1.3\%$ ($n = 100$ beads) of the silica beads were removed. For the below-ground experiment, we found a similar result, $59 \pm 4.4\%$ ($n = 600$ seeds) of the seeds removed versus $16 \pm 7.8\%$ ($n = 100$ beads) of silica beads ($F = 14.42$, df = 1, 59, $p < 0.001$).

Species effect

Species identity had a significant effect on total removal both above-ground ($F = 13.08$, df = 11, 219, $p < 0.001$; Fig. 1a) and below-ground ($F = 5.11$, df = 5, 45, $p < 0.001$; Fig. 1b). For the above-ground experiment, *Z. ekmanii* had the highest average seed removal ($85.5 \pm 5.4\%$), and *Trema*

micrantha (L.) Blume black seed morph had the lowest average seed removal (16.5 ± 6.1%). In contrast, for the below-ground experiment, *T. micrantha* black seed morph had the highest average seed removal (81 ± 9.2%) and *Jacaranda copaia* (Aubl.) D. Don. had the lowest average seed removal (27 ± 8.8%). There was no significant correlation between species removal rates above- and below-ground (Spearman's $r = 0.26$, $p = 0.66$). Species alone, as well as models that included species, tended to account for most of the variation in the data for both above-ground (Fig. 2a) and below-ground (Fig. 2b) experiments.

Effect of seasonality

We tested if there was a seasonality effect on seed removal for the above-ground experiment and found no differences between the numbers of seeds removed during the dry season (39 ± 3.8%) and the wet season (46 ± 3.8%; without species: $F = 1.92$, df = 1, 226, $p = 0.17$; with species as a random effect: $F = 2.39$, df = 1, 227, $p = 0.12$).

Effect of primary dispersal mode

Animal-dispersed seeds had higher mean seed removal rates than wind-dispersed species for both above-ground ($F = 13.7$, df = 1, 226, $p < 0.003$; Fig. 3a) and below-ground ($F = 5.13$, df = 1, 47, $p = 0.028$; Fig. 3b) experiments. However, including species as a random effect masks this effect for both above-ground ($F = 2.32$, df = 1, 8, $p = 0.17$) and below-ground ($F = 3.12$, df = 1, 2, $p = 0.22$) experiments.

Effect of dormancy

We found an effect of dormancy type on mean seed removal for both above-ground ($F = 10.58$, df = 2, 226, $p < 0.001$; Fig. 4a) and below-ground ($F = 3.91$, df = 2, 47, $p = 0.027$; Fig. 4b) experiments. In the above-ground experiment, physically dormant seeds ($n = 5$ species) had a slightly higher mean seed removal rate than physiologically dormant seeds ($n = 3$ species) and greater removal rate than quiescent seeds ($n = 4$ species). In the below-ground experiment, physically ($n = 3$ species) and physiologically dormant seeds ($n = 1$ species) were removed in greater amounts than quiescent seeds ($n = 2$

species). However, including species as a random effect masks this effect for both above-ground ($F = 1.80$, df = 2, 8, $p = 0.23$) and below-ground ($F = 2.38$, df = 2, 2, $p = 0.30$) experiments.

Seed mass

Neither dispersal mode (ANOVA: $F = 0.26$, df = 1, $p = 0.62$) nor dormancy type (ANOVA: $F = 1.78$, df = 2, $p = 0.22$) had a significant effect on seed mass. Seed mass was not correlated with above-ground (Spearman's $r = 0.36$, $p = 0.26$) or below-ground (Spearman's $r = 0.14$, $p = 0.80$) removal.
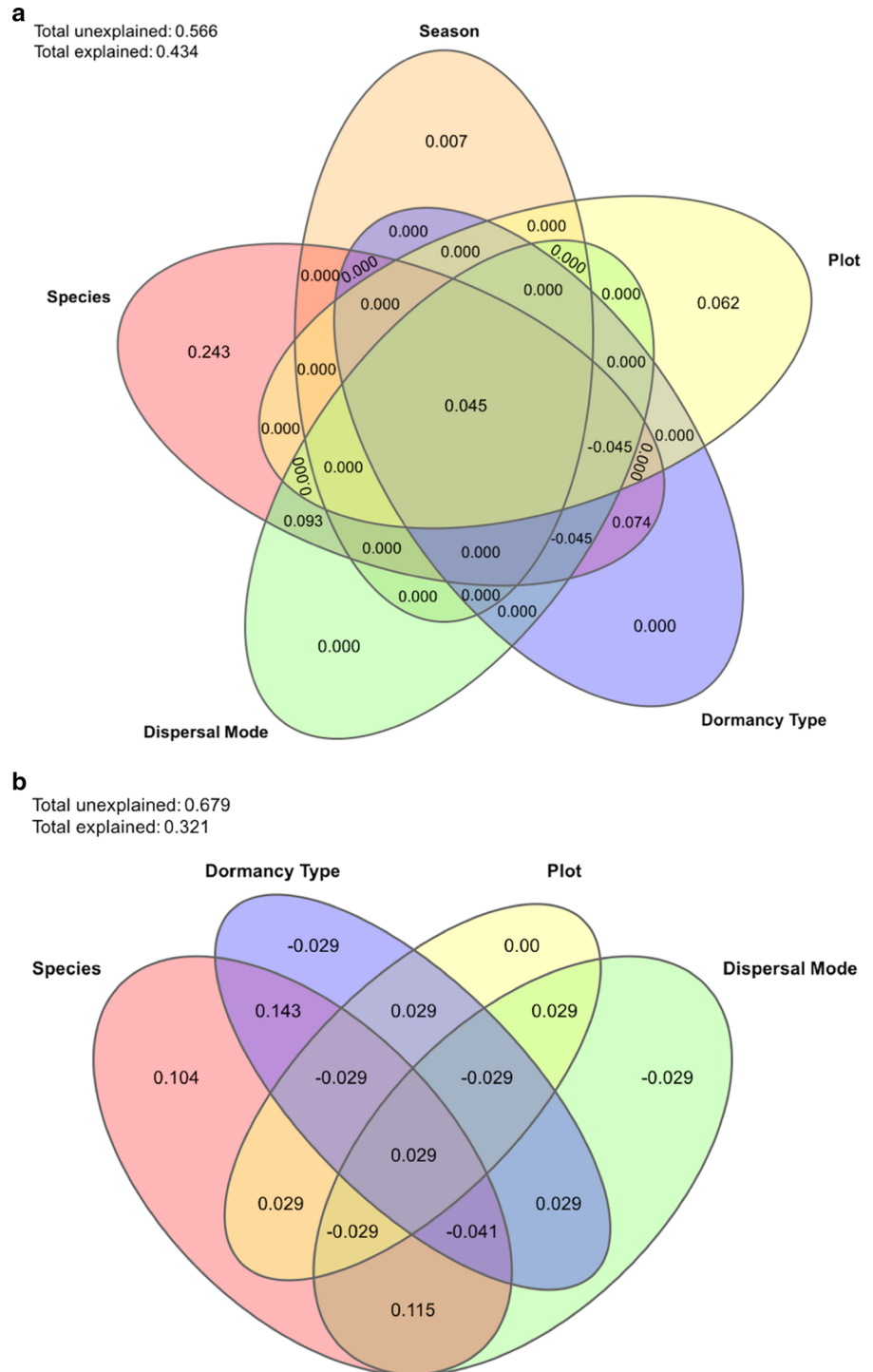
Persistence

Seed persistence in the soil seed bank after being buried within 2 cm of topsoil for 18 months was not correlated with either above-ground removal (Pearson's $r = 0.27$, $p = 0.39$) or below-ground removal (Pearson's $r = 0.57$, $p = 0.24$).

Discussion

In this study, we examined the secondary removal rates of seeds of 12 common Neotropical pioneer trees in the dry and wet seasons from the soil surface at five locations on Barro Colorado Island in the Republic of Panama. We found no effect of seasonality on seed removal for the above-ground experiment. Consistent with both our prediction and previous results (Fornara and Dalling 2005a), seed dispersal mode influenced the removal rates. However, seeds adapted for primary dispersal by animal vectors did not on average have higher removal rates than seeds adapted for wind dispersal when species identity was included as a random effect in our model. A similar effect to dispersal mode was seen with dormancy type. Species identity accounted for a majority of the variance in removal rates captured by the models. With additional species sampling, it is possible that the amount of variation attributed to species identity would be minimized and generalizations based on seed characteristics would emerge.

In addition to above-ground removal rates, we investigated the removal rates of six species buried 2 cm beneath the soil surface. Clear seasonal changes in density of viable seeds present in the soil have been

a Total unexplained: 0.566
Total explained: 0.434

b Total unexplained: 0.679
Total explained: 0.321

documented (Fornara and Dalling 2005b), particularly for seeds in upper 3 cm of soil (Dalling et al. 1997). These seasonal dynamics occur beneath closed canopy forest between fruiting seasons and can therefore be

attributed to either seed movement or seed mortality. While we did not measure below-ground seed removal in the dry season, our wet season results indicate that seeds remain highly mobile below-ground even over
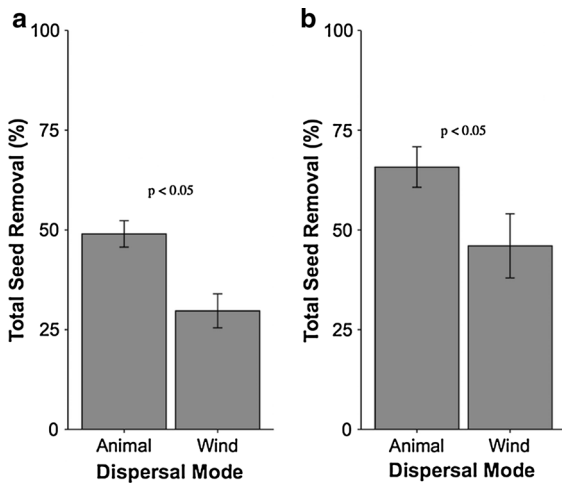
Fig. 3 Average seed removal by dispersal mode for above-ground (**a**) and below-ground (**b**) experiments. *Error bars* correspond to SE
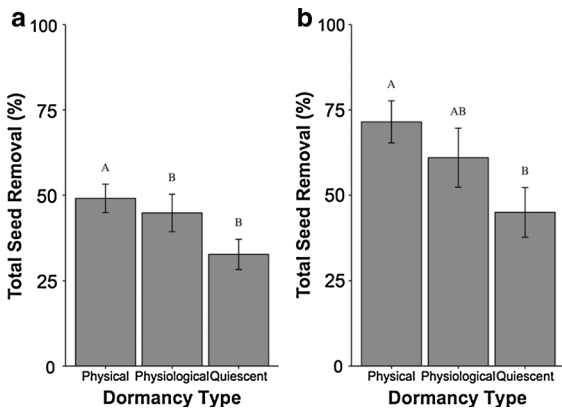
Fig. 4 Average seed removal by dormancy type for above-ground (**a**) and below-ground (**b**) experiments. *Error bars* correspond to SE. *Letters* denote significant differences as determined by Tukey's HSD means separation tests from models without species identity as a random effect

short periods (4 weeks), perhaps providing a mechanism for seasonal changes in seed density. It is unclear whether this mobility extends to deeper soil layers. For seeds larger than those used in this study (5–17.5 mm), Estrada and Coates-Estrada ([1991](#)) found that rodents were able to detect and eat an average of 92% of seeds located in the one to two and a half centimeter depth range, though their ability to detect seeds rapidly decreases with increasing depth. Previous work, however, has shown that surface removal of seeds of some species used in this study can be attributed to invertebrates rather than vertebrates (Fornara and

Dalling [2005a](#)). We also found no support for our hypothesis that long-term persistence in the seed bank is contingent on avoiding seed predation as seed removal rates were uncorrelated with seed persistence capacity of 18 months.

We found that when variation associated with species identity was not accounted for, our results matched our predictions as species adapted for primary dispersal by animals had a higher average removal rate than species adapted for primary dispersal by wind both when seeds were placed on the soil surface and buried in the soil (see also Fornara and Dalling [2005a](#)). This dispersal mode effect persists even though animal-dispersed seeds were not presented in dung, excluding possible secondary dispersal by dung beetles (Andreson and Levey [2004](#)). However, it is possible that adaptations to attract animals during the first dispersal event may also be acting at the secondary dispersal level. For example, even though seeds were cleaned of fruit pulp, small pieces of pulp may have remained adhered to the seed. Seed movement due to wind gusts, rain, or falling debris was kept to a minimum through the use of seed shelters leaving mainly animals as the vector for seed removal in both seasons. As we found no effect of seasonality on above-ground seed removal, secondary dispersal activity may be relatively uninfluenced by season or, alternatively, the identity of dispersers may have changed between seasons while net removal rates remained similar. However, comparison of models with and without species identity demonstrates that dispersal mode is not as important an indicator of secondary removal potential as species identity, though this result is only significant for the above-ground experiment. This result is further supported by the variance partitioning where species identity accounts for most of the variance in seed removal both in the above- and below-ground experiments.

Similar to the results of dispersal mode, there is an effect of dormancy type on seed removal when not including species. However, our hypothesis that physically dormant seeds would have the lowest removal rates based on the decreased diffusion of olfactory cues to seed predators (Paulsen et al. [2013](#)) was not supported. Instead, physically dormant seeds were removed in the greatest numbers both in above- and below-ground experiments, with physiologically dormant and quiescent seeds having the lowest removal rates for the above-ground and quiescent

seeds for the below-ground experiments. However, species identity again masked this effect indicating that the predictive power of functional and life-history traits is weak relative to species-specific characteristics, such as seed chemical and physical traits. Seed polyphenols, important for seed defense, have been shown to be ubiquitous though variable in concentration among tropical tree species, correlating negatively with seed size and seed physical defenses (Gripenberg et al. 2017, but see Tiansawat et al. 2014). While pioneer species tend to have fewer of these defensive compounds (Gripenberg et al. 2017), it is possible that there is variation among pioneer species that could relate to differences in species-specific removal rates by deterring removal agents, though future work will need to be done to say this conclusively. Alternatively, seeds may contain attractive chemicals that act as behavioral releasers to elicit seed removal responses in organisms (e.g., 1,2-diolein acts as a behavioral releaser in elaiosome-bearing systems by eliciting removal responses in ants, Marshall et al. 1979). However, future studies will need to investigate the chemistry of these species to know how chemistry influences secondary removal.

Species identity did not always influence removal the same way above- and below-ground, as the rank order of removal rates was different between above- and below-ground experiments. For the above-ground experiment, *Z. ekmanii* experienced the greatest amount of removal (85.25 ± 5.4%), while *T. micrantha* black seed morph experienced the lowest (16.5 ± 6.1%). In contrast, *T. micrantha* black seed morph experienced the highest average total below-ground seed removal (81 ± 9.2%). This suggests that different seed removal agents and seed traits affect removal rates above- and below-ground. For above-ground removal rates, we found more removal for *Apeiba membranacea* Aubl. and *Luehea seemannii* Triana and Planch than previously recorded on BCI for both the same time frame and approximately time of year (75.5 ± 6.4 and 17 ± 6.5% this study compared to 35 ± 3 and 5 ± 8% for *A. membranacea* and *L. seemannii*, respectively, in May–June 2001, Fornara and Dalling 2005a). It is possible that more pulp remained on *A. membranacea* seeds post cleaning, making seeds more attractive for removal, while not removing the wings on the *L. seemannii* seeds may have led to gusts of air removing them from the petri dishes, though this is unlikely. Additionally, studies investigating other

Cecropia species have found variable rates of seed removal at the genus level (*C. longipes* 47.0 ± 9.7%, this study; *Cecropia peltata* L. 17 ± 3%, Fornara and Dalling 2005a; *Cecropia obtusifolia* Bertol. and *O. pyramidale* together 40% of seeds removed in 48 h, Garcia-Orth and Martinez-Ramos 2008).

Ants (Formicidae) were the only taxa observed removing seeds during our observation periods. Out of 158 daytime (between 10:00 and 16:00) intervals that had a recorded change in hourly seed count, ants were present at 47.5% (75/158) of these daytime seed count changes and were observed removing seeds 38% (60/158) of the time (S.A. Ruzi, unpubl. data). Both *Z. ekmanii* and *O. pyramidale* stand out for their high overall removal rates (85.25 ± 5.4 and 66.9 ± 8.0%, respectively). *Zanthoxylum ekmanii* seeds are dispersed from capsules and do not contain any pulp or other conspicuous agent that could attract ants, yet they had the highest removal rate in the above-ground experiment. Preliminary data from ongoing projects suggest that there are chemicals on the seed coat of *Z. ekmanii* seeds that elicit the seed removal response for one of the common ants *Ectatomma ruidum* (Roger) observed removing these seeds (S.A. Ruzi, unpubl. data). *Ectatomma ruidum* have also been observed moving *Z. ekmanii* seeds into their colony, though whether the seeds are ingested, cached, or later removed remains unclear (S.A. Ruzi, unpubl. data). *Ochroma pyramidale* has a swollen spongy area in the funicle region whose function is unclear (Vazquez-Yanes and Perez-Garcia 1976). Ants in the genus *Trachymyrmex* were observed to approach *O. pyramidale* seeds, remove and carry away the funicle leaving the rest of the seed in the petri dish, though they did occasionally remove the entire seed (S.A. Ruzi, pers. obs.). Other ant genera (*Solenopsis* and *Pheidole*) have also been recorded as removing *Ochroma* seeds (Garcia-Orth and Martinez-Ramos 2008), though removal of the funicle was not mentioned. It is possible that other seed species traits are driving this pattern, such as seed-specific chemical profiles that ants cue in on to remove seeds.

Although ants were the only taxa observed removing seeds from the soil surface, it is difficult to tell whether ants were the taxa responsible for the removal of seeds within the topsoil. The common ants observed removing seeds are known to forage at the soil surface (*E. ruidum*, Franz and Wcislo 2003; *Trachymyrmex*, Leal and Oliveira 2000). It is likely that the ant

community below-ground consists of different species than those that forage at the soil surface. For example, soil probes captured a significantly different assembly of ants than sampling methods traditionally used to sample ground, leaf litter, and arboreal ants in Amazonian Ecuador (Wilkie et al. 2007). This difference in ant community could account for the difference in the observed rank removal rates between above- and below-ground experiments if different ant species have preferences for different seed species; however, further investigation will be needed to determine if this is the case.

While species identity accounts for most of the variance in seed removal both above- and below-ground (0.243 and 0.104, respectively) in the current models, there is still a large amount of variance unaccounted for in both the above-ground (0.566) and below-ground (0.679) experiments. Other factors that potentially influence seed removal rates, but that were not explicitly studied, include the incorporation of seeds into the soil by rain, and habitat variables that alter foraging patterns of the removal agents. While direct removal of seeds by rainfall from the above-ground experiment was controlled for using seed shelters, rainfall could have affected the below-ground experiment, though the amount of vertical movement will also depend on the soil structure, material, and pore size (Chamber and MacMahon 1994). Habitats with higher moisture levels tend to have higher foraging activity as it reduces the risk of desiccation (Kaspari and Weiser 2000). Litter cover also alters the size of the ants most likely to forage (smaller ants forage below the leaf litter, Kaspari and Weiser 2000), potentially affecting ant species interactions that could influence dispersal (Horvitz and Schemske 1986). The presence or abundance of other seed or plant species could also have indirect effects on focal species by attracting seed removal vectors (apparent competition, Holt and Kotler 1987; Garb et al. 2000; Veech 2000) or alternatively reducing seed removal rates if vectors preferentially remove other seed species. In an attempt to reduce these confounding effects, the experiment locations were selected to be >20 m away from adult conspecifics of the seed species. For the below-ground experiment, however, seeds of conspecifics were buried in mesh bags within the plots sampled (see Zalamea et al. 2015) and could have attracted density-dependent removal agents increasing our observed below-ground removal rates.

Conclusions

Overall, our results suggest that seeds show a striking amount of variation in removal rates both above- and below-ground. Our results indicate that seed removal rates are primarily associated with traits that vary at the species level. These may include characteristics such as chemical cues or physical seed structures that attract ants. We found that the incorporation of seeds into the topsoil did not lead to the loss of seed mobility. Future studies should determine whether the high mobility in the topsoil can be generalized to all Neotropical pioneer species, whether mobility remains high over longer periods of time, and how this mobility is correlated with seed fate to ultimately understand how this influences the recruitment patterns of Neotropical pioneer species.

Compliance with ethical standards

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