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Recruitment limitation of dominant tree species with varying seed masses in a subtropical evergreen broad-leaved forest

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Abstract: Recruitment limitation has been hypothesized to promote the maintenance of high species diversity in forests by slowing down competitive exclusion. However, the difference of recruitment limitation for tree species with varying seed masses, which is a common phenomenon in tropical or subtropical forests, is largely unknown. In this study we conducted a seed sowing experiment for five dominant tree species with varying seed mass (a proxy of dispersal ability) in a subtropical evergreen broad-leaved forest at different successional stages to test the hypothesis that the determinants of species recruitment vary with their seed masses in Heishiding Nature Reserve (Guangdong Province, China). The effects of seed predators, soil pathogens, light conditions, plant litter, seed additions, and the presence of adult conspecific trees on the performance of seeds and seedlings for the five species were examined. We particularly investigated the effects of habitat hazards and seed size on the relative importance of dispersal limitation and establishment limitation. The results show that all five sowing species experienced recruitment limitation at the microsite level, although the causes of the limitation of these species varied between pathogen infection, animal predation, litter covering and shading. Seedling recruitment of the wind-dispersed, small-seeded species was mostly limited by microsite condition, while large-seeded species were mostly limited by dispersal ability.

Nomenclature: Anon. (1974-1999).

Introduction

Recruitment limitation has been documented to promote the maintenance of high species diversity in forests by slowing down competitive exclusion (Hubbell et al. 1999, Makana and Thomas 2004). Supportive evidence has showed that resource competition among seedlings may be irrelevant to their recruitment within the low density of tree seedlings in Neotropical forests (Paine et al. 2008).

The tolerance-fecundity tradeoff enables stable coexistence of large numbers of species in heterogeneous habitats (Muller-Landau 2008, 2010). The recruitments of many species are sustained to a certain degree by seedling banks (Cuevas and Arroyo 1999). The failure for seeds to germinate is normally caused by seed predators, light condition, litter or soil moisture (LePage et al. 2000, Pages et al. 2003, Scarpa and Valio 2008). While seed size is indicative of dispersal ability, it is not a direct measure of colonization ability (Eriksson and Jakobsson 1998), for example, it has been observed that seedling establishment of shade-tolerant species in tropical rain forests is independent of seed size (Svenning and Wright 2005). Another problem is that how seeds of long-lived trees compete and what they compete for are yet unclear. Although large-seeded species can have an advantage in germination and seedling survival, it may not be because they are more competitive but because large seeds have a stronger reserve effect (Westoby et al. 1996), even it is similar with small seeds in other ways. An alternative argument is that the advantage of large seeds is not due to their competitiveness but their ability to tolerate environmental hazards (e.g., shade and drought) during the phase of seed germination to the initial stage of seedling.

Dispersal and establishment are very different processes and are influenced by different species and environmental attributes. Dispersal limitation describes the failure for seeds (propagules) of a given species to reach sites suitable for their germination and growth, while establishment limitation refers to the inability of the seeds of a given species, once they have reached a site, to establish. Field observations and seed sowing experiments have shown that host-specific herbivores (e.g., soil pathogens), light conditions, litter and soil moisture are the primary agents affecting seed survival and seedling establishment (Janzen 1970, Connell 1971, Augspurger 1984, Clark and Clark 1984, Schupp 1992, Packer and Clay 2000, Dalling and Hubbell 2002, Klironomos 2002, Pages et al. 2003, McEuen and Curran 2004, Bell et al. 2006, Dupuy and Chazdon 2008, Petermann et al. 2008). If seed addition results in establishment at unoccupied sites, a corollary is that the area contains unoccupied suitable sites but there is seed dispersal limitation. If seed addition

Table 1. Characteristics of the sowing sites.

Site	Aspect	Slope	Elevation (m)	Soil moisture (%)	Depth of litter(cm)	Transparency (%)
Site I	northwest	18°	230	20.34±0.54	5.3±0.48	14.96±0.38
Site II	northwest	22°	280	13.26±0.36	4.2±0.36	16.57±0.51
Site III	north	25°	350	21.46±0.80	3.8±0.41	11.63±0.46

Table 2. Species that were used in the seed sowing experiment in this study, along with their respective seed sizes. *Notes*: ^{S} only seedlings were observed; ^{Sa} only saplings were observed.

Species	Family	Shade tolerance	Thousand seed weight (g)	Dispersal mode	Which experiment sites they have naturally grown?
Schima superba	Theaceae	Intolerant	7.60±0.54	Wind	Site I, II
Engelhardia fenzelii	Juglandaceae	Tolerant	12.22±0.86	Wind	Site II, III
Castanopsis carlesii	Fagaceae	Tolerant	493.18±23.48	Gravity/animal	Site III
Castanopsis fissa	Fagaceae	Intolerant	931.93±98.26	Gravity/animal	Site II^{S}, III^{S}
Cryptocarya concinna	Lauraceae	Tolerant	640.14±47.04	Gravity	Site I^{Sa}, II^{Sa}, III

does not increase seedling establishment, establishment limitation is at work. Of the sowing experiments reported in the literature, most are concerned with dispersal limitation in grasslands with small-seeded species (Turnbull et al. 2000, Rees et al. 2001, Moles and Westoby 2002). Few studies investigate establishment limitation of tree species. For those that do experiment with tree seed/seedling establishment, they either focus exclusively on testing the Janzen-Connell hypothesis (Augspurger 1984, Clark and Clark 1984, Schupp 1992, Packer and Clay 2000, Bell et al. 2006) or do not test the effect of habitat hazards on establishment (Pages et al. 2003, McEuen and Curran 2004, Dupuy and Chazdon 2008), or they find no relationship between seed size and seedling establishment (Svenning and Wright 2005). Clearly, the coexistence of species with varied seed size is still poorly understood.

The objective of this study is to test the hypothesis that the determinants of species recruitment in a subtropical forest vary with their seed masses, by conducting a seed sowing experiment for five dominant tree species with a wide range of variation (> 120 times difference) in seed size in a subtropical evergreen broad-leaved forest in China. We tested and separated the effects of soil pathogens, seed predation, light conditions and plant litter on seedling establishment. Our results revealed that recruitment limitation play a major role in maintaining tree diversity in species-rich forest communities.

Materials and methods

Study sites and selection of species

The study sites were located in Heishiding Nature Reserve (23°27'N, 111°53'E, 150-927 m altitude), South China. The reserve is right on the Tropic of Cancer and has a southern subtropical monsoon climate with a mean annual temperature of 19.6°C and an average annual rainfall of 1743.8 mm. The vegetation is a typical subtropical evergreen broad-leaved forest (Liu et al. 2012a,b).

Three circular sites (with size ~35 m in radius for each) were established along a successional gradient from young (site I), intermediate (site II) to old growth (site III) forests. The three sites were similar in elevation, aspect, slope and soil conditions (Table 1). The sites were selected so that each would have a newly formed gap with gap size varying between 200 and 300 m^2. The composition of the dominant species varied from the young to the old-growth site; e.g., the pioneer pine species *Pinus massoniana* dominates the young stand, *P. massoniana* and *Schima superba* dominate the middle-aged site, while shade-tolerant species (*Engelhardia fenzelii*, *Ixonanthes chinensis*, *Ormosia glaberrima* and *Cryptocarya concinna*) dominate the old growth site. Five tree species varying in seed mass and shade tolerance (Table 2) were selected for sowing experiments. Of the five species, *S. superba* and *Castanopsis fissa* are the dominant pioneering species, and the other three species are the dominant late successional species. Note that although *C. fissa* and *Castanopsis carlesii* in Table 2 do not dominate our study sites (importance value <50%), they are dominant species in the region. *S. superba* and *E. fenzelii* produce mature seeds in October, and their seeds both have morphologies suitable for wind dispersal. Both *C. carlesii* and *C. fissa* produce starchy seeds in November, and their seeds suffer from a high mortality resulting from seed attacks by seed-borers and predation by animals, particularly rodents. The seeds of *C. concinna* have a long maturation period from October to December.

From October to November 2007, the seeds of four species (*S. superba*, *E. fenzelii*, *C. fissa* and *C. concinna*) used in this experiment were collected from the nearby Dinghushan National Nature Reserve (112°34'¬23°10', 120-1008 m altitude), while the seeds of *C. carlesii* were collected from the Heishiding Natural Reserve. They were collected from one adult individual of each species, which grow in the overstory.

The lower subtropical evergreen broad-leaved forest, with similar dominant tree species, distribute in both nature reserves.

Field experiment and data analysis

We conducted a factorial experiment with treatments including light condition (gap vs. shaded understory), plant litter (litter removal vs. intact litter), seed predation (enclosure protection vs. control), pathogens (sterilization vs. control) and seed addition (seeds added vs. control). We set up 32 1.5 m × 1.5 m quadrats in each site (i.e., each successional stage), with 16 quadrats located in the central area of a gap and 16 located in a nearby forest. The quadrats were widely spaced (>1m) to avoid fungicide contamination. Within each quadrat, nine 0.4 m × 0.4 m subquadrats separated by a 0.1 m buffer were further established.

Half of the 32 quadrats in each site were selected for seed sowing (eight from gap plots and eight from forest plots). In each of these 16 quadrats, five of the nine 0.4 m × 0.4 m subquadrats were randomly chosen for seed sowing, with the restriction of assigning one species to one subquadrat only. The seeds of each species were sown evenly within each subquadrat and only the seeds that were collected within one week before sowing were used. The sowing density for *S. superba* and *E. fenzelii* was 60 seeds/subquadrat, and 50 for *C. concinna*, *C. fissa* and *C. carlesii*, according to the natural densities observed in the field in 2007, which was a seed mast year.

The vegetation dispersed in the gap quadrats consisted of mainly the lianas and ferns, and grasses and large seedlings were rare. The shrubs and herbs in the gap quadrats and forest quadrats were carefully removed to minimize disturbance on the litter layer, while the seedlings of tree species with height less than 50 cm were retained. The regrowth of shrubs and herbs was quite limited due to the lack of propagules. For the quadrats with litter removal treatment, litter was removed every month until the end of the experiment. For the quadrats with enclosure protection, we used PVC tubes and wire-mesh (1 mm^2) to build a mesh enclosure with a height of 80 cm to protect the seeds in the quadrats from animal predation.

Half of the 32 quadrats (eight from gap plots and eight from forest plots, all randomly chosen) were sterilized monthly with the fungicide Captan WP (50% Captan by weight) which is a broad-spectrum fungicide. The fungicide was applied using a hand-held mister at the recommended concentration of 0.4% (Zeng et al. 2004) in 5L of solution (Captab and water). To make it comparable with the sterilized quadrats, the control quadrats (without sterilization) were also treated but with the same amount of water (5L). Note that although the fungicide is effective in killing pathogens, it can also kill other fungi, e.g., mycorrhizal fungi. No fungicide (or pathogencide) is available that exclusively kills a specific group of pathogens. With that caveat, we refer to the fungicide treatment as "pathogen" (sterilization vs control) in this study.

At the time of sowing, two seed bags (each with 30 seeds) for each species were placed in the 16 control quadrats (i.e., the quadrats without seeds addition, eight in gaps and eight in the nearby forest) in each site for testing seed vitality on the soil surface. The seed bags (size=15 cm × 15 cm) were made of wire-mesh (1 mm^2). The vitality of the seeds was determined using TTC (2,3,5-Triphenyl Tetrazolium Chloride) before being sown in October and November 2007. Their vitality was tested again in August and December 2008 (i.e., after being placed in the field for 9-14 months). We followed the method of Roth and Vander Wall (2005) for testing seed vitality.

Seed germination was surveyed monthly after sowing, from October 2007 to December 2008. The number of seedlings and their height were recorded, and the dead seedlings were counted and removed. We also recorded the number and the species of naturally dispersed seeds in each quadrat.

Because our interest was to investigate the effects of seed size, seed predation, pathogens, light condition, plant litter, and the number of conspecific adult trees on the rates of seedling establishment (proportion of seeds germinated to seedlings), a logistic regression model was employed. To account for the variation within species and sites, a mixed effect logistic regression model with species and sites being considered as random effects was used. In this,

$Y_{ijl} \sim \text{Binomial}\ (1, p_{ijl})$: describes the number of seedlings not surviving to the end of the experiment;

$$\text{logit}\,(p_{ijl}) = \log\,(p_{ijl}/(1-p_{ijl})) = \beta_0 + [\beta_1\text{seedsize} + \beta_2\text{pathogen} + \beta_3\text{light} + \beta_4\text{seedsize} \times \text{pathogen} + \beta_5\text{seedsize} \times \text{light} + \beta_6\text{pathogen} \times \text{light} + \beta_7\text{seedsize} \times \text{pathogen} \times \text{light}]_{\text{fixed.part}} + [\gamma_1 \times \text{species}_i + \gamma_2 \times \text{site}_j]_{\text{random.part}}$$

Random effects: $\text{species}_i \sim N(0,\sigma_1^2)$, $\text{site}_j \sim N(0,\sigma_2^2)$,

where i denotes species, j denotes site, and l denotes individual seedlings. This mixed model considers both the effects of individual covariates and their interactive terms. The model was estimated in R version 2.1.0 (http://www.r-project.org/) using the function "lmer" of the "lme4" package (Bates 2010).

The above model was also applied to analyze the effects of pathogens, predation, light etc. on seedling establishment for each species. For the analysis of individual species, seed size had to be dropped off because each species has only one (averaged) seed size. Also, for the ease of comparison, we did not include interactive terms for the individual species analysis because the interactions could not be estimated for some of the species due to too few seedling survivals (e.g., *E. fenzelii*) or lack of conspecific adult trees (e.g., no *C. fissa* adult trees were found in any of the study sites.)

The nonparametric Mann-Whitney test was used to compare the difference in seedling establishment between the different contrasts of treatments and seedling survival between sites.

Results

Seed size and colonization limitation

The analysis of seed vitality of the seed bags on the soil surface showed that none of the un-germinated seeds of the five sowing species survived to December 2008 (13-14 months after sown; Table 3). Clearly, the small-seeded species (*S. superba* and *E. fenzelii*) have much lower survivorship. We also observed that no new individuals of the five species germinated in the sown quadrats after December 2008. This excludes the possibility that the sown seeds in the experiment might have formed seed banks to confound the results of our sowing experiment.

In the sown quadrats, the seed germination rates of the two small-seeded species were relatively low (*S. superba* 5.49±1.22%, *E. fenzelii* 1.04±0.21%) and most seedlings died (90% for *E. fenzelii*, 72.78% for *S. superba*) before October 2008 (i.e., 12 months after seed sowing). Seed addition had no significant effect on the seedling establishment of these two small-seeded species across all sites, with or without their adults (Fig. 1a). This suggests that colonization of *S. superba* and *E. fenzelii* was driven mostly by establishment limitation through microsite filtering. In contrast, seed addition had a highly significant effect on seedling establishment for the three large-seeded species (*C. carlesii, C. fissa* and *C. concinna*), except for *C. carlesii* in site III which had a weak effect (P=0.064) (Fig. 1a). This result suggests that colonization of the three large-seeded species was constrained mostly by dispersal limitation. In contrast, without seed addition, all species except *C. concinna* had a poor establishment (Fig. 1b). The poor establishment under the natural condition (Fig. 1b) can result from either microsite filtering or dispersal limitation or both. These two mechanisms can be separated by a seed addition experiment as shown in Fig. 1a., or we can track the natural seed rain to answer the question.

The small- and large-seeded species are not only different in their colonization mode (establishment limitation versus dispersal limitation), but are also significantly different in survivorship (Table 3 and Fig. 1). This result is further confirmed by the analysis of the generalized linear mixed effect model for "all species" which shows that the coefficient (β) for seed size is significantly smaller than 0, indicating that smaller-seeded species have a higher seedling mortality, or lower seedling establishment rate (Table 4).

Effect of site conditions on seedling establishment

Many habitat hazards can affect the dispersal-establishment tradeoff observed in Fig. 1a and Table 4. As revealed by Table 4, the small-seeded and large-seeded species are different in the way by which they are affected by site factors. Fungicide treatment did not alter the seedling establishment for the two wind-dispersed, small-seeded species (*S. superba* and *E. fenzelii*). Seed predators and light are the two major factors limiting the establishment of *S. superba*: enclosure and gap opening both have negative effects on mortality, i.e., positive effect on its seedling establishment (Table 4). No factors were found to have significant effects on the seedling establishment of *E. fenzelii* due to its very low seedling establishment (only 34 seedlings germinated during the experiment period, and 7 seedlings survived to the end of the experiment).

Table 3. Vitality performance of seeds placed on the soil surface. Vitality is measured by the percentage ± standard errors of seeds surviving to a time. 480 seeds of each species were tested.

Species	Seed vitality (%)		
	Before sowing (Oct. and Nov. 2007)	Aug. 2008	Dec. 2008
Engelhardia fenzelii	53.30±2.79	0	0
Schima superba	43.30±2.11	0	0
Castanopsis fissa	76.70±3.65	1.26±0.74	0
Castanopsis carlesii	90.00±2.58	1.47±0.64	0
Cryptocarya concinna	80.00±3.16	11.48±2.84	0

The dispersal of the three large-seeded species was commonly limited by seed predators (Table 4), with pathogens

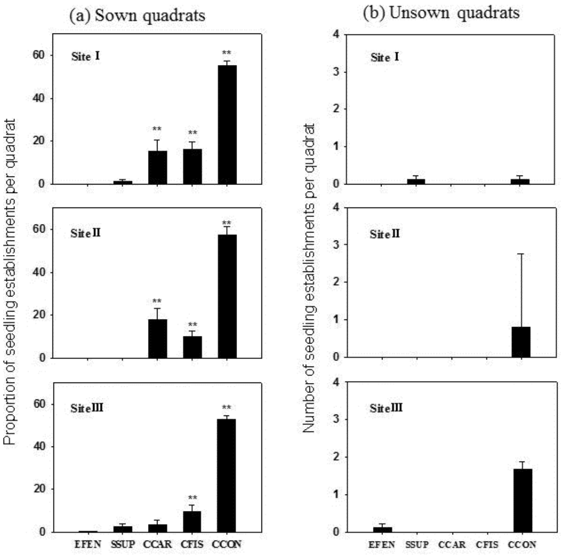

Figure 1. Seedling establishment per quadrat for the five sowing species at the end of experiment. (**a**) is for sown quadrats and (**b**) is for unsown quadrats. Because the number of seeds in each unsown quadrat is unknown, the proportion of seedling establishment could not be estimated and thus the number of seedlings is presented here; while for the sown quadrats the proportion of seedling establishments is presented to account for the difference in sowing densities (50 and 60 seeds per quadrats). The Mann-Whitney test compares the difference in the number of seedling establishments between seed addition and control. * $P \leq 0.05$; ** $P \leq 0.01$. Species codes are: CCON: *C. concinna*, CFIS: *C. fissa*, CCAR: *C. carlesii*, SSUP: *S. superba,* and EFEN: *E. fenzelii*.

Table 4. Mixed effect logistic regression model testing for the effects of factors on seedling establishment. β is the coefficient of logistic regression. “All species” is the mixed effect model including all five species across all sites by which species and sites are considered as random effects. This “all species” analysis allows a test for the effect of seed size on seedling establishment. The mixed effect model for each individual species can only consider site as a random effect because each species is assumed to have one seed size. –: $p<0.1$, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Note that to model seedling mortality, a dead seedling was coded as 1 while a live seedling was coded as 0. The treatments (pathogen, predation, light and litter) were coded as two-level factors (e.g., sterilization vs control for pathogen), with the first level (e.g., sterilization) being $x=1$ and second level (e.g., control) $x=0$. Therefore, the negative coefficient β_{pathogen} means “sterilization” treatment led to a lower mortality (higher survival of seedlings). “—” indicates data were not available for estimating the effect.

Variables (β)	All species	*Schima superba*	*Engelhardia fenzelii*	*Castanopsis fissa*	*Castanopsis carlesii*	*Cryptocarya concinna*
Intercept	6.763***	7.127***	7.391***	3.980***	5.489***	0.0340
Seed size	-0.00583**	--	--	--	--	--
Pathogen (sterilization vs control)	-0.332**	0.150	-0.695	-0.657***	-0.334*	-0.0138
Predation (enclosure protection vs control)	-2.400***	-2.659***	-0.695	-1.889***	-4.145***	-0.397***
Light (gap vs understory)	-0.559*	-1.539***	17.357	0.124	0.233	0.193*
Litter (removal vs intact)	-0.208	-0.884	-0.695	-0.693***	-0.993***	-0.384***
Adult tree	0.279	2.272	--	--	1.892***	0.155•
Seed size×Predation	0.00141***					
Seed size×Light	0.000785*					
Seed size×Adult tree	-0.00223***					
Pathogen×Adult tree	0.414**					
Predation×Adult tree	1.011***					
Litter×Adult tree	0.742***					
Pathogen×Light	0.410***					
Pathogen×Litter	-0.332**					
Predation×Light	0.373**					
Predator×Litter	-0.286*					
Light×Litter	-0.456***					

having only a relatively minor effect (except for *C. fissa*). For all three species, litter removal caused seedling establishment to increase significantly. Light has only a weak limiting effect on *C. concinna*. It is worth mentioning (although results are not shown) that the effect of each factor on seedling establishment between sown and control quadrats can be different along the three sites (successional stage; see Table 2). For example, the effect of predation on *C. concinna* is highly significant in Site I but not in Site III. This means that the abiotic and biotic effects on establishment can vary across sites contingent on specific site conditions.

The coefficient of the number of conspecific adult trees in each site ($\beta_{\text{adulttree}}$) is always positive, although the degree of significance varies from species to species (Table 4). This means the presence of adult trees has a tendency to increase seedling mortality (or reduce seedling survival), although the effect is only significant in two large-seeded species (*C. carlesii* and *C. concinna*; Table 4). In comparing the difference in seedling establishment for *S. superba, E. fenzelii* and *C. concinna* across sites, the Mann-Whitney test did not reveal a difference between the sites with and without their adults ($P>0.05$). In contrast, the seedling establishment rate of *C. carlesii* in site III with its adults was significantly less than that in site I and site II without its adults (Mann-Whitney test; site I vs site III: $P=0.018$; site II vs site III: $P=0.0029$; but site I vs site II: $P=0.344$).

Discussion

Different species ecological functional traits are considered pivotal to the maintenance of species assemblages (Kneitel and Chase 2004, Muller-Landau 2008). Seed size is a key trait underlying many forms of tradeoffs in plant communities, e.g., the competition-colonization tradeoff (Rees et al. 2001), the seed size-seed number tradeoff (Westoby et al. 1996), and the tolerance-fecundity tradeoff (Muller-Landau 2010). In this study, we examined the effects of seed predators, soil pathogens, light conditions, plant litter, seed additions, and the presence of adult conspecific trees on the performance of seeds and seedlings for the five tree species with varying seed masses. Our results showed that colonization of small-seeded species was severely affected by microsite limitation, while colonization of large seeded species was more strongly affected by dispersal limitation (Fig. 1 and Table 4). The reason for the poor germination is unknown, although it could be that pathogen infection made the seeds unviable. With such low germination rates, no statistical analysis was able to infer the effects of the treatments.

We showed that seed predation, pathogen infection, light conditions and plant litter were among the key factors that limit microsite establishment of seedlings. However, the effects of these factors on species of different seed size were different. For example, animal predation and plant litter were the two most significant factors that limited seedling establishment for all of the species except *E. fenzelii,* which had too few germinated seedlings for a reliable analysis (Table 4), while light was the least important among all the factors; it only had a significantly positive effect on seedling establishment of *S. superba* (Table 4). The result that litter removal caused seedling establishment to increase was consistent with the observation of Dalling and Hubbell (2002).

Our study showed that pathogens only significantly affected the dominance of two of the five species (*C. fissa* and *C. carlesii*) (Table 4). In the case of *C. carlesii*, the seedling establishment rate in sites with its adults was significantly less than that in sites without its adults (the Mann-Whitney test shown in the Results). The positive $\beta_{\text{adulttree}}$ across all species in Table 4 suggest a clear tendency that the presence of conspecific adult trees elevates seedling mortality, although the effect is not always significant (but note the significant effect of the interactive term between pathogen and adult tree in the case of "all species"). All these indicate that the Janzen-Connell hypothesis plays a presumable role in regulating the dominance of the five species studied.

In the study of tree diversity, particularly in tropical forests, much effort has been devoted to understanding the density-dependent regulation by host-specific soil pathogens (Augspurger 1984, Augspurger and Kelly 1984, Clark and Clark 1984, Packer and Clay 2000, Bell et al. 2006, Freckleton and Lewis 2006, Liu et al. 2012a), with much less attention paid to the effect of seed predation by animals, although the original Janzen-Connell hypothesis stressed the effect of all types of antagonistic organisms (Janzen 1970, Connell 1971). Among those that studied the effect of seed predation, the results were not consistent. Some did not find the distance- or density-dependent Janzen-Connell effect on seed survival (Hulme 1997, Alcántara et al. 2000, Blendinger and Díaz-Vélez 2010), while others showed that seed predation can produce a density-dependent, density-independent or even inverse density-dependent effect on seed survival, subject to the relative distance of seed dispersal to predation distance (Nathan and Casagrandi 2004). The lack of a consistent effect of seed predation on diversity is probably due to the fact that few seed predators are host-specific. Our study showed that animal predation was widespread in the studied forest and its adverse effect on seeds and seedling establishment could be stronger than the effect of soil pathogens.

But how does animal predation contribute to species coexistence? The answer is not clear because seed herbivores are unlikely to be host-specific, a prerequisite for the Janzen-Connell hypothesis. Thus, in order to invoke seed predation to explain species coexistence, the assumption of host-specificity must be relaxed because most seed predation is not host specific (Hulme 1997). We suggest that a positive relationship between seed size and predation be sufficient to regulate tree populations. By this relationship, large-seeded species are suppressed by high predation as to avoid competitive exclusion of small-seeded species. In our experiment, the dispersal limitation of the two large-seeded Fagaceae (*C. fissa* and *C. carlesii*) is mostly caused by seed predators (Table 4). Their large starchy seeds appeal to high animal predation, particularly by rodents. This high mortality can reduce the dominance of the species, thus promoting coexistence.

In summary, we have experimentally demonstrated that the determinants of species recruitment vary with their seed masses in the subtropical forests at different successional stages. The high mortality in the dispersal-establishment stage is not an outcome of competition but is caused by habitat hazards, including animal predation, pathogen infection and plant litter. All five sowing species in our experiment experienced strong seed limitation, with small-seeded species limited by microsite establishment and large-seeded species limited by dispersal. The separation of seed recruitment into dispersal process (seed size as a proxy) and establishment process (seedling survival) reveals that recruitment limitation can be used to explain tree species diversity in forest communities as long as there are agents (pathogens and seed predators) or habitat factors that check the population of superior competitors as to reduce their dominance. Competition is not the only force, and may not even be an important one, for regulating seed survival and seedlings establishment. Indeed, it is not clear what seeds and seedlings of long-lived trees compete for under natural conditions. The duration of experiments is rather short (typically up to 3 years) compared to the life cycle of tree species. The effects of abiotic and biotic agents on the survival of trees at different life stages can change over time.

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References

Alcántara, J.M., Rey, P.J., Sánchez-Lafuente, A.M. and Valera, F. 2000. Early effects of rodent post-dispersal seed predation on the outcome of the plant-seed disperser interaction. *Oikos* 88: 362-370.

Augspurger, C.K. 1984. Seedling survival of tropical tree species–interactions of dispersal distance, light-gaps, and pathogens. *Ecology* 65: 1705-1712.

Augspurger, C.K. and Kelly, C.K. 1984. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. *Oecologia* 61: 211-217.

Bates, D. 2010. *lme4: Mixed-effects modeling with R.* Springer, New York.

Bell, T., Freckleton, R.P. and Lewis, O.T. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecol. Lett.* 9: 569-574.

Blendinger, P.G. and 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.

Clark, D.A. and Clark, D.B. 1984. Spacing dynamics of a tropical rain forest tree: evaluation of the Janzen-Connell model. *Am. Nat.* 124: 769-788.

Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Boer, P.J.D. and Gradwell, G.R. (eds.), *Dynamics of Populations*. PUDOC, Wageningen, pp. 298-312.

Cuevas, J.G. and Arroyo, M.T.K. 1999. Absence of a persistent seed bank in *Nothofagus pumilio* (Fagaceae) in Tierra del Fuego, Chile. *Revista Chilena De Historia Natural* 72: 73-82.

Dalling, J.W. and Hubbell, S.P. 2002. Seed size, growth rate and gap microsite conditions as determinants of recruitment success for pioneer species. *J. Ecol.* 90: 557-568.

Dupuy, J.M. and Chazdon, R.L. 2008. Interacting effects of canopy gap, understory vegetation and leaf litter on tree seedling recruitment and composition in tropical secondary forests. *Forest Ecol. Manag.* 255: 3716-3725.

Eriksson, O. and Jakobsson, A. 1998. Abundance, distribution and life histories of grassland plants: a comparative study of 82 species. *J. Ecol.* 86: 922- 933.

Freckleton, R.P. and Lewis, O.T. 2006. Pathogens, density dependence and the coexistence of tropical trees. *Proc. R. Soc. Lond. B*. 273: 2909-2916.

Hubbell, S.P., Foster, R.B., O'Brien, S.T., Harms, K.E., Condit, R., Wechsler, B., Wright, S.J. and de Lao, S.L. 1999. Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. *Science* 283: 554-557.

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

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. *Am. Nat.* 104: 501-528.

Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67-70.

Kneitel, J.M. and Chase, J.M. 2004. Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecol. Lett.* 7: 69-80.

LePage, P.T., Canham, C.D., Coates, K.D. and Bartemucci, P. 2000. Seed abundance versus substrate limitation of seedling recruitment in northern temperate forests of British Columbia. *Can. J. Forest Res.* 30: 415-427.

Makana, J.R. and S.C. Thomas 2004. Dispersal limits natural recruitment of African mahoganies. *Oikos* 106: 67-72.

McEuen, A.B. and Curran, L.M. 2004. Seed dispersal and recruitment limitation across spatial scales in temperate forest fragments. *Ecology* 85: 507-518.

Moles, A.T. and Westoby, M. 2002. Seed addition experiments are more likely to increase recruitment in larger-seeded species. *Oikos* 99: 241-248.

Muller-Landau, H.C. 2008. Colonization-related trade-offs in tropical forests and their role in the maintenance of plant species diversity In: Carson, W.P. and Schnitzer, S.A. (eds.), *Tropical Forest Community Ecology*. Wiley-Blackwell, West Sussex, pp. 182-195.

Muller-Landau, H.C. 2010. The tolerance–fecundity trade-off and the maintenance of diversity in seed size. *Proc. Nat. Acad. Sci. USA* 107: 4242-4247.

Nathan, R. and Casagrandi, R. 2004. A simple mechanistic model of seed dispersal, predation and plant establishment: Janzen–Connell and beyond. *J. Ecol.* 92: 733-746.

Packer, A. and Clay, K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* 404: 278-281.

Pages, J.P., Pache, G., Joud, D., Magnan, N. and Michalet, R. 2003. Direct and indirect effects of shade on four forest tree seedlings in the French Alps. *Ecology* 84: 2741-2750.

Paine, C.E.T., Harms, K.E., Schnitzer, S.A. and Carson, W.P. 2008. Weak competition among tropical tree seedlings: Implications for species coexistence. *Biotropica* 40: 432-440.

Petermann, J.S., Fergus, A.J.F., Turnbull, L.A. and Schmid, B. 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89: 2399-2406.

Rees, M., Condit, R., Crawley, M., Pacala, S. and Tilman, D. 2001. Long-term studies of vegetation dynamics. *Science* 293: 650-655.

Roth, J.K. and Vander Wall, S.B. 2005. Primary and secondary seed dispersal of bush chinquapin (Fagaceae) by scatterhoarding rodents. *Ecology* 86: 2428-2439.

Scarpa, F.M. and Valio, I.F.M. 2008. Relationship between seed size and litter effects on early seedling establishment of 15 tropical tree species. *J. Tropical Ecol.* 24: 569-573.

Schupp, E.W. 1992. The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *Am. Nat.* 140: 526-530.

Svenning, J.C. and Wright, S.J. 2005. Seed limitation in a Panamanian forest. *J. Ecol.* 93: 853-862.

Turnbull, L.A., Crawley, M.J. and Rees, M. 2000. Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88: 225-238.

Westoby, M., Leishman, M. and Lord, J. 1996. Comparative ecology of seed size and dispersal. *Philos. Trans. R. Soc. London B*. 351: 1309-1318.

Liu, X., Liang, M., Etienne, R.S., Wang, Y., Staehelin, C. and Yu, S. 2012a. Experimental evidence for a phylogenetic Janzen-Connell effect in a subtropical forest. *Ecol. Lett.* 15: 111-118.

Liu, Y., Yu, S., Xie, Z-P and Staehelin, C. 2012b. Analysis of a negative plant-soil feedback in a subtropical monsoon forest. *J. Ecol.* 100: 1019-1028.

Zeng, M., Li, D.G. and J. Yuan. 2004. Effect of the pesticide on the arbuscular mycorrhizal fungi in the soil of citrus orchard. *Mycosystema* 23: 429-433.

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