

Influence of soil pathogens on early regeneration success of tropical trees varies between forest edge and interior

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Abstract Soil fungi are key mediators of negative density-dependent mortality in seeds and seedlings, and the ability to withstand pathogens in the shaded understory of closed-canopy forests could reinforce light gradient partitioning by tree species. For four species of tropical rainforest trees—two shade-tolerant and two shade-intolerant—we conducted a field experiment to examine the interactive effects of fungal pathogens, light, and seed density on germination and early seedling establishment. In a fully factorial design, seeds were sown into 1 m² plots containing soil collected from underneath conspecific adult trees, with plots assigned to forest edge (high light) or shaded understory, high or low density, and fungicide or no fungicide application. We monitored total seed germination and final seedling survival over 15 weeks. Shade-intolerant species were strongly constrained by light; their seedlings survived only at the edge. Fungicide application significantly improved seedling emergence and/or survival for three of the four focal species. There were no significant interactions between fungicide and seed density, suggesting that pathogen spread with increased aggregation of seeds and seedlings did not contribute to

pathogen-mediated mortality. Two species experienced significant edge-fungicide interactions, but fungicide effects in edge vs. interior forest varied with species and recruitment stage. Our results suggest that changes to plant-pathogen interactions could affect plant recruitment in human-impacted forests subject to fragmentation and edge-effects.

Keywords Density-dependence · Edge-effects · Light · Plant–soil feedback · Tropical forest

Introduction

Processes governing seedling establishment contribute substantially to the composition and diversity of plant communities (Harms et al. 2000; Wright 2002; Bagchi et al. 2014). In closed-canopy forests, low light conditions and natural enemies are primary stresses faced by seeds and seedlings (Augsburger 1984b; Augsburger and Kelly 1984; Montgomery and Chazdon 2002). The growth-mortality trade-off in relation to light availability allows local coexistence of species via resource niche partitioning (Kobe 1999; Montgomery and Chazdon 2002; Balderrama and Chazdon 2005). In addition, density-dependent natural enemy attack can prevent any one species from becoming dominant in the community and is strongest during the earliest phases of seed-to-seedling transition (Freckleton and Lewis 2006; Bagchi et al. 2014). Although both light-gradient partitioning and negative density-dependence are niche-based processes contributing to community diversity, only a handful of studies have examined how light and pathogens act in concert to influence plant recruitment (Augsburger 1984a; McCarthy-Neumann and Kobe 2008; McCarthy-Neumann and Ibáñez 2013). Discerning interactions among coexistence mechanisms is fundamental to a better understanding

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of community assembly, especially for predicting how forests will respond to natural or anthropogenic environmental change (McCarthy-Neumann and Ibáñez 2012).

Light is a critical determinant of seedling survival. Seed germination and seedling establishment improve for most species at higher light levels, although substantially less so for shade-tolerant species (Kitajima and Poorter 2008; Valladares and Niinemets 2008). Shade-tolerant species are unable to exploit high light conditions due to lack of plasticity in their growth response (Valladares and Niinemets 2008). In comparison, shade-intolerant species can exploit high light availability by rapid photosynthesis and tissue growth, but succumb to lack of light in the shaded understory of closed-canopy forest (McCarthy-Neumann and Kobe 2008; McCarthy-Neumann and Ibáñez 2013). Consequently, seedling establishment rates for shade-intolerant species tend to increase substantially more than shade-tolerant species between high and low light (Myers and Kitajima 2007; Valladares and Niinemets 2008; Wright et al. 2010).

Additionally, in the shaded understory of closed-canopy forests, fungal pathogens are key agents of mortality in seeds and seedlings (Gilbert and Hubbell 1996; Packer and Clay 2000; Bell et al. 2006; Mangan et al. 2010; Bagchi et al. 2014). Fungal pathogens mediate negative density-dependence (NDD), a phenomenon where individuals suffer higher mortality when surrounded by high densities of conspecifics (Bell et al. 2006; Freckleton and Lewis 2006; Bagchi et al. 2010). Because pathogen infection tends to be lower in tree-fall gaps than under closed canopies (Augsburger 1984a; Augsburger and Kelly 1984), NDD might lower in light than shade. However, species' shade tolerance may itself influence species' susceptibility to pathogens—for example, shade-tolerant species had lower mortality from pathogens than shade-intolerant species in a Costa Rican forest, thus reinforcing species' shade-tolerance (McCarthy-Neumann and Kobe 2008). In contrast, Pringle et al. (2007) found that increased shade-tolerance increased seed susceptibility to pathogen attack. Thus, how species' shade tolerance influences pathogen-mediated mortality of seed and seedlings in light vs. shade remains unclear.

Tolerance of soil pathogens could link light-gradient partitioning to density-dependent mortality of seeds and seedlings (McCarthy-Neumann and Kobe 2008; Kobe and Vriesendorp 2011), providing a mechanism for why species vary in the strength of NDD they experience (Comita et al. 2010; Lebrija-Trejos et al. 2016). In high light conditions, seedling establishment could benefit either from lowered pathogen activity per se in the warmer, drier conditions relative to shade (Augsburger and Kelly 1984), or rapid growth and lignification of the seedling through increased photosynthesis (and thereby better withstand pathogen attack). If pathogen infection is greater in shade, species' survival in increasing shade could be partly determined by

their tolerance of soil pathogens (McCarthy-Neumann and Kobe 2008; Kobe and Vriesendorp 2011). Conversely, some endosymbiotic fungi have been shown to become pathogenic in high light and may thus decrease recruitment (Álvarez-Loayza et al. 2011). Either way, soil fungi might be a key factor regulating seedling establishment in high light vs. deep shade.

Understanding the interaction between light and pathogen-mediated NDD is especially relevant for comprehending changes to tree communities in fragmented and disturbed forests. Tree communities in fragmented forests undergo drastic compositional changes in adult trees (Laurance et al. 2006; Benchimol and Peres 2015) and recruiting seedlings (Benitez-Malvido and Martinez-Ramos 2003; Benitez-Malvido and Lemus-Albor 2005). Dispersal limitation through loss of seed dispersers is one possible driver of these compositional shifts (Magrath et al. 2014). However, edge-effects—altered habitat conditions at fragment boundaries—are also well documented in fragmented forests, resulting in increased light availability, lower soil moisture, lower humidity, and increased impacts of wind and rain (Laurance and Yensen 1991; Didham and Ewers 2012). These changes in habitat conditions affect post-dispersal processes governing recruitment, which could influence future composition of tree communities in forest fragments.

Although both pathogens and light availability are key determinants of seedling recruitment (Augsburger 1984a), only a handful of studies have examined their simultaneous role in the context of edge-effects in fragmented forest (Benitez-Malvido et al. 1999; Benitez-Malvido and Lemus-Albor 2005). Moreover, very few experimental studies have manipulated both light and pathogens to examine their relative influence on tree seedling recruitment (McCarthy-Neumann and Kobe 2008; McCarthy-Neumann and Ibáñez 2013). In this study, we conducted an *in situ* field experiment in a fragmented tropical rain forest to examine interactive effects of pathogens and seed density on early seedling establishment at forest edge vs. interior. Using four focal tree species that varied in life-history strategy, we asked:

1. Does the strength of pathogen-mediated mortality during seedling establishment vary between forest edge (high light) and interior (shade)?
2. Do shade-tolerant and shade-intolerant species differ in the strength of pathogen-mediated mortality experienced during seedling establishment?
3. How does seedling establishment for shade-tolerant vs. shade-intolerant species vary between forest edge and interior in relation to seed density and pathogens?

We hypothesized that fungicide application would increase establishment rates to a larger extent in interior forest than at the forest edge. In addition, we expected that

gains in seed germination and seedling establishment with fungicide application would be higher for shade-intolerant species, particularly within interior forest. Finally, we expected increases in seedling establishment with fungicide application to be more prominent in high density treatments, especially for shade-intolerant species and within interior forest.

Methods

Study site

We conducted this study within a fragmented forest landscape in the Western Ghats biodiversity hotspot, south India (12°56'N and 75°39'E). Located in Karnataka state, the study area of ~ 3600 ha is a heterogeneous matrix of tea, abandoned coffee, roads, grassland, and fragments of remnant evergreen forest. Of this, about 60% is forest (~ 2300 ha). Until the late 1980s, the region was selectively logged for timber species such as *Mesua ferrea*, *Hopea canarensis*, *Elaeocarpus tuberculatus*, *Dimocarpus longan*, and *Actinodaphne bourdillonii*, until banned by the Supreme Court in 1996. Sporadic logging occurred up to 2002, and cutting of small stems for fuelwood and construction continues to the present day. The study area is contiguous with larger patches of Reserve Forest to the north and south, and a human-dominated landscape of coffee plantations and villages in the east.

Focal species

We experimentally assessed the relative importance of pathogens, and seed density on seed germination and early seedling establishment at forest edge vs. interior in a three-way fully factorial design under controlled field conditions. We chose four evergreen forest tree species based on known natural history of their shade-tolerance. *Toona ciliata* M. Roem. (Meliaceae) and *Macaranga peltata* Roxb. Mueller (Euphorbiaceae) are shade-intolerant. *Olea dioica* Roxb. (Oleaceae) and *Heritiera papilio* Bedd. (Malvaceae) are more shade-tolerant; seedlings and saplings can persist in the shaded understory and are also found in gaps and forest edges. The species also represent a gradient in seed mass [dry mass: *Toona ciliata* (0.014 g) < *Macaranga peltata* (0.034 g) < *Olea dioica* (0.27 g) < *Heritiera papilio* (1.29 g)]. All seeds were collected from forest fragments in April 2016 during peak fruiting (March–May), dried, weighed, and sown in early May 2016. We only selected seeds without any visible insect holes or fungal attack, and checked seed viability before sowing using the flotation test. Seeds were sown fresh after collection, not stored for long periods, and sprayed with an insecticide on their surface.

Experimental design

In a forest patch at the northern edge of the estate, three experimental blocks were set up at one edge and one interior forest location to capture high light and shaded conditions, respectively. The interior location was c. 80 m away from the edge and had a complete canopy with no large canopy openings (> 5 m diameter) within 50 m. In contrast, blocks at the edge location were placed just outside the forest edge so that they received full sunlight for most of the day. Each block contained a total of 16 adjacent 1 m² plots, with each plot assigned to a single focal species. We demarcated plots using 0.5 mm metal wire mesh to prevent access by mammalian seed predators. In each plot, existing leaf litter, vegetation, and top soil were first cleared and we sprayed the substrate with fungicides and an insecticide for 10 days prior to layering with experimental soil. Despite our precautions, it is possible that the original substrate beneath the experimental soil might have had microbes that recolonized the experimental soil. Next, we collected soils from underneath the trees from which seeds were collected because these soils would have high concentrations of pathogens likely to infect seeds and seedlings. Sampled trees were at least 30 m away from the nearest conspecific.

For each species, we bulked soils from under multiple conspecifics (Mangan et al. 2010; McCarthy-Neumann and Ibáñez 2013), sieved out preexisting seeds and debris, and layered each plot with 10 cm of this soil, since field and greenhouse observations of newly germinated seedlings showed that young seedlings of the four species had roots extending 2.5–5 cm. Thus, seeds and germinating seedlings were exposed to any soil pathogens present near conspecific adults. To avoid contamination from conspecifics in the vicinity, the experimental locations were laid at least 30 m away from conspecific adults of the study species. Seeds were then sown randomly within each plot at a depth of 5 mm into this conspecific soil. Each species was separately sown at low density (10 seeds/m²) and high density (100 seeds/m²). For *Macaranga* we also had an additional density treatment of 500 seeds/m², to represent commonly encountered local seed densities of this species (Krishnadas, unpublished data). Half of every block was randomly assigned to one of two fungicide treatments—sprayed (hereafter fungicide application) or not sprayed (control).

For the fungicide treatment we used a combination of Amistar (Syngenta Ltd; active ingredient, azoxystrobin) and Ridomil Gold (Syngenta Ltd; active ingredients, mancozeb and metalaxyl). Amistar has broad-spectrum systemic activity against multiple plant pathogenic fungi, and Ridomil protects plants against oomycetes and fungi. Fungicides were applied with a hand mister following the manufacturers' guidelines (Amistar: 0.01 g and Ridomil: 0.5 g, each dissolved in 100 ml water per 1 m² plot). Control plots were

sprayed with 100 ml of water. Both fungicides have been shown to have low toxicity to non-target organisms and minimal inhibitory effects on arbuscular mycorrhizal root colonization in temperate grasslands, crops, and in seedlings of two Neotropical tree species (Gripenberg et al. 2014 and references therein). To prevent fungicide run-off into control plots, we made sure that the ground was leveled and fungicide treated plots were placed together within each block. If there was a slight slope, we placed fungicide plots on the lower end of the slope. Furthermore, plots with the same treatment were within 0.25 m of each other, but we placed the fungicide half of a block nearly a meter away from the controls.

We applied fungicides and monitored germination and seedling recruitment weekly from May 2016 until mid-August 2016, before the heavy monsoon rains set in. To avoid mortality due to desiccation, plots were watered twice each week during the experiment. Seeds started germinating about 3 weeks after sowing. At each census, the number of emergent seedlings was calculated. A seedling was included (as having emerged) in the census only when it had at least two fully developed leaves in addition to its cotyledons, and tagged with a wooden toothpick to monitor survival into the next census. From these censuses, we estimated the cumulative number of seeds recruiting into seedlings per species per treatment combination over 15 weeks (henceforth, cumulative seedling emergence). Because seedlings died after emergence, we also modeled the final number of seedlings alive after the last census at 15 weeks (henceforth, final seedling establishment).

Data analysis

We tested the effects of forest edge/interior, fungicide treatment, seed density, and their interactions on cumulative seedling emergence (proportion of seeds that germinated during the experiment) and final seedling establishment (proportion of seeds that germinated and survived until the end of the experiment) using generalized linear mixed-effects models with binomial errors (Bolker et al. 2009) using the glmmADMB package (Fournier et al. 2012) in R (R Core Team 2017). We modeled the data separately per species to discern species-level responses to edge/interior, pathogens, and seed density at both phases of early recruitment. Block was included as a random effect because of potential variation arising from their spatial location. Individual plots were included as a random-effect to account for overdispersion observed during preliminary analysis (<http://glmm.wikidot.com/faq>). Because light, fungal pathogens, and seed density are all known to affect seed-to-seedling transition, we set up candidate models that tested (1) three-way interaction among edge/interior, density, and fungicide, (2) all two-way interactions among all variable pairs, (3) two variables interact and

the third has an additive effect, and (4) three variables have an additive effect. The six candidate models for the above hypotheses (Supplementary material Appendix 1, Tables A1 and A2) were compared and the top-ranked model that best fit the observed data was selected in an information theoretic approach using small-sample corrected AICc (Bolker et al. 2009). In addition, we ran models for all species combined and tested for interactions with seed mass. Results of the full model for each species, as well as the all species model with seed mass, are presented in Supplementary Material Appendix 1 (Tables A3, A4, and A5).

Results

We planted a total of 5280 seeds for all treatment combinations of the four focal species, plus an additional 3000 seeds for the highest density category for *Macaranga* (500 seeds/m²). Of the 5280 seeds, 1177 (22%) seedlings emerged and 603 (11%) survived to the end of the experiment.

Cumulative seedling emergence

For shade-intolerant *Toona*, probability of seedling emergence was markedly lower in interior compared to edge and fungicide increased seedling emergence more in interior than edge (Fig. 1, Supplementary material Appendix 1). The top-ranked model for seedling emergence of *Toona* included interior, fungicide treatment, density, and an interaction between fungicide and forest interior (Fig. 2). Furthermore, seedling emergence was lower at high density compared to low density, independent of fungicide or shade treatments. Seedling emergence for *Macaranga*, the other shade-intolerant species, was influenced only by light (Figs. 1, 2). The top-ranked model for shade-tolerant *Olea* showed seedling emergence to increase significantly with fungicide treatment, but with no effect of edge/interior or density (Fig. 2). For the other shade-tolerant species, *Heritiera*, fungicide and an interaction between density and edge/interior best explained seedling emergence. *Heritiera* emergence improved with fungicide application as expected, but unexpectedly also at high density in forest interior (Fig. 2, Supplementary material Appendix 1, Fig. 1). In the all species model, fungicide significantly improved seedling emergence; smaller-seeded species had significantly lower emergence in forest interior and high density, but had higher emergence with fungicide (Table A5).

Final seedling establishment

None of the seedlings of shade-intolerant *Toona* or *Macaranga* survived to the end of the experiment in forest interior (Fig. 3), so we could not estimate parameter for edge/interior.

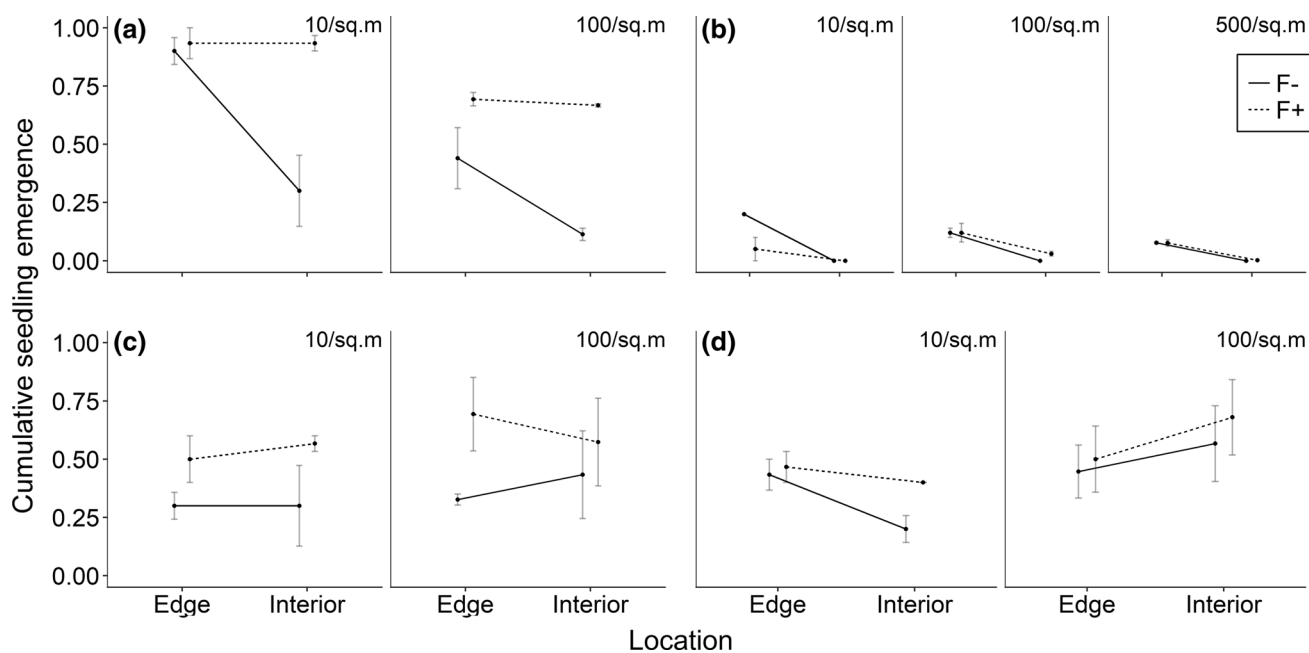
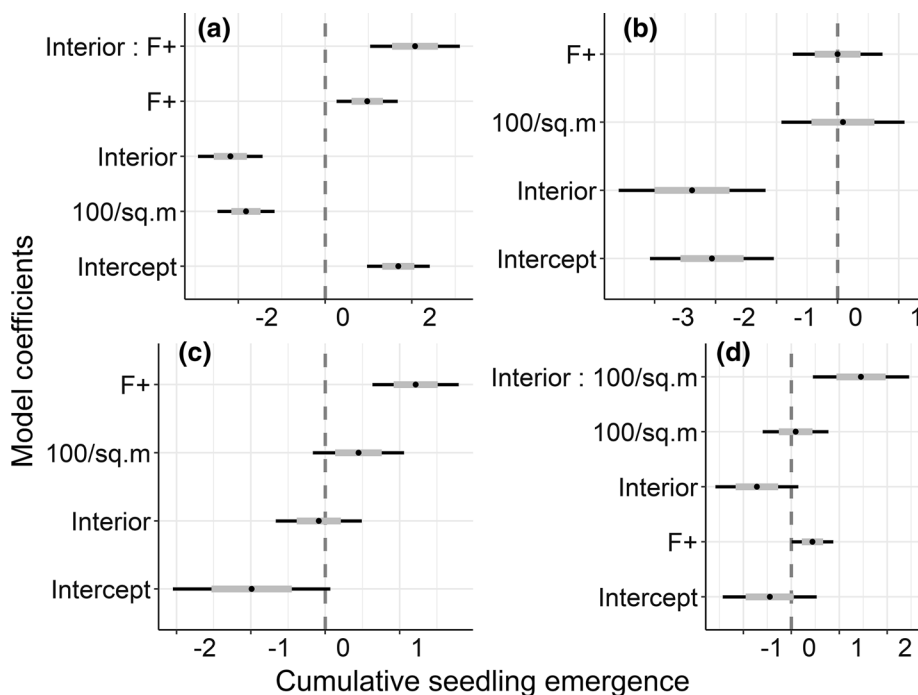


Fig. 1 Observed proportions (± 1 SE) of seedlings that emerged during the experiment in edge vs. interior plots in response to fungicide treatment and seed density for four tree species: **a** *Toona ciliata*, **b** *Macaranga peltata*, **c** *Olea dioica*, and **d** *Heritiera papilio*. Seeds were planted into plots in factorial combinations of light (light/shade) and seed density (10 and 100 seeds/m²; 100 seeds/m² for *Macaranga*)

and fungicide treatment (F \pm). Dotted and solid lines indicate control and fungicide treatment, respectively. *Toona* and *Macaranga* are shade-intolerant; *Olea* and *Heritiera* are considered shade tolerant. The four species also represent an increasing gradient in seed size (*Toona* < *Macaranga* < *Olea* < *Heritiera*)

Fig. 2 The effect of forest edge, fungicide, seed density and their interactions on cumulative seedling emergence for four tropical tree species **a** *Toona ciliata*, **b** *Macaranga peltata*, **c** *Olea dioica*, and **d** *Heritiera papilio*, estimated using generalized linear mixed-effects models with binomial errors. Experimental blocks and individual plots were included as random effects. Gray bars represent ± 1 SE and black lines represent 95% confidence intervals. For every species, the intercept represents estimates of cumulative emergence in high light, low density, without fungicide



For *Toona*, tested for effects of density and fungicide for the seedlings grown at edge and found that seedling establishment was not affected by density, but was significantly

greater with fungicide application in the top-ranked model (Fig. 4, Supplementary material Appendix 1, Fig A2). For *Macaranga*, seedlings survived only at the highest density in

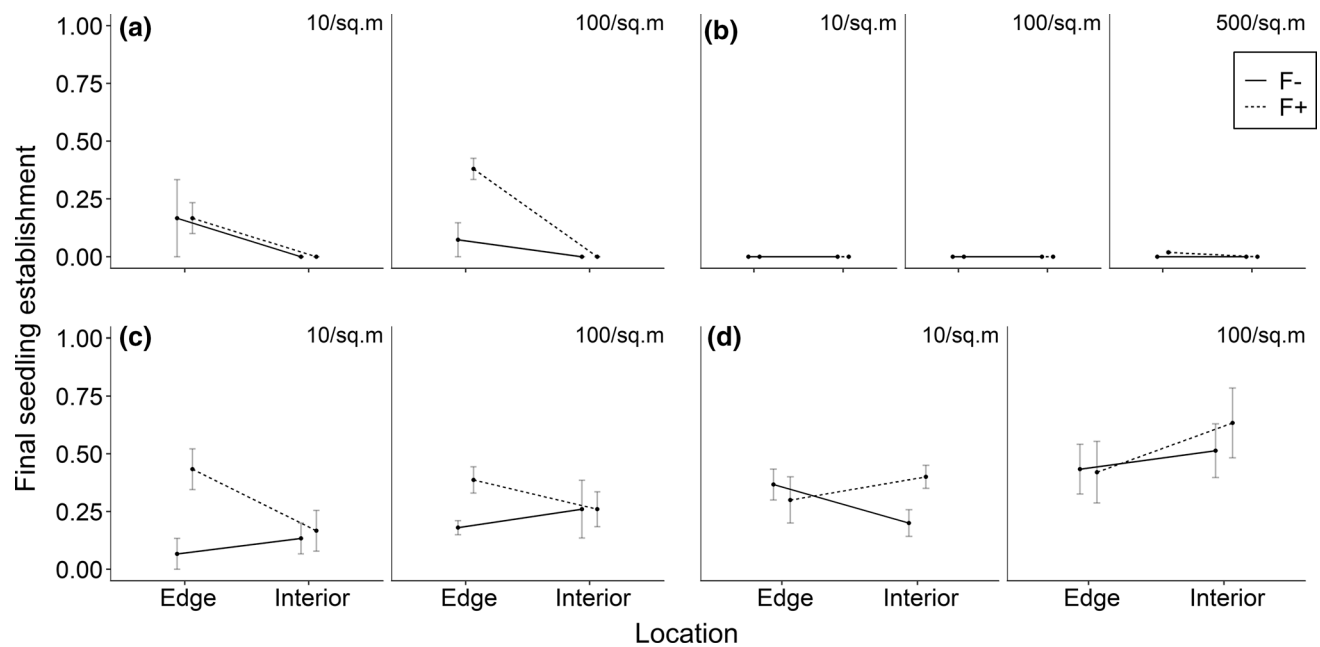


Fig. 3 Observed proportions (± 1 SE) of final seedling establishment during the experiment in edge vs. interior plots in response to fungicide treatment and seed density for four tree species: **a** *Toona ciliata*, **b** *Macaranga peltata*, **c** *Olea dioica*, and **d** *Heritiera papilio*. Seeds were planted into plots in factorial combinations of light (light/shade) and seed density (10 and 100 seeds/m²; 100 seeds/m² for *Macaranga*)

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edge plots treated with fungicide, and so model parameters could be estimated for final seedling establishment. The top-ranked model for shade-tolerant *Olea* included significant effects of fungicide application and an interaction between forest interior and fungicide (Fig. 4). Fungicide application increased final seedling establishment for *Olea*, although this improvement was significantly lower in interior forest (Fig. 4). For *Heritiera*, the top-ranked model included only additive effects of edge/interior, fungicide, and density. Specifically, seedling establishment increased at higher density and in forest interior, but fungicide treatment, even though included in the best fit model, did not significantly improve seedling establishment (Fig. 3, Supplementary material Appendix 1). In the all-species model, larger-seeded species had significantly higher survival in interior forest, and smaller-seeded species had significantly higher survival with fungicide (Table A5).

Discussion

Pathogen-driven negative density dependence (NDD) and light-dependent survival are two key mechanisms contributing to species coexistence in diverse plant communities (Wright 2002; Freckleton and Lewis 2006). However, few studies have examined their roles simultaneously

(Augsburger 1984a; McCarthy-Neumann and Ibáñez 2013). For four tropical tree species, we found that the influence of soil fungi on seed-to-seedling transition was contingent on whether seeds/seedlings were located in the high light environment of forest edge or shaded conditions of the forest interior, but to varying degrees, demonstrating that coexistence mechanisms interact to determine regeneration niches of tree seedlings. Moreover, fungicide effects varied between cumulative seedling emergence and post-germination early seedling survival, being stronger earlier during recruitment. Although fungicide application improved recruitment, interspecific differences were prominent, as seen in other forests (McCarthy-Neumann and Kobe 2008; McCarthy-Neumann and Ibáñez 2013; Gripenberg et al. 2014).

Recruitment gain from fungicide application in light vs. shade

Fungicide application significantly increased probability of seedling emergence and final seedling establishment for three and two of the four focal species, respectively, suggesting that fungal pathogens were a common cause of mortality during recruitment. We had expected recruitment gains with fungicide application to be more marked in interior forest, based on previous studies that found higher pathogen attack in shade compared to high light. For example,

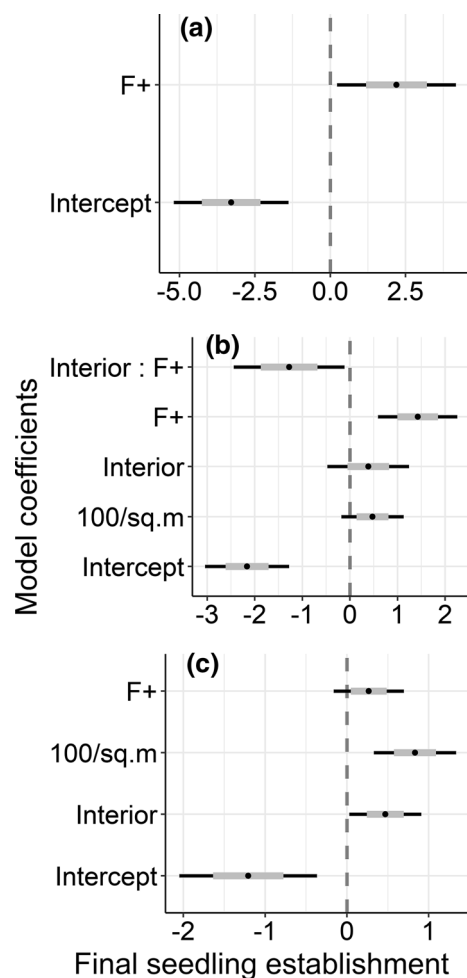


Fig. 4 The effect of forest edge, fungicide, seed density and their interactions on final seedling survival for four tropical tree species **a** *Toona ciliata*, **b** *Olea dioica*, and **c** *Heritiera papilio*, estimated using generalized linear mixed-effects models with binomial errors. Experimental blocks and individual plots were included as random effects. Gray bars represent ± 1 SE and black lines represent 95% confidence intervals. For *Macaranga*, only seedlings in high light at the highest density with fungicide application finally survived and hence model parameters could not be calculated. For *Toona*, no seedlings survived in interior forest and only edge plots were used for analysis. For every species, the intercept represents estimates of final seedling establishment in high light, low density, without fungicide

Augsburger and Kelly (1984) reported that incidence of disease in young seedlings was lower in forest gaps compared to shaded understory for 16 out of 18 neotropical tree species. However, in our study, only one species, *Toona*, experienced significantly higher gains from fungicide in interior forest compared to edge. Thus, it's possible that pathogen attack is not universally lower in high light compared to forest understory.

For another species in our study, *Olea*, the effect of fungicide on final seedling establishment was actually greater at edges compared to forest interior. Greater recruitment gains

from fungicide application at edge could occur if high light alters plant-fungi interactions. For example, in the Neotropical palm species *Iriartea deltoidea*, Álvarez-Loayza et al. (2011) found that endophytic fungi were beneficially symbiotic in shade, but turned pathogenic in high light, thus reducing seedling survival in direct light. Whether conversions of fungi from pathogenic to endosymbiotic are widespread among species merits future examination, but their existence suggests that interactive effects of microbes and light are highly complex.

An important caveat to our results is that seedlings can die of multiple factors in field-studies, e.g. above and below-ground herbivory by insects. While we cannot account for below-ground effects, we did not notice any above-ground herbivory on the experimental seedlings. Desiccation at forest edges is another source of seedling mortality. Because we watered plots to avoid desiccation, differences between interior and edge were most likely due to light and not soil moisture. Differences in pathogen impacts between forest edge and interior may be even more pronounced under natural conditions because of drier soils and lower relative humidity at edges.

Variation among species in fungicide effects

Differences in susceptibility to pathogens are suggested to reinforce species' shade tolerance, with both low light and pathogens restricting recruitment of shade-intolerant species in the shaded forest understory (McCarthy-Neumann and Kobe 2008). In our study, comparison of shade-intolerant *Toona* vs. shade-tolerant *Heritiera* supported this idea. For *Toona*, the large difference in seedling emergence between forest edge vs. interior was erased with fungicide application (Fig. 1) but *Heritiera* showed no difference between edge and interior. However, all *Toona* seedlings in interior forest subsequently died out, even with fungicide, indicating that ultimately light, and not pathogens, restricts this species to light gaps. In addition, seedling emergence of the other shade-intolerant species, *Macaranga*, was unaffected by fungicide, suggesting that light dependence primarily underlies its inability to recruit in interior forest. Thus, pathogen susceptibility did not reinforce shade intolerance for *Macaranga*. However, our experiment only had four species, and inclusion of additional species spanning a range of shade-tolerance is needed to fully understand the role of pathogens in light vs. shade.

Also, we found that pathogens impacted recruitment of shade-intolerant *Toona* at forest edges. The clear benefit from fungicide application at edge plots suggests that seedling recruitment of *Toona* would be most successful with both dispersal into light gaps and escape from pathogens. For shade-intolerant *Macaranga*, seedlings finally survived only in the highest density plots (i.e. those with 500 seeds).

However, those survivors were only from high light, fungicide treated plots, suggesting that *Macaranga* is strongly limited by light, but pathogens could cause mortality even when recruiting in gaps or edges. Due to the low baseline seedling survival rate of this species, however, additional replicates would be needed to test this statistically.

Greater carbohydrate reserves in larger seeds might support metabolic needs post germination, which could improve seedling survival compared to smaller seeds and also assist in recovery from pathogen attack (Myers and Kitajima 2007). However, in our study, larger-seeded *Olea* had similar increase with fungicide (12%) as the smallest-seeded *Toona* (13%), whereas small-seeded *Macaranga* only gained as much as the largest-seeded *Heritiera* (5 and 6%, respectively). Our results contrast with a study of 16 neotropical tree species that found larger-seeded species to be more susceptible than smaller-seeded species to attack by fungal pathogens (Pringle et al. 2007). Furthermore, when we analyzed all species together using seed mass as a covariate, larger-seed size was associated with a weaker effect of fungicide (Table A5). However, with four species we cannot generalize patterns to entire communities that span a range of seed sizes. Extending this study to include additional species covering a wider range of traits would provide a better idea of whether pathogens differentially affect species during seedling recruitment based on species' life-history trade-offs.

Interactions of seed density and fungicide

We predicted that higher seed densities would lead to increased pathogen abundance and spread within plots. Hence, recruitment would decrease at higher seed densities, but fungicide application would eliminate negative effects of high density. However, cumulative seedling emergence diminished at high densities only for shade-intolerant *Toona*. Further, even for this species, gains in seedling emergence with fungicide did not increase at high density, indicating that mortality from fungi was independent of density. Moreover, final seedling establishment for *Toona* was unrelated to density and strongly dependent on light. For *Macaranga*, also shade-intolerant, there was no statistically significant effect of density by itself or in interaction with fungicide (Fig. 1b). This lack of a density effect for *Toona* and *Macaranga* could facilitate successful recruitment despite high local densities when colonizing high light locations.

The two shade-tolerant species differed from each other in their high-density recruitment patterns. Although *Olea* recruitment improved with fungicide, lack of an interaction between fungicide and density indicates that propensity for fungal disease may not increase with seed density for this species, at least up to the density we examined. Such a pattern suggests that fungal pathogens would cause mortality

in *Olea* seeds and seedlings growing in soil from beneath conspecific adults, but high initial seed density per se may not exacerbate seedling mortality. The other shade-tolerant species (*Heritiera*) had higher seedling emergence and survival at high density compared with low density, more so in interior forest. Moreover, contrary to expectation, fungicide application did not lead increase recruitment in high density plots. Patterns seen in *Heritiera* could occur if mycorrhizal fungi were important for recruitment of this species and higher densities of hosts increased mycorrhizal activity (Willis et al. 2013). Further, if endosymbiotic fungi have reduced pathogenicity in low light, as has been observed in a Neotropical palm (Álvarez-Loayza et al. 2011), plant species with such associations might recruit better at higher densities when in shade. It remains to be investigated whether positive plant-fungi interactions increase seedling survival up to a threshold conspecific density, and survival declines only beyond this threshold with addition of conspecific neighbors (García-Guzmán and Heil 2014).

The signal of pathogen-mediated NDD can be noisy at mean natural seedling densities in observational studies, becoming more apparent when densities are manipulated to higher levels (Bell et al. 2006). Our experimental "high density" treatments were 5–80% higher than median observed seedling densities observed for these species in our study area (Krishnadas, unpublished data). Also, seedling densities in natural forest are usually higher close to conspecific adult trees, such that the density effect may actually be due in large part to distance to large adults or high densities of existing juvenile plants near conspecific adults. Since we used soils from beneath conspecific adult trees, the soil pathogen load likely simulates presence of a large conspecific adult. However, moving soil from under trees into a different location or bulking soils from different sites might alter the microbial community from their natural composition. Thus, the observed responses in our study are to a microbial community unique to our experiment, and may not exactly mirror what seedlings experience under a conspecific adult.

Recruitment stage-dependent response to light and fungicide

Differences between cumulative seedling emergence and final seedling survival corroborate previous results that light-fungicide interactions vary in intensity at different stages of plant recruitment (Gripenberg et al. 2014). The relative importance of pathogens for recruitment between forest edge and interior (high and low light) varied even within the short phase of early seed-to-seedling transition. Light is a vital germination cue for seedling emergence (Valdadares and Niinemets 2008), but light levels appeared to be more critical for seedling survival rather than emergence in our study. Once seed reserves are exhausted, low-light

conditions of closed-canopy forest would impose photosynthetic stress and create metabolic deficits in seedlings (Poorter and Kitajima 2007), lowering survival even in the absence of pathogens (Kobe and Vriesendorp 2011). Accordingly, we found that seedlings of shade-intolerant species suffered massive mortality in interior forest after germination, whereas the shade-tolerant, larger-seeded species did not experience such prominent thinning.

Gains from fungicide application were more marked during seed germination than seedling survival. Seedling vulnerability to fungal disease diminishes following germination (Freckleton and Lewis 2006), possibly due to lignification, at which point light may become a more critical requirement (Valladares and Niinemets 2008). This could explain why fungicide did not improve seedling survival beyond the early stages in insufficient light. Moreover, benefits to seedlings from fungicide application at the edge could have resulted from greater photosynthetic or metabolic gains in high light compared to shaded conditions of interior forest. Therefore, the shade-intolerant species likely benefited at edges from their ability to better utilize available light for rapid growth (Valladares and Niinemets 2008).

Conclusion

Previous studies suggest that pathogens can mediate light gradient partitioning by causing greater mortality of shade-intolerant species in shade (McCarthy-Neumann and Ibáñez 2013). In our study—using forest edge and interior as proxies for high light and shade—we found that fungicides decreased mortality of shade-intolerant species even at edges, but no seedlings survived in shade regardless of fungicide application. In the forest interior, shade-tolerant species recruited better than shade-intolerant species but the former would also benefit from dispersal to locations with lower pathogen pressure. However, our inferences are limited because we conducted the experiment over a short 15-week phase of early recruitment and in a single year.

A combination of biotic and abiotic factors governs recruitment differences among tree species (Augsburger 1984b; Harms et al. 2000; Wright 2002; McCarthy-Neumann and Kobe 2008; Comita et al. 2010). In human-impacted forests, fragmentation and edge-effects alter abiotic factors like light, soil moisture, and temperature, (Laurance and Yensen 1991; Didham and Ewers 2012), which can in turn alter biotic processes that regulate plant recruitment. Interactions between light and plant–soil feedbacks, both positive and negative, influence plant recruitment and species distributions (McCarthy-Neumann and Kobe 2008; McCarthy-Neumann and Ibáñez 2012), yet remain understudied in human-impacted forests. Understanding interactions among coexistence mechanisms—usually examined

independently—would enable deeper insights into the dynamics of diverse ecological communities when subject to human impacts.

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