Seedling performance in a dioecious tree species is similar near female and male conspecific adults despite differences in colonization by arbuscular mycorrhizal fungi

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Abstract

Patterns of seedling growth and survival near conspecific plants have important consequences for species diversity in plant communities, but the factors causing intraspecific variation in seedling performance are unclear. Greater seed production or size of adult plants could both drive the local accumulation of specialized antagonist species in the environment and affect conspecific seedling performance. Experiments with dioecious species, in which only female individuals produce seeds, decouple these factors in areas of high conspecific density. To assess whether conspecific seedling performance is reduced in the environments associated with seed-producing female trees relative to male or heterospecific trees, we conducted shadehouse and field experiments with a dioecious tropical tree species, *Virola surinamensis* (Myristicaceae), on Barro Colorado Island, Panama. The shadehouse experiment isolated the effect of soil microbial communities on seedling performance and allowed us to quantify colonization by mutualistic arbuscular mycorrhizal (AM) fungi, while the field experiment allowed us to assess seedling performance in a context that included the effects of nearby conspecific trees. Seedling biomass at the end of the 8-mo shadehouse experiment was similar between experimental plants exposed to soil microbial communities from underneath female conspecific, male conspecific, and heterospecific adult trees. However, seedling colonization by AM fungi was higher in male soil microbial communities. Similarly, at the end of the 7-mo field experiment, survival and biomass did not differ among experimental seedlings grown in female, male, and heterospecific environments (but biomass was reduced near larger conspecific adults). Together, our experiments did not support the hypothesis that conspecific seedling performance is reduced in female environments relative to male environments, despite reductions in colonization by mutualistic AM fungi in female soil microbial communities. Thus, intraspecific variation in the

biotic interactions between conspecific seedlings, established adults, and mutualist species may not translate directly to patterns of tropical tree seedling survival.

Introduction

In many ecological communities, whether a given species recruits successfully depends on its identity and abundance, as well as the composition and traits of neighboring individuals, and has important ecological and evolutionary consequences. Recruitment is often suppressed by the density or proximity of conspecific neighbors (Janzen, 1970; Connell, 1971; Chesson, 2000), a form of negative conspecific density (or distance) dependence (CDD). This phenomenon has been demonstrated in a variety of plant ecosystems and may be caused by a combination of (i) the accumulation of host-specific natural enemies (reviewed by Carson & Schnitzer, 2008; Comita et al., 2014; Hülsmann et al., 2021; Song et al., 2021) or (ii) intraspecific competition for shared resources (Suzuki et al., 2003; Pujol & Mckey, 2005; Adler et al., 2018) in areas of high conspecific density. Patterns of negative CDD may be driven by plant–soil feedbacks between established plants and host-specific pathogenic soil microbes that negatively influence seedling performance (reviewed by Kulmatiski et al., 2008; van der Putten et al., 2013). Variation in negative CDD is often quantified among co-occurring plant species and attributed to differences in species' life history strategies and related traits (Kobe & Vriesendorp, 2011; Lebrija-Trejos et al., 2016; Zhu et al., 2018; Jia et al., 2020; Zang et al., 2021), species relative abundance (Comita et al., 2010), or mycorrhizal associations (Bennett et al., 2017; Jiang et al., 2020; Jevon et al., 2022). Though experiments have shown that variation in seedling performance near conspecifics may also occur within species (Packer & Clay, 2003; Hood et al., 2004; Bell et al., 2006; Eck et al., 2019), the factors that contribute to intraspecific variation in conspecific neighbor effects are rarely studied (Freckleton & Lewis, 2006; but see Liu et al., 2015). Elucidating the factors causing variation in seedling recruitment and performance within species' populations may allow better predictions of how negative CDD influences important ecological and evolutionary dynamics in plant communities.

Variation in the composition and abundance of host-specific natural enemies in the soil near parent or other established plants is a prime driver of performance and recruitment success (Packer & Clay, 2003; Reinhart & Clay, 2009; Kotanen, 2010; Chanthorn et al., 2013; Liang et al., 2016; Chen et al., 2019). Despite this, the influence of variation among individuals (such as in genotype, size, or functional traits) on the local accumulation of natural enemies remains poorly understood. For example, differences in the fruit or seed crop could lead to variation in natural enemy abundance (Hubbell, 1980). Because of limited dispersal distances, many seeds fall beneath or close to parent trees (Swamy et al., 2011). The presence of conspecific seeds and seedlings has been shown to contribute to density-dependent mortality in tropical tree seedlings (Harms et al., 2000; Wright et al., 2005; Queenborough et al., 2007a; Comita et al., 2010; Metz et al., 2010; Lebrija-Trejos et al., 2014), presumably by increasing host-specific enemy densities (or competition among seedlings for shared resources; but see Paine et al., 2008). Thus, seed production is one potential factor that may impact enemy densities and/or conspecific seedling performance or recruitment near adult plants, though this hypothesis has rarely been tested (but see Hood et al., 2004).

Though the strength of negative CDD on performance and recruitment varies among life stages (Piao et al., 2013; Zhu et al., 2015), it is unclear whether the size and/or age of established plants influences conspecific seedling performance (Uriarte et al., 2004). Because larger individuals have older and more extensive root systems, higher pathogen densities or more specialized pathogen communities could accumulate in the soils near larger individuals. Because larger or older individuals may also be more fecund (Aarssen & Jordan, 2001), teasing apart the effects of adult size from adult reproduction is challenging in many species. Dioecious plant species, in which male flowers that produce pollen and female flowers that produce seeds occur on separate individuals, offer the opportunity to decouple the effects of the size versus seed production of established plants on seedling performance in their soils.

The negative effects of variation in natural enemy conditions on seedling performance are well documented, but positive effects from mutualistic microbes (such as mycorrhizal fungi) may also influence patterns of seedling performance and CDD (Bever, 2002; Mangan et al., 2010a; Bachelot et al., 2015; Liang et al., 2015; Bachelot et al., 2017; Bennett et al., 2017; Jevon et al., 2022). For example, arbuscular mycorrhizal (AM) fungal association may help seedlings counter conspecific distance- and density-dependent mortality due to pathogens (Liang et al., 2015; Bachelot et al., 2017), via either increases in plant growth or nutritional status (Smith & Read, 2008; reviewed by Delavaux et al., 2017) or activation of plant defensive pathways (Newsham et al., 1995; Pozo & Azcón-Aguilar, 2007). Like enemies, the accumulation of mutualist species in soil is subject to host availability, and variation in mutualist-mediated CDD could result from variation in the size or seed production of nearby host individuals.

Despite recent evidence of intraspecific variation in CDD, the factors driving variation in conspecific seedling performance within plant populations remain unclear. To address this issue, we focused our study on a dioecious tropical tree species, isolating the effect of seed production on seedling performance from other potential factors in the vicinity of conspecifics. We conducted shadehouse and field experiments with *Virola surinamensis* (Myristicaceae) on Barro Colorado Island, Panama, to answer the following questions: 1) does seedling performance vary in the soil environment associated with female conspecifics versus soil associated with male conspecifics? 2) does the size or seed production of conspecific adults influence seedling performance in their associated soil? and 3) does seedling colonization by mutualistic AM fungi vary between female and male soil environments? We hypothesized that: i) seedling performance would be reduced in the soil environments of female conspecifics, because repeated inputs of conspecific seeds and/or seedlings into female soils over time should cause greater accumulation of host-specific soil pathogens, ii) seedling performance would be reduced in the soil environments of larger conspecific trees, because their larger root systems will have accumulated higher densities of host-specific soil pathogens; iii) seedling performance would be reduced in the soil environments of conspecific trees that produce more seeds, because the input of greater numbers of conspecific seeds into the soil should increase the accumulation of host-specific pathogens, and iv) seedling colonization by AM fungi would be higher in female soils, because the repeated input of conspecific seeds and/or seedlings over time in their soils present host resources for AM fungal growth.

The shadehouse experiment allowed us to isolate the effect of soil microbial communities on seedling performance, while the field experiment allowed us to assess seedling performance in female and male conspecific environments in an ecologically relevant context. To investigate the factors influencing seedling performance more fully in conspecific soils, we quantified colonization by mutualistic AM fungi in the experimental seedlings, seed production by focal conspecific adults, and nutrient conditions in our experimental soil treatments. With this study, we aim to better understand the sources of intraspecific variation in plant–soil feedbacks and CDD, allowing better predictions of how these phenomena influence species diversity and evolution in plant communities.

Materials and Methods

Study site and species

Our study focused on *Virola surinamensis* (Rol. ex Rottb.) Warb. (Myristicaceae), a dioecious tropical tree species occurring on Barro Colorado Island (BCI), Republic of Panama (9°09' N, 79°51' W). BCI is a 15.6 km^2 moist tropical lowland forest (Croat, 1978) receiving ~2600 mm of rainfall per year (punctuated by a distinct dry season; Windsor, 1990). *Virola surinamensis* is native to tropical and subtropical wet lowland forests in Central America and Amazonia. It is a shade-tolerant (Howe, 1990), drought-sensitive (Fisher *et al*., 1991) canopy tree, and occurs commonly in slope and stream habitats on BCI (Harms *et al*., 2001).

The minimum reproductive size for *V. surinamensis* is ~ 30 cm diameter at 1.3 m above ground (DBH) (Riba-Hernández *et al*. 2014). Adult *V. surinamensis* are spatially aggregated (Condit *et al*., 2000; Riba-Hernández *et al*., 2014) but the sexes are distributed randomly relative to one another (Riba-Hernández *et al*. 2014). Flowers are fly-pollinated (Jardim & Mota, 2007). On BCI, flowering occurs in the dry season (~ Jan.) and seed production in the following wet season (~ July) (Zimmerman *et al*., 2007). Seeds are large (~ 2 cm) and borne singly inside a heavy, inedible capsule: ~ 62 % of seeds are dispersed away from the maternal tree by animals (Howe & Vande Kerckhove, 1981). Herbivores predate many seeds and/or seedlings below maternal crowns, but seedlings experience higher survival if dispersed > 20 m away from the maternal tree (Howe, 1990).

Soil inoculation shadehouse experiment

To isolate the effect of soil microbes on the performance of seedlings near female and male conspecific trees, we conducted a soil microbial inoculation experiment in a shadehouse on BCI. Seeds were collected from beneath the canopy of 11 fruiting (female) *V. surinamensis* on

BCI during the peak of the species' fruiting in 2014 (between June – July). We also selected five reproductive-size male *V. surinamensis* (because flowers could not be sexed in the field, maleness was assumed because of lack of seed production during the study years, 2014 – 2016). All trees were 50 – 100 cm DBH and were located by exploring a ~ 3.5 km^2 area of BCI (see Fig. 1A for a map). Selected trees were located ~ 30 m to ~ 2 km apart. Seeds from each maternal source were surface-sterilized (10 % bleach for 1 m, rinse, 70 % ethanol for 30 s, rinse), air dried, and germinated in a shadehouse in autoclaved BCI soil (collected from the forest edge near the shadehouse). One-month post-germination, a minimum of 12 healthy seedlings per maternal seed source were randomly selected for inclusion in the experiment (more seedlings were selected if available).

We then collected samples of the soil microbial community beneath the canopy of each of the source trees to use as inocula in the experiment. To create one soil microbial inoculum per tree, we collected soil at a depth of 10 cm from three randomly-selected points within 3 m of each trunk (to roughly correspond to fine roots, where soil microbes are likely to be most active), then coarsely sieved and homogenized the soil from these three points. To act as a control, we also created soil microbial inocula from beneath the canopy of five reproductive-size heterospecific trees within the study area (Fig. 1A). Heterospecific trees (abbreviated as H1 – H5) were chosen to represent a range of common species and families (H1: *Spondias mombin* L. (Anacardiaceae); H2: *Ormosia macrocalyx* Ducke (Fabaceae); H3: *Anacardium excelsum* (Bertero & Balb. ex Kunth) Skeels (Anacardiaceae); H4: *Platypodium elegans* Vogel (Fabaceae); H5: *Protium tenuifolium* (Engl.) Engl. (Burseraceae)). Each seedling was assigned to the soil inoculum of only one of the 21 adult trees in the study (i.e., the 11 female conspecific trees, five male conspecific trees, or five heterospecific trees). Thus, seedlings from each maternal seed source were assigned at random to each of three soil inoculum treatments: female conspecific soil inoculum (*n* = 144 seedlings), male conspecific soil inoculum (*n* = 60 seedlings), and heterospecific soil inoculum (*n* = 57 seedlings). More seedlings were planted in female soil inoculum because the experiment was designed to test concurrently for the effect of relatedness on seedling performance in maternal soils (Eck et al., 2019). All soil inocula were collected in September 2014 and used in the experiment within two days.

In a shadehouse on BCI, 261 experimental seedlings were transplanted into individual 2-L pots in September 2014 (at ~ 1–2 mo of age). Each pot contained 20 % by volume of soil microbial inoculum from the seedling's assigned tree and 80 % by volume of a common soil medium (a steam-sterilized 1:1 mixture of BCI field soil:potting sand). Inoculum was placed in the center of the potting medium in each pot using a 'core' method. Relatively small volumes of field soil inoculum were used to minimize the potential impact of differences in soil nutrients among inocula on seedling performance. Seedling pots were placed across four shadehouse benches in a balanced, randomized design (see Fig. 1B for a photograph of the shadehouse experiment). Shadehouse benches were covered with two layers of 80 % shade cloth (to mimic shady understory conditions) and were shielded from rainfall with a roof of clear plastic lining. Stem height, the number of leaves, and the length and width of each leaf were measured for each seedling immediately after transplant. Seedlings remained in their experimental treatments for ~ 8 mo and were amply watered three times per week. In May 2015, the 250 surviving seedlings were harvested. Final oven-dried biomass, stem height, leaf area, and foliar damage were measured for each seedling. Foliar damage was measured as the number of leaves showing any sign or symptom of disease or predation and the % damage by area was visually estimated on each damaged leaf. Initial oven-dried biomass was estimated for each experimental seedling based on stem height at the time of transplant using an allometric model ($F_{(1,42)} = 338.1$, $p < 0.001$, $R^2 = 0.887$). Because dry biomass measurements require seedling harvest (and thus could not be obtained for actual experimental seedlings), this linear regression model was built based on measurements of height and dry biomass of a randomly harvested sample of potential experimental seedlings at the beginning of the experiment. Seedlings with missing initial or final biomass data were excluded from all models.

To quantify colonization by AM fungi in the surviving seedlings, we used the magnified root intersect method (McGonigle *et al*., 1990) described in Eck *et al*. 2019. To test whether nutrient availability during the experiment varied among soil inocula or was linked to seedling performance, we also quantified soil nutrient conditions in each soil inocula at the end of the experiment using the method described in Eck *et al*. 2019. Finally, to explore the effect of the tree size and seed production of conspecific soil inoculum source trees on seedling performance in their soil microbial communities, we measured DBH and quantified seed production by the *V. surinamensis* trees providing soil inocula in the experiment using the method described in Queenborough *et al*. 2007b (see Supplementary Methods for details).

Field experiment

To test whether seedling performance was reduced beneath female conspecific trees relative to male conspecific and heterospecific trees in an ecologically-relevant setting, we conducted a field experiment in the BCI forest. We collected seeds from beneath the canopy of six female *V. surinamensis* during the June–July 2015 fruiting season. Because of seed availability and germination rates, two of the female trees from the shadehouse experiment were also used in the field experiment (V5 and V11). Additional females were located by exploring the same ~ 3.5 km^2 area of BCI as in the shadehouse study (see Fig. 3A for a map). All five male *V. surinamensis* and four of the heterospecific trees studied in the shadehouse experiment were included in the field experiment (H2 was replaced with a new heterospecific tree, H6: *Sterculia apetala* (Jacq.) H. Karst). Seeds from each maternal source were surface-sterilized, air dried, and germinated in autoclaved BCI forest soil in a shadehouse under two layers of 80 % shadecloth.

One month post-germination, a minimum of 18 healthy seedlings per maternal seed source were randomly selected for inclusion in the field experiment. Within each seed source, seedlings were randomly assigned to one of three experimental field treatment groups: beneath a male conspecific adult, beneath a female conspecific adult, or beneath a heterospecific adult. Seedlings were randomly assigned to be transplanted beneath one adult tree within their treatment group, and into one of 3–6 seedling plots (1 m^2) beneath the canopy of their assigned tree. Plot locations were randomized with respect to direction and distance from the base of the focal tree (1–4 m). Plots contained 3–5 experimental seedlings, each of which were randomly assigned to a 25-cm^2 position within the plot. Because the field experiment was designed to concurrently test for the effect of conspecific relatedness on seedling performance (Eck *et al*., 2019), female trees had, on average, more plots per tree and more seedlings per plot than male trees.

The 249 experimental seedlings were transplanted at ~ 1 mo of age into their field treatment plots. Immediately after transplant (August 2015), stem height, the number of leaves, and the length and width of each leaf were measured for each seedling. Each seedling was also stem-tagged with a unique identification number. All seedlings were censused every 1–2 mo, during which time survival was recorded, and the stem height and number of leaves were measured for all surviving seedlings. We also recorded instances of seedling stem breakage and uprooting (likely caused by mammalian herbivores). Seedlings that disappeared (with or without their tags being found) were recorded as dead during the census of their disappearance. After ~ 7 mo in the field experimental treatments (late March 2016), the 40 surviving seedlings were harvested and their total oven-dried biomass, stem height, and total leaf area were measured.

Statistical analyses

Seedling performance in the shadehouse experiment

To test whether seedling performance was reduced in female conspecific soil microbial communities relative to male and heterospecific soil microbial communities in the shadehouse experiment (Q1), we built a series of linear mixed-effects models (LMMs). We focused on growth (i.e., biomass) of seedlings that survived until the end of the experiment because survival rates in the experiment were high (95.8 %). First, we modelled seedling oven-dried biomass at harvest in all conspecific and heterospecific soils. Fixed effects in this model included soil microbial treatment (female conspecific, male conspecific, or heterospecific) and estimated initial seedling biomass. Maternal seed source, soil inoculum source, and shadehouse bench were included as crossed random effects.

Next, we tested whether seedling biomass at harvest was explained by the size of the conspecific tree whose soil microbial community the seedling was grown in (Q2). Because we only expect size to affect seedling performance in conspecific soils (due to the accumulation of host-specific microbes), we tested this using a separate LMM that excluded seedlings planted in heterospecific soils. Because dioecious species may increase in size without increasing in seed production, this allowed us to decouple tests of size from seed production (Fig. S2 & Table S1). Fixed effects in this model included the size (DBH) of the conspecific soil inoculum source and estimated initial seedling biomass; maternal seed source, soil inoculum source, and shadehouse bench were included as crossed random effects. Then, we tested whether seedling biomass at harvest was explained by the amount of seed production by female conspecific trees (Q2) using a third LMM (excluding seedlings planted in male conspecific and heterospecific soils). Fixed effects in this model included a log-transformed estimate of the total number of seeds produced by the female conspecific soil inoculum source and estimated initial seedling biomass; maternal seed source, soil inoculum source, and shadehouse bench were included as crossed random effects.

To better investigate the factors contributing to seedling growth, we then analyzed whether seedling biomass at harvest was related to i) seedling colonization by AM fungi or ii) nutrient conditions in the soil inocula at the end of the experiment (Supplementary Methods). We also tested whether nutrient conditions at the end of the experiment varied among the female conspecific, male conspecific, and heterospecific soil microbial treatments (Supplementary Methods). As another measure of seedling performance, we tested whether seedling foliar damage was i) higher in female conspecific soil inocula (relative to male conspecific and heterospecific soil inocula) or ii) related to the size or seed production of the conspecific soil inoculum source (Supplementary Methods). All statistical analyses in our study were conducted in the R statistical environment (R Core Team 2022). All mixed-effects models in our study were constructed using the lme4 package (Bates et al., 2015). P values for LMM predictors were obtained using the lmerTest package (Kuznetsova et al., 2016).

Seedling colonization by AM fungi

To test whether seedling colonization by AM fungi in the shadehouse experiment was higher in female conspecific soil microbial inocula relative to male conspecific and heterospecific soil inocula (Q3), we built a generalized linear mixed-effects model (GLMM). The proportion of root intersects colonized by any AM fungal structure was modelled in each seedling that survived until the end of the shadehouse experiment using the glmmTMB package (Brooks et al., 2017). Fixed effects in this model included soil microbial treatment and estimated initial seedling biomass. To check whether patterns of AM fungal colonization were related to nutrient conditions in the soil inocula at the end of the experiment, we included soil nutrient conditions as a fixed effect in this model (Supplementary Methods). To account for potential variation between the two researchers that quantified AM fungal colonization in the seedlings, we also included observer as a fixed effect in the model. Random effects in the model included maternal seed source, soil inoculum source, and shadehouse bench. Model family was set to beta-binomial (to counter overdispersion) and weights were set to the number of root intersects quantified in each seedling.

Next, we tested whether seedling colonization by AM fungi was explained by the size of the conspecific soil inoculum source tree using a second GLMM. Fixed effects in this model included the size (DBH) of the conspecific soil inoculum source, estimated initial seedling biomass, observer, and soil nutrient conditions. Like in the model above, maternal seed source, soil inoculum source, and shadehouse bench were included as random effects. Finally, we tested whether seedling colonization by AM fungi was explained by the seed production of female conspecific soil inoculum sources using a third GLMM. Fixed effects in this model included a log-transformed estimate of the total number of seeds produced by the female soil inoculum source, estimated initial seedling biomass, observer, and soil nutrient conditions. Random effects in this model included maternal seed source, soil inoculum source, and shadehouse bench. Because preliminary analyses using the proportion of root intersects colonized by arbuscules yielded similar results, we focus here on analyses utilizing data on all visible AM fungal structures. P values for GLMM predictors were obtained using the car package (Fox & Weisberg, 2019).

Seedling performance in the field experiment

To test whether seedling performance in the field was reduced in female conspecific environments relative to male conspecific and heterospecific environments, we built a series of mixed-effects models analyzing seedling survival and growth. First, the survival of seedlings in all conspecific and heterospecific environments was modelled at the end of the field experiment as a binary response variable using a GLMM (with family set to binomial). Because including seedlings that were damaged in the field (likely by mammalian herbivores) did not affect the results of the model, we included whether a seedling experienced stem breakage and/or uprooting (at any point during the field experiment) as a binary fixed effect in the model. Additional fixed effects in the model included field environment (female conspecific, male conspecific, or heterospecific) and estimated initial seedling biomass. Maternal seed source and the identity of the adult tree each seedling was planted near were included as random effects.

Next, we tested whether seedling survival was explained by the size (DBH) of the conspecific adult they were planted near using a separate GLMM (excluding seedlings in heterospecific field environments). Estimated initial seedling biomass and whether a seedling was damaged by mammalian herbivores were also included as fixed effects in this model, while maternal seed source and focal adult identifier were included as random effects. Because the number of experimental seedlings varied among field plots, we also included the number of experimental seedlings in the plot as a fixed effect in this model (this factor could not be included in the previous model because it was correlated with field environment). In preliminary analyses, we included the exact distance between the seedling and the focal adult (in m) as a fixed effect; because seedling performance was not related to the distance to the focal adult at this small spatial scale (within 1 – 4 m of the trunk and beneath the canopy), this factor was removed from all final field models.

As another measure of seedling performance in the field, we then tested whether the biomass of the seedlings that survived until the end of the field experiment varied among female conspecific, male conspecific, and heterospecific field environments. First, we modelled the oven-dried harvest biomass of the seedlings that survived until the end of the experiment in all conspecific and heterospecific environments using a LMM. Explanatory factors in the model included field environment (female conspecific, male conspecific, or heterospecific), estimated initial seedling biomass, and whether the seedling was damaged by mammalian herbivores. Maternal seed source and the identity of the focal adult each seedling was planted near were included as random effects. Finally, we tested whether seedling biomass was explained by the size of the conspecific adult they were planted near using a separate LMM (excluding seedlings in heterospecific field environments). Explanatory factors in the model included the DBH of the conspecific adult, estimated initial seedling biomass, seedling damage by mammalian herbivores, and the number of experimental seedlings in the field plot. Like in the model above, maternal seed source and focal adult identifier were included as random effects in the model.

Results

Seedling performance in the shadehouse experiment

At the end of an 8-mo shadehouse experiment, we found that *V. surinamensis* seedlings grown in a soil microbial community associated with a female conspecific tree had similar biomass as seedlings grown in a soil microbial community associated with a male conspecific tree or a heterospecific tree (Fig. 1C & Table S2; $F = 0.87$, $p = 0.44$; $n = 246$ seedlings). Seedlings grew substantially during the experiment: the average biomass of seedlings at the end of the experiment was 2.58 ± 1.08 g versus an estimated 0.40 ± 0.14 g at the beginning of the experiment. We also did not find a relationship between seedling biomass and the size (Fig. 1D & Table S3; $F = 0.32$, $p = 0.59$, $n = 184$ seedlings) of the conspecific soil inoculum source, or the number of seeds produced by female conspecific soil inoculum sources (Fig. 1E & Table S4; $F = 1.75$, $p = 0.23$, $n = 130$ seedlings). Soil nutrient conditions at the end of the experiment were similar among seedlings grown in the female conspecific, male conspecific, and heterospecific soil microbial treatments (Fig. S4 & Table S5; $F = 0.98$, $p = 0.40$, $n = 21$ soil inocula), and we did not find a relationship between seedling biomass and nutrient conditions in the soil inocula (Fig. S5 & Table S6; $F = 2.12$, $p = 0.16$; $n = 246$ seedlings). Similarly, we found that the incidence of seedling foliar damage was not affected by the soil microbial treatment (Fig. S6 & Table S7; $p = 0.82$; $n = 241$ seedlings), nor related to the size of the conspecific soil inoculum source (Fig. S7A & Table S8; $p = 0.45$, $n = 184$ seedlings) or seed production by female conspecific trees (Fig. S7B & Table S9; $p = 0.35$; $n = 130$ seedlings).

Seedling colonization by AM fungi

Most of the seedlings (82.5 %) were colonized by AM fungi after 8-mo of growth in the shadehouse experiment. We found that seedlings grown in a soil microbial community associated with a male conspecific tree had higher proportions of colonization by AM fungi in their roots than seedlings grown in a soil microbial community associated with a female conspecific tree or a heterospecific tree (Fig. 2A & Table S10; $p = 0.04$; $n = 230$ seedlings). This result was consistent across soil inoculum sources: in four out of the five male *V. surinamensis* trees in our study, the predicted mean proportions of seedling colonization by AM fungi were higher than the means predicted for any of the 11 female *V. surinamensis* trees (Fig. 2B). We found that seedling colonization by AM fungi was not related to the size of the conspecific tree providing the soil inocula (Fig 2C & Table S11; $p = 0.37$; $n = 174$ seedlings) or the number of seeds produced by female conspecific soil inoculum source trees (Fig. 2D & Table S12; $p = 0.55$; $n = 121$ seedlings). We did not find a relationship between seedling biomass and seedling colonization by AM fungi at the end of the experiment (Fig. S8 & Table S13; $F = 0.91$, $p = 0.34$; $n = 230$ seedlings). We found a marginally significant effect of nutrient conditions in the female conspecific soil inocula at the end of the experiment on seedling colonization by AM fungi in those inocula (Table S12; $p = 0.08$; $n = 121$ seedlings).

Seedling performance in the field experiment

At the end of the field experiment (Figs. 3A & 3B), we found that seedling survival was similar among seedlings planted in female conspecific environments, male conspecific environments, and heterospecific environments in the field (Fig. 3C & Table S14; $p = 0.79$; $n = 249$ seedlings). Seedling mortality was high with only 16.1 % of the experimental seedlings alive after 7 mo. We found a marginally significant positive effect of the size of the conspecific tree the seedling was planted near on seedling survival (Fig. 3D & Table S15; $p = 0.08$, $n = 198$ seedlings). In contrast, we found that seedlings in field plots that contained higher densities of experimental seedlings at the beginning of the experiment had lower survival (Fig. 3E & Table S15; *p* = 0.01; *n* = 198 seedlings).

As with survival, the harvest biomass of seedlings that survived until the end of the field experiment was similar among female conspecific, male conspecific, and heterospecific field environments (Fig. 4A & Table S16; *F* = 1.05, *p* = 0.38; *n* = 40 seedlings). We found a significant negative effect of the size of the conspecific tree the seedling was planted near on seedling biomass in the field (Fig. 4B & Table S17; *F* = 5.14, *p* = 0.05; *n* = 32 seedlings). Seedling biomass at the end of the field experiment was not related to the initial density of experimental seedlings in the field plots (Table S17; *F* = 2.95, *p* = 0.11; *n* = 32 seedlings).

Discussion

Though recent studies have focused on quantifying patterns of CDD among co-occurring species and determining whether such patterns are caused primarily by natural enemies, mutualists, or competition with neighbors (Chanthorn et al., 2010; Kotanen, 2010; Lebrija-Trejos et al., 2014), less is known about the patterns and causes of intraspecific variation in conspecific seedling performance. In parallel shadehouse and field experiments with a dioecious tropical tree species in Panama, we examined variation in conspecific seedling performance beneath established conspecific adult trees and tested whether seedling growth and survival were negatively impacted by seed input or size of the conspecific adult and whether such effects were mediated by soil microbes. We did not find evidence in support of the hypothesis that seed production by female trees decreases seedling performance in female soils. In the field and in the shadehouse, seedling survival and growth were similar among seedlings grown in female conspecific, male conspecific, and heterospecific soils. However, unexpectedly, seedling colonization by AM fungi was more frequent in the roots of seedlings grown in male soils than in the roots of seedlings grown in female or heterospecific soils. Together, our results suggest that though plant–mycorrhizal interactions differ between seedlings in male and female soils, these differences do not necessarily translate to differences in seedling growth or survival.

Traits that vary among individuals within populations are often overlooked in community ecology but are an important source of intraspecific variation in ecological dynamics (Freckleton & Lewis, 2006). In our study, we asked whether intraspecific variation in factors such as size or seed production of established plants could underlie variation in the performance of conspecific seedlings in their soils. Though we hypothesized that variation in seed production by adult trees would cause variation in the accumulation of host-specific natural enemies, and consequently, variation in conspecific seedling performance in their environments, we did not find evidence in support of this hypothesis. Seedling survival, growth, and leaf damage in conspecific soils were independent of the amount of seeds produced by females and of the stark contrast in seed production between male and female adults of the species. In a shadehouse experiment with another dioecious tropical tree species in Ghana, Hood et al. (2004) found reductions in survival and higher disease incidence for seedlings grown in female relative to male soils, indicating that such differences may occur in other species. Because we did not quantify enemy densities in our study, we cannot rule out the possibility that seed production could cause short- or long-term changes to the density or composition of natural enemy communities near established plants. However, our study suggests that any such changes, if they occur, do not have a strong influence on conspecific seedling performance in our study species. Effects of seed production on conspecific seedling performance via natural enemies (or mutualists) could still occur in other species, emerge over longer time periods or during later developmental stages, or could be critically determined during or immediately after seed germination. In contrast, we found that increases in the number of experimental conspecific seedlings in our field plots negatively affected seedling survival, in line with other studies showing the importance of conspecific seedling neighbors in determining seedling performance (Harms et al., 2000; Queenborough et al., 2007a; Comita et al., 2010; Metz et al., 2010; Lebrija-Trejos et al., 2014).

We also asked whether the size of established conspecifics might influence the performance of conspecific seedlings in their soils. By studying a dioecious species, we were able to decouple the influences of size and seed production, because male trees are as large as females but do not produce fruit. In the field, we found some effects of the size of the conspecific adult a seedling was growing near on its survival. Specifically, seedling survival was slightly higher in the soils of larger conspecific trees relative to smaller ones. In contrast, seedling growth was reduced in the soils of larger conspecific trees relative to smaller ones. Growth–defense tradeoffs are common in plants (reviewed by Huot et al., 2014); together, these findings could indicate a tradeoff in which seedlings in the soils of larger conspecific adults invest energetic resources in defense (thus aiding survival), while divesting resources away from growth. This could occur if natural enemy conditions are more limiting in the soils of larger conspecific adults relative to smaller ones. However, we did not observe any effects of conspecific tree size in the shadehouse experiment, suggesting that any differences in seedling performance due to conspecific size are not caused by soilborne microbes. Thus, such effects may be driven by other types of host-specific enemies (e.g., foliar pathogens or insect herbivores) or stronger competition for resources near larger adult trees. Future studies that quantify enemy abundances and seedling–enemy interactions, as well as resource competition, in the environments of conspecific trees that vary in size are necessary to conclude whether adult size is an important factor influencing conspecific seedling performance.

Plant genotype and the level of genetic relatedness between seedlings and nearby adults may also influence seedling performance in conspecific soils (Browne & Karubian, 2016; Eck et al., 2019). In our study species, shadehouse seedlings that were grown in the soil microbial community associated with their maternal tree had lower biomass than seedlings that were grown in the soil microbial community of an unrelated conspecific female adult (Eck et al., 2019). This finding demonstrates that the shadehouse experimental approach utilized in our study is capable of detecting differences in conspecific soil effects. Together, these shadehouse results suggest that genetic relatedness to the seedling, rather than the size or seed production of nearby adults, determines the effect of conspecific soil microbial communities on seedling performance. Lack of differences in seedling growth between male and female conspecific soil microbial inoculum sources in our study could be because i) we did not account for relatedness of the soil donor tree to the focal seedling and ii) the soil donor trees likely had varying levels of relatedness to the experimental seedlings. In addition, though negative plant-soil feedbacks have also been documented in our study species (Mangan et al., 2010b), we did not find differences between *V. surinamensis* seedling performance in conspecific soils versus heterospecific soils in our study. However, because plant-soil feedbacks arise from pairwise interactions between co-occurring species (rather than from comparisons of the performance of one species in conspecific versus heterospecific soils), our result does not necessarily contradict prior studies demonstrating negative plant-soil feedbacks in *V. surinamensis*. Thus, host-specific soil-borne pathogens could still be a key factor determining seedling performance in our study species. Furthermore, a study of a subtropical tree community at the Heishiding Nature Reserve in China found a positive relationship between the density of conspecific adults and soil pathogens that led to higher conspecific seedling mortality (Liang et al., 2016); thus, the density of conspecific adults may have a larger effect on enemy densities than the size or seed production of just the nearest conspecific adult.

Mutualistic AM fungi are also thought to be important in influencing CDD and seedling performance (Bever, 2002; Mangan et al., 2010b; Bachelot et al., 2015; Liang et al., 2015; Bachelot et al., 2017; Bennett et al., 2017; Jevon et al., 2022). We hypothesized that the repeated input of conspecific seeds and/or seedlings near female trees would result in more opportunities for the growth of AM fungi, and subsequently, higher colonization rates by AM fungi in seedlings grown in female conspecific soils relative to male soils. Contrary to our expectations, colonization by AM fungi was higher in seedlings grown in male conspecific soils relative to female conspecific soils. This result suggests that another mechanism may be affecting AM fungal abundance, such as the release of AM fungal growth (or seedling association with AM fungi) in male soils from pathogen suppression in female soils (or other constraints). Though nutrient conditions were similar among seedlings grown in female and male soil inocula at the end of the experiment, nutrient conditions were more strongly linked to AM fungal colonization rates in female soils than in conspecific soils in general. In the dioecious tropical tree *Milicia regia* (Moraceae), Hood et al. (2004) found differences in AM fungal colonization depending on whether seedlings were grown in conspecific versus distant soils, but not between female versus male soils, suggesting that these dynamics could vary among plant species. In addition, in our study differences in AM fungal colonization did not translate to differences in seedling performance in the soil microbial communities associated with male conspecific versus female conspecific trees. Though negative impacts of the soil microbial communities near adults on conspecific seedlings appear to be more common than positive impacts, especially in AM fungal-associated trees (Mangan et al. 2010a, Liu et al. 2012, McCarthy-Neumann & Ibáñez 2013, Bennett et al. 2017, Eck et al., 2019), AM fungal benefits to seedlings are strong enough to balance the negative impact of host-specific pathogens in at least some tree species (Mangan et al., 2010b; Liang et al. 2015). Because AM fungal-associating tree species tend to show stronger negative CDD than EM fungal-associating tree species (Jiang et al., 2020; Delavaux et al., *in prep.*), this could indicate that the impact of AM fungi are relatively weak in these systems.

The advantages and disadvantages of the dioecious reproductive system and how dioecious species are maintained in plant communities is an important topic of current research (Bruijning et al., 2017). Our study adds to this discussion by suggesting that male environments may provide some advantages over female environments for conspecific seedlings, mainly in colonization by mutualist AM fungi. Sex ratios in dioecious tree species tend to be male-biased (Queenborough et al., 2007b), though the sex ratio is uniform in *V. surinamensis* (Riba-Hernandez et al. 2014). The distributions of adult *V. surinamensis* are aggregated, with males and females occurring equally frequently within patches (Riba-Hernandez et al., 2014). In our study, AM fungal colonization in the soil inocula of female conspecific trees was not related to the quantity of seeds the female produced. This indicates that the influence of seed production on seedling-AM fungal dynamics in adult soils is likely more qualitative, i.e., related to broader qualities that may differ among female and male environments, rather than quantitative, i.e., related specifically to variation in the quantity of seeds produced. In the context of dioecy, higher colonization by AM fungi (or other mutualist advantages) in some conspecific sites could cause reductions in negative CDD within species and act as a selection force on species coexistence (Montesinos et al., 2007). However, because we did not find differences in seedling growth or survival near male and female trees, our study suggests overall tolerance of seedlings to female seed production (and any associated environmental changes). This tolerance could help seedlings withstand any potentially negative impacts of aggregated seed production (e.g., higher enemy densities).

The causes and consequences of interspecific and intraspecific variation in negative CDD continue to be an important area of future research (Comita & Stump, 2020). The demographic patterns predicted by Janzen (1970) and Connell (1971) have been shown to occur in a variety of plant communities worldwide (reviewed by Carson & Schnitzer 2008; Terborgh, 2011; Comita et al., 2014), and theoretical studies demonstrate that they can enhance local species richness (Adler & Muller-Landau, 2005; but see Stump & Comita, 2018) and structure the relative abundance of species in plant communities (Mangan et al., 2010b). Recently, studies expanding on the Janzen–Connell hypothesis to consider intraspecific effects within populations and have found that within-species variation may also contribute to the maintenance of species diversity and the evolution of plant traits, such as seed dispersal (Schupp et al., 1992; Liu et al., 2015; Browne & Karubian, 2016; Marden et al., 2017; Eck et al., 2019). Variation in conspecific seedling survival due to variation in plant-enemy or plant-mutualist interactions within species could help to enhance plant species diversity by enabling species to differ in the distributions of their responses to the environment (Clark, 2010). Our study does not provide evidence of variation in negative CDD within species, but instead shows variation in biotic interactions between conspecific seedlings and adults and mutualists. Intraspecific variation in negative conspecific effects was not explained by differences in seed inputs or size of established conspecific adults. Understanding the factors that influence conspecific seedling survival near adult plants will help us better understand how natural enemies and mutualists structure plant diversity and abundance.

Figures

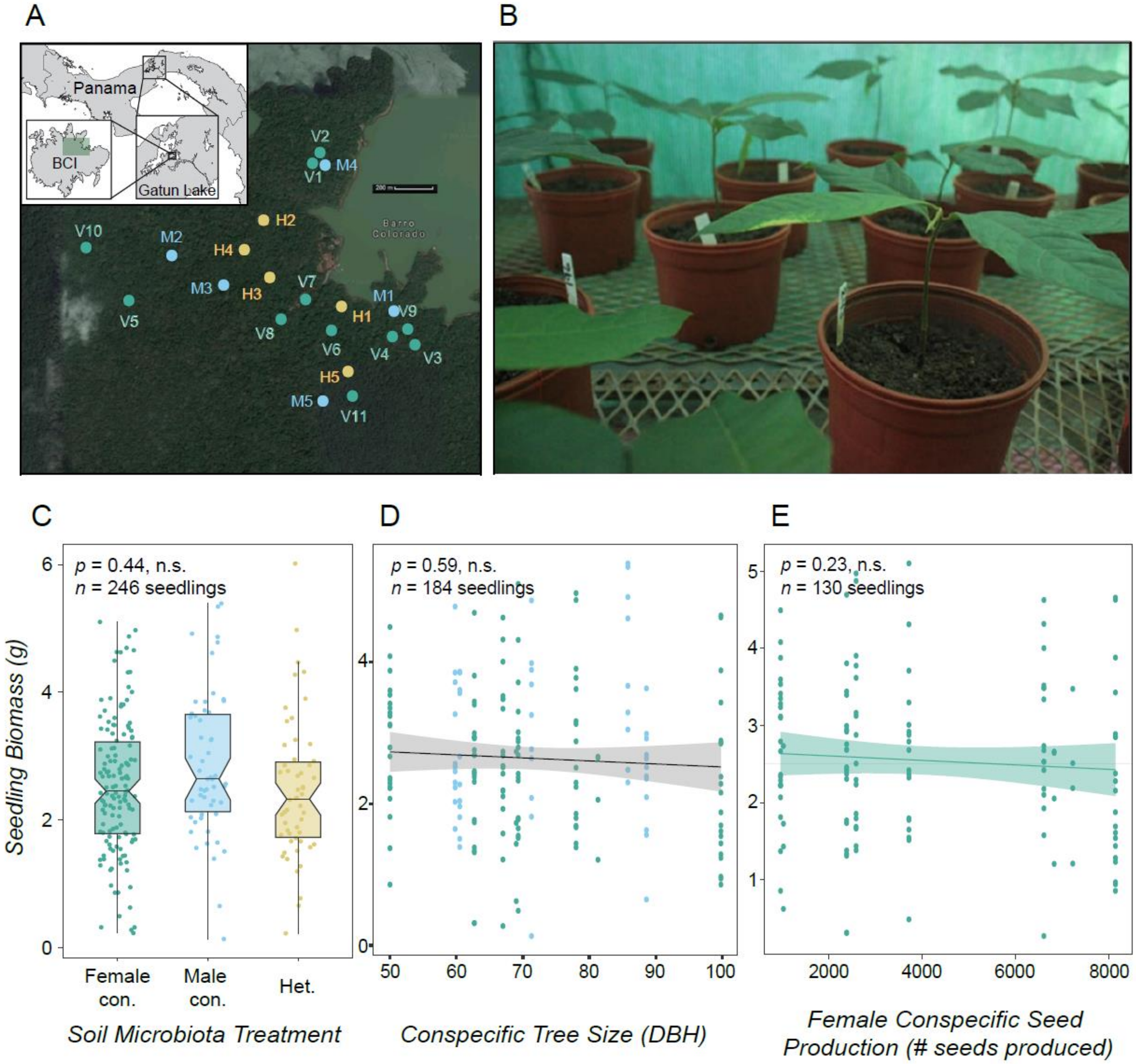

Figure 1: In a shadehouse experiment, seedling biomass was similar in female conspecific and male conspecific soil microbial communities. *Panel A: Virola surinamensis* and heterospecific trees used as soil inoculum sources in the shadehouse experiment are located on Barro Colorado Island (BCI), Panama (the study area is shaded in green on the BCI map). Female *V. surinamensis* are marked in green (V1 - V15), male *V. surinamensis* in blue (M1 - M5), and heterospecific trees in yellow (H1 – H5). Map data: Google, ©2017. Map inset modified from Baldeck *et al*., 2014. *Panel B:* In a shadehouse on BCI, we planted *V. surinamensis* seedlings in three soil microbial community inoculation treatments: female conspecific soil, male conspecific soil, or heterospecific soil. *Panel C*: At the end of the 8-mo shadehouse experiment, the biomass of *V. surinamensis* seedlings grown in the soil microbial community associated with female conspecific trees was similar to seedlings grown in the soil microbial community associated with male conspecific trees or heterospecific trees (Table S2).

Panel D: Seedling biomass at the end of the shadehouse experiment was not related to the size of the conspecific tree whose soil microbial community the seedlings were grown in (*Panel D*; Table S3) or the amount of seed production by the female conspecific tree whose soil microbial community the seedlings were grown in (*Panel E*; Table S4). In *C*, box belts show the median values, box notches represent a 95 % confidence interval for comparing medians, box hinges correspond to the first and third quartiles, and box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. In *D* & *E*, shaded areas represent 95 % confidence interval bands surrounding the best fit regression lines.

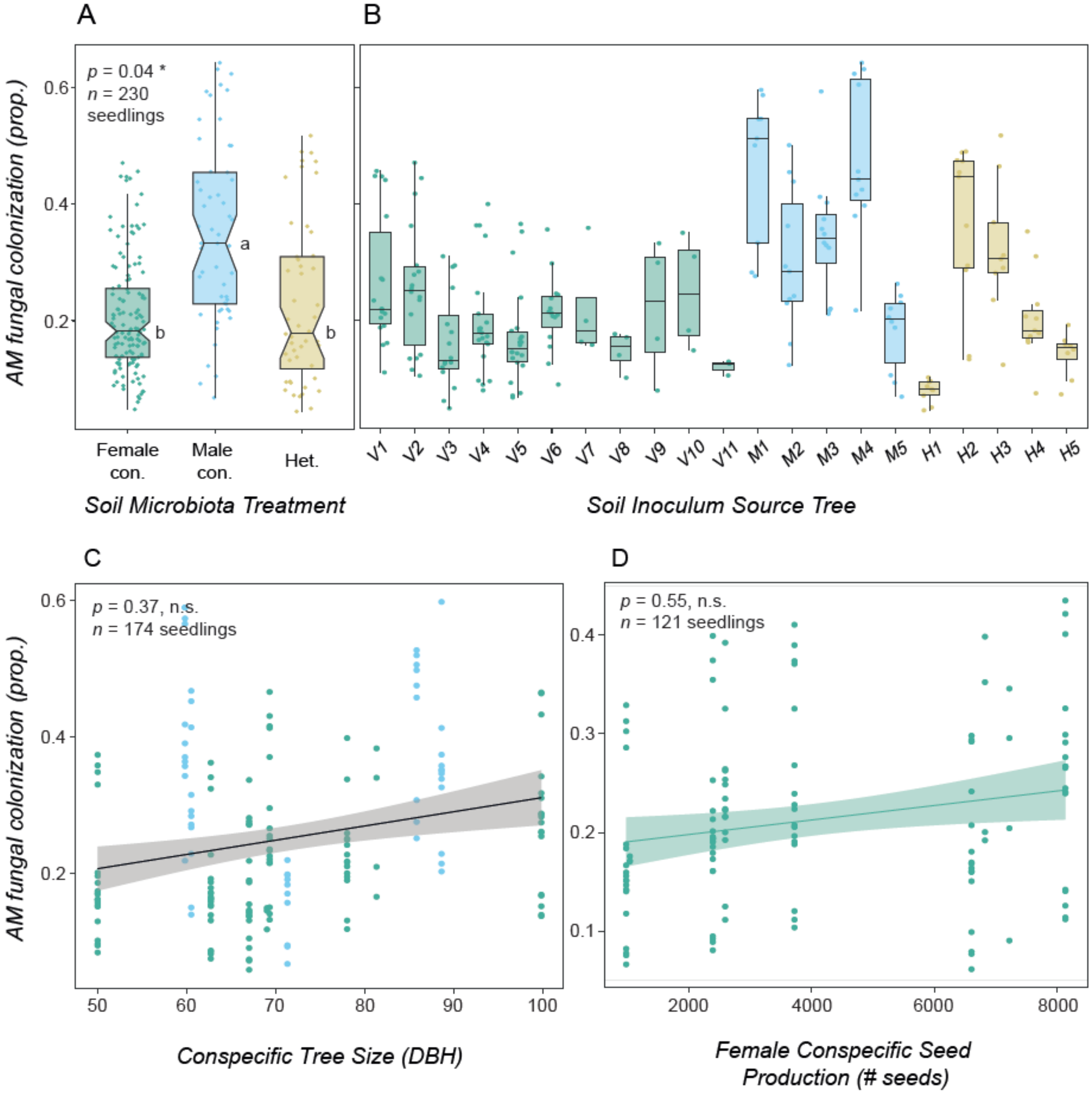

Figure 2. Seedling colonization by AM fungi was higher in the soil microbial communities associated with male conspecific trees in a shadehouse experiment. *Panel A:* At the end of the 8-mo shadehouse experiment on Barro Colorado Island (Panama), colonization by AM fungi was higher in *Virola surinamensis* seedlings grown in the soil microbial communities associated with male conspecific trees relative to seedlings grown in soil microbial communities associated with female conspecific trees or heterospecific trees (Table S10). *Panel B*: In four of the five male soil microbial communities tested, the median predicted values for AM fungal colonization were higher than in any of the 11 female soil microbial communities tested. *Panel C:* At the end of the shadehouse experiment, AM fungal colonization was similar in seedlings grown in the soil microbiota from larger and smaller conspecific soil inoculum source trees (Table S11). *Panel D*:

AM fungal colonization at the end of the shadehouse experiment was also similar in seedlings grown in the soil microbiota from female conspecific trees that produced more or less seeds (Table S12). In *A* & *B*, box belts show the median predicted values, box hinges correspond to the first and third quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges (predicted values are plotted to show patterns after accounting for covariates). In *A*, box notches represent a 95 % confidence interval for comparing predicted medians. In *C* & *D*, shaded areas represent 95 % confidence interval bands surrounding the best fit regression lines.

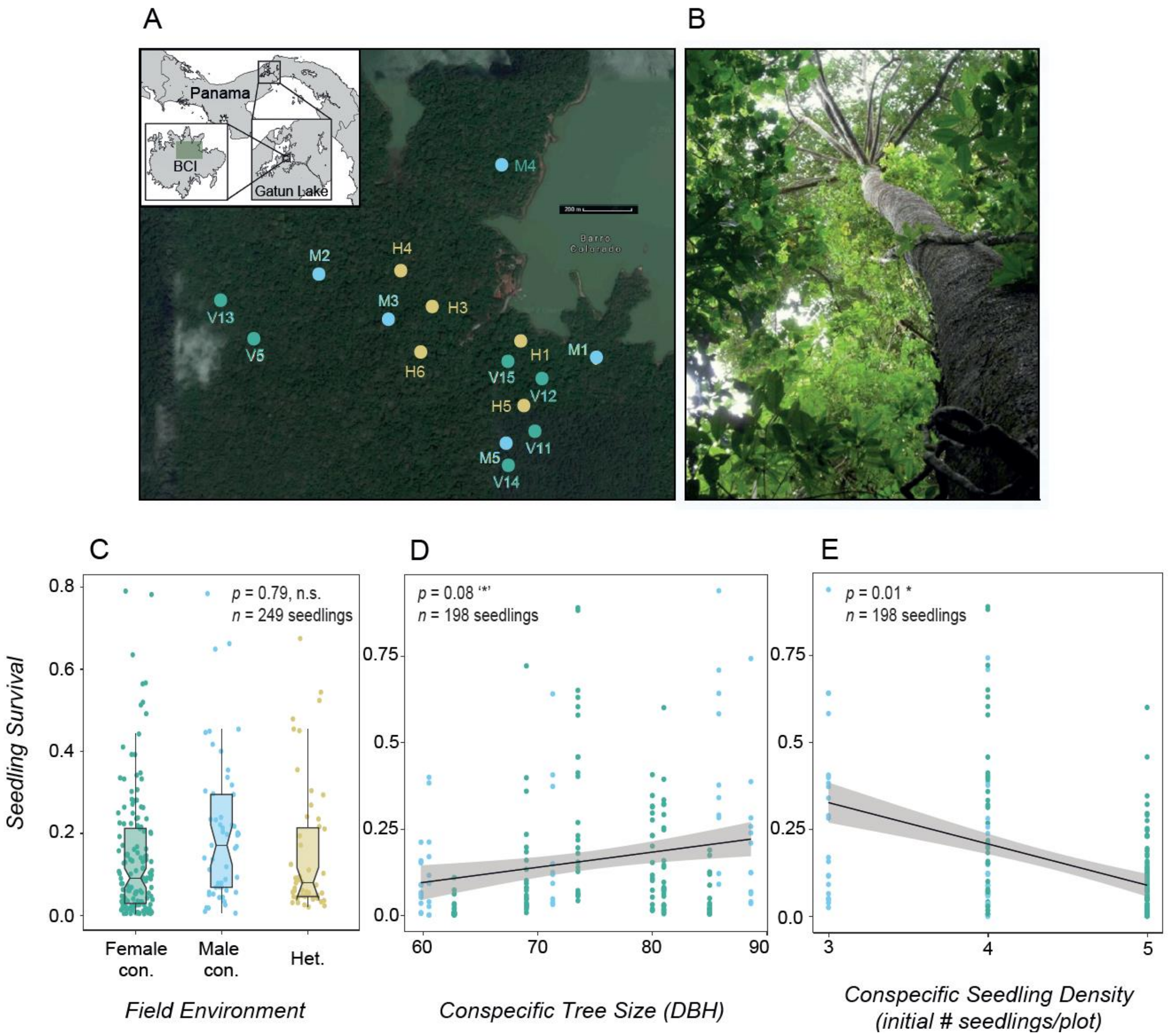

Figure 3. In a field experiment, seedling survival was similar in female conspecific and male conspecific environments but was influenced by other factors. *Panel A: Virola surinamensis* and heterospecific trees used as soil inoculum sources in the field experiment are located on Barro Colorado Island (BCI), Panama (the study area is shaded in green on the BCI map). Female *V. surinamensis* are marked in green (V5, V11 - V15), male *V. surinamensis* in blue (M1 - M5), and heterospecific trees in yellow (H1, H3 – H6). Map data: Google, ©2017. Map inset modified from Baldeck *et al*., 2014. *Panel B:* In a field experiment on BCI, we planted *V. surinamensis* seedlings in three field treatments: female conspecific environments, male conspecific environments, or heterospecific environments. *Panel C*: At the end of the 7-mo field experiment, the survival of *V. surinamensis* seedlings was similar among female conspecific, male conspecific, and heterospecific field environments (Table S14). *Panel D*: At the end of the field experiment, we found a marginally significant positive effect of the size of the adult conspecific tree on the survival of experimental seedlings (Table S15). *Panel E*: In the field experiment, seedling survival decreased as the number of experimental conspecific seedlings in the field plots increased (Table S15). In *C*, predicted values are plotted as points, box belts show the median predicted values, box notches represent a 95 % confidence interval for comparing

predicted medians, box hinges correspond to the first and third quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. In *D* & *E*, shaded areas represent 95 % confidence interval bands surrounding the best fit regression lines.

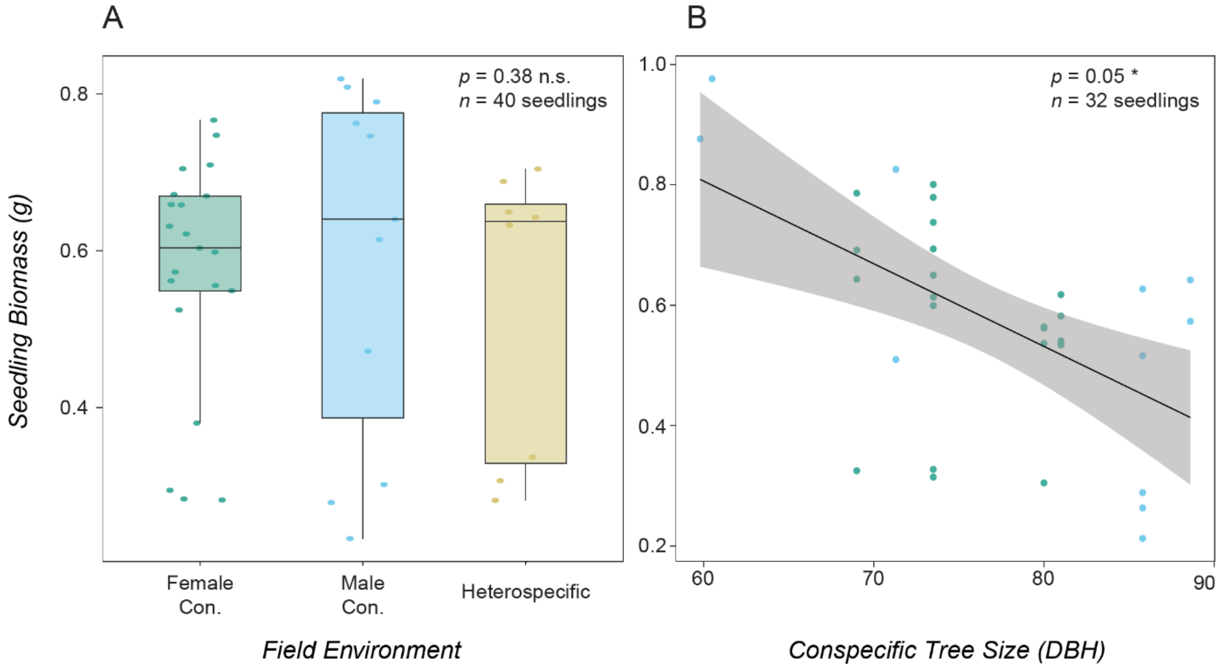

Figure 4. In a field experiment, seedling biomass was similar among female conspecific and male conspecific environments but was influenced by tree size. *Panel A:* At the end of a 7-mo field experiment on Barro Colorado Island (Panama), the biomass of the *Virola surinamensis* seedlings that survived until the end of the experiment was similar among seedlings growing in female conspecific, male conspecific, and heterospecific field environments (Table S16). *Panel B:* We found a negative effect of conspecific tree size on seedling biomass at the end of the field experiment (Table S17). In *A*, predicted values are plotted as points, box belts show the median predicted values, box notches represent a 95 % confidence interval for comparing predicted medians, box hinges correspond to the first and third quartiles, while box whiskers extend to the largest and smallest value no further than 1.5 × the interquartile range from the hinges. In *B*, the shaded area represents 95 % confidence interval bands surrounding the best fit regression line.

Author Contributions

JLE and LSC designed the experiments, with help from SAQ and CSD. JLE, CSD, and DMW collected the data. JLE, LSC, and SAQ analyzed the data. JLE wrote the first draft of the manuscript. All authors approved the final version of the manuscript.

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References

- Aarssen, L. W. & Jordan, C. (2001). Between-species patterns of covariation in plant size, seed size and fecundity in monocarpic herbs. *Ecoscience* 8(4): 471-477.
- Adler, F. R., & Muller-Landau, H. C. (2005). When do localized natural enemies increase species richness? *Ecology Letters* 8, 438-447.
- Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., Meiners, J. M., Tredennick, A. T., Veblen, K. E. (2018). Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecology Letters* 21(9): 1319-1329.
- Bachelot, B., Uriarte, M., & McGuire, K. (2015). Interactions among mutualism, competition, and predation foster species coexistence in diverse communities. *Theoretical Ecology* 8, 297-312.
- Bachelot, B., Uriarte, M., McGuire, K. L., Thompson, J., & Zimmerman, J. (2017). Arbuscular mycorrhizal fungal diversity and natural enemies promote coexistence of tropical tree species. *Ecology* 98(3): 712-720.
- Baldeck, C. & Asner, G., Martin, R., Anderson, C., Knapp, D., Kellner, J. & Wright, S. J. (2014). Operational tree species mapping in a diverse tropical forest with airborne imaging spectroscopy. *PloS one, 10*. 10.1371/journal.pone.0118403.
- Bates, D., Maechler, M., Bolker, B., & Walker, W. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67(1)*, 1-48.
- Bell, T, Freckleton, R. P., & Lewis, O. T. (2006). Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters* 9, 569-574.
- Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* 355, 181-184.
- Bever, J. D. (2002). Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proc. R. Soc. Lond. B* 269, 2595-2601.
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M. & Bolker, B. M. (2017). glmmTMB balances speed and

flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9(2), 378-400. doi:10.32614/RJ-2017-066

- Browne, L, & Karubian, J. (2016). Frequency-dependent selection for rare genotypes promotes genetic diversity of a tropical palm. *Ecology Letters* 19, 1439-1447.
- Bruijning, M., Visser, M. D., Muller-Landau, H. C., Wright, S. J., Comita, L. S., Hubbell, S. P., de Kroon, H., & Jongejans, E. (2017). Surviving in a cosexual world: a cost-benefit analysis of dioecy in tropical trees. *American Naturalist* 189(3), 297-314.
- Carson, W. P., Anderson, J. T., Leigh, E. G., & Schnitzer, S. A. (2008). Challenges associated with testing and falsifying the Janzen-Connell hypothesis: a review and critique. Tropical Forest Community Ecology (eds W. P. Carson & S. A. Schnitzer), pp. 210-241. Wiley-Blackwell, Chichester, UK.
- Chanthorn, W., Caughlin, T., Dechkla, S., & Brockelman, W. Y. (2013). The relative importance of fungal infection, conspecific density and environmental heterogeneity for seedling survival in a dominant tropical tree. *Biotropica* 45(5), 587-593.
- Chen, L., Swenson, N. G., Ji, N., Mi, X., Ren, H., Guo, L. & Ma, K. (2019). Differential soil fungus accumulation and density dependence of trees in a subtropical forest. *Science* 366: 124-128.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* 31, 343-346.
- Clark, J. S. (2010). Individuals and the variation needed for high species diversity in forest trees. *Science* 327, 1129-1132.
- Comita, L. S., Muller-Landau, H. C., Aguilar, S., & Hubbell, S. P. (2010). Asymmetric density dependence shapes species abundances in a tropical tree community. *Science* 329, 330-332.
- Comita, L. S., Queenborough, S. A., Murphy, S. J., Eck, J. L., Xu, K., Krishnadas, M., Beckman, N., & Zhu, Y. (2014). Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance-dependent and density-dependent seed and seedling survival. *Journal of Ecology* 102, 845-856.
- Comita, L.S. & Stump, S. M. (2020). Natural enemies and the maintenance of tropical tree diversity: recent insights and implications for the future of biodiversity in a changing world. *Annals of the Missouri Botanical Garden* 105(3), 377-392.

- Condit, R., Ashton, P. S., Baker, P., Bunyavejchewin, S., Gunatilleke, S., Gunatilleke, N., Hubbell, S. P., Foster, R. B., Itoh, A., LaFrankie, J. V., Lee, H. S., Losos, E., Manokaran, N., Sukumar, R., & Yamakura, T. (2000). Spatial patterns in the distribution of tropical tree species. *Science*, *288*, 1414-1418.
- Connell, J. H. (1971). On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. Dynamics of Populations (eds. den Boer, P. J. & Gradwell, G. R.) 298-312, Center for Agricultural Publication and Documentation, Wageningen.
- Croat, T. B. (1978). Flora of Barro Colorado Island. Stanford University Press, Stanford, CA, USA.
- Delavaux, C. S., Smith-Ramesh, L. M. & Kuebbing, S. E. (2017). Beyond nutrients: a meta-analysis of the diverse effects of mycorrhizal fungi on plants and soils. *Ecology* 98(8), 2111-2119.
- Eck, J. L., Stump, S. M., Delavaux, C. S., Mangan, S. A., & Comita, L. S. (2019). Evidence of within-species specialization by soil microbes and the implications for plant community diversity. *PNAS* 116(15): 7371-7376.
- Fisher, B. L., Howe, H. F., & Wright, S. J. (1991). Survival and growth of *Virola surinamensis* yearlings: water augmentation in gap and understory. *Oecologia*, 86(2), 292-297.
- Fox, J. & Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition. Thousand Oaks, CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/
- Freckleton, R. P. & Lewis, O. T. (2006). Pathogens, density dependence and the coexistence of tropical trees. *Proceedings: Biological Sciences* 273(1604), 2909-2916.
- Harms, K. E., Wright, S. J., Calderon, O. Hernandez, A., & Herre, E. A. (2000). Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature* 404, 494-495.
- Harms, K. E., Condit, R., Hubbell, S. P., & Foster, R. B. (2001). Habitat associations of trees and shrubs in a 50-ha neotropical forest plot. *Journal of Ecology*, *89*, 947-959.
- Hood, L. A., Swaine, M. D., & Mason, P. A. (2004). The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. *Journal of Ecology* 92, 816-823.

- Howe, H. F. (1990). Survival and growth of juvenile *Virola surinamensis* in Panama: effects of herbivory and canopy closure. *Journal of Tropical Ecology, 6(3),* 259-280.
- Howe, H. F. & Vande Kerckhove, G. A. (1981). Removal of wild nutmeg (*Virola surinamensis*) crops by birds. *Ecology, 62(4),* 1093-1106.
- Hubbell, S.P. (1980). Seed predation and the coexistence of tree species in tropical forests. *Oikos*, 35, 214–229.
- Hülsmann, L., Chisholm, R., & Hartig, F. (2021). Is variation in conspecific negative density dependence driving tree diversity patterns at large scales? *Trends Ecol. Evol.*, 36(2), 151-163.
- Janzen, D. H. (1970). Herbivores and the number of tree species in tropical forests. *American Naturalist* 104, 501-528.
- Jardim, M. A. G. & da Mota, C. G. (2007). *Virola surinamensis* (Rol.) Warb. (Myristicaceae) floral biology. *Revista Arvore, 31(6),* https://doi.org/10.1590/S0100-67622007000600020
- Jevon, F. V., De La Cruz, D., LaManna, J. A., Lang, A. K., Orwig, D. A., Record, S., Kouba, P. V., Ayres, M. P. & Matthes, J. H. (2022). Experimental and observational evidence of negative conspecific density dependence in temperate ectomycorrhizal trees. *Ecology* 103(11):e3808. doi: 10.1002/ecy.3808
- Jia, S., Wang, X., Yuan, Z., Lin, F., Ye, J., Lin, G., Hao, Z. & Bagchi, R. (2020). Tree species traits affect which natural enemies drive the Janzen-Connell effect in a temperate forest. *Nature Communications* 11(286). https://doi.org/10.1038/s41467-019-14140-y
- Jiang, F., Zhu, K., Cadotte, M. W., & Jin, G. (2020). Tree mycorrhizal type mediates the strength of negative density dependence in temperate forests. *Journal of Ecology* 108(6):2601-2610.
- Kobe, R.K. & Vriesendorp, C.F. (2011). Conspecific density dependence in seedlings varies with species shade tolerance in a wet tropical forest. *Ecol. Lett.*, 14, 503–510.
- Kotanen, P. M. (2010). Effects of fungal seed pathogens under conspecific and heterospecific trees in a temperate forest. *Can. J. Bot*. 85, 918-925.
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2016). lmerTest: Tests in linear mixed effects models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest

- Lebrija-Trejos, E., Wright, S. J., Hernández, A., & Reich, P. B. (2014). Does relatedness matter? Phylogenetic density-dependent survival of seedlings in a tropical forest. *Ecology* 95(4), 940-951.
- Lebrija-Trejos, E., Reich, P. B., Hernández, A. & Wright, S. J. (2016). Species with greater seed mass are more tolerant of conspecific neighbors: a key driver of early survival and future abundances in a tropical forest. *Ecology Letters*, 19(9), 1071-1080.
- Liang, M., Liu, X., Etienne, R. S., Huang, F., Wang, Y., & Yu, S. (2015). Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology* 96(2), 562-574.
- Liang, M., Liu, X., Gilbert, G. S., Zheng, Y., Luo, S., Huang, F., & Yu, S. (2016). Adult trees cause density-dependent mortality in conspecific seedlings by regulating the frequency of pathogenic soil fungi. *Ecology Letters* 19, 1448-1456.
- Liu, X., Liang, M., Etienne, R. S., Wang, Y., Staehelin, S., & Yu, S. (2012). Experimental evidence for a phylogenetic Janzen-Connell effect in a subtropical forest. *Ecology Letters* 15, 111-118.
- Liu, X., Etienne, R. S., Liang, M., Wang, Y., Yu, S. (2015). Experimental evidence for an intraspecific Janzen-Connell effect mediated by soil biota. *Ecology* 96(3), 662-671.
- Mangan, S. A., Herre, E. A., & Bever, J. D. (2010a). Specificity between Neotropical tree seedlings and their fungal mutualists leads to plant-soil feedback. *Ecology* 91(9), 2594-2603.
- Mangan, S. A., Schnitzer, S. A., Herre, E. A., Mack, K. M. L., Valencia, M. C., Sanchez, E. I., & Bever, J. D. (2010b). Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466, 752-755.
- Marden, J. H., Mangan, S. A., Peterson, M. P., Wafula, E., Fescemyer, H. W., Der, J. P., DePamphilis, C. W., & Comita, L. S. (2017). Ecological genomics of tropical trees: how local population size and allelic diversity of resistance genes relate to immune responses, cosusceptibility to pathogens, and negative density dependence. *Molecular Ecology* doi: 10.1111/mec.13999
- McCarthy-Neumann, S., & Ibáñez, I. (2013). Plant-soil feedback links negative distance dependence and light gradient partitioning during seedling establishment. *Ecology* 94(4), 780-786.

- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, *115*, 495-495.
- Metz, M. R., Sousa, W. P., & Valencio, R. (2010). Widespread density-dependent seedling mortality promotes species coexistence in a highly diverse Amazonian rain forest. *Ecology* 91(12), 3675-3685.
- Montesinos, D., Verdú, M., & García-Fayos, P. (2007). Moms are better nurses than dads: gender biased self-facilitation in a dioecious Juniperus tree. *Journal of Vegetation Science* 18, 271-280.
- Newsham, K. K., Fitter, A. H., & Watkinson, A. R. (1995). Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* 83, 991-1000.
- Packer, A. & Clay, K. (2003). Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees. *Ecology*, 84(1), 108-119.
- Paine, C. E., Harms, K. E., Schnitzer, S. A. & Carson, W. P. (2008). Weak competition among tropical tree seedlings: implications for species coexistence. *Biotropica* 40(4), 432-440.
- Piao, T., Comita, L.S., Jin, G. & Kim, J.H. (2013). Density dependence across multiple life stages in a temperate old-growth forest of northeast China. *Oikos*, 172, 107–117.
- Pozo, M. J. & Azcón-Aguilar, C. (2007). Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10, 393-398.
- Pujol, B. & Mckey, D. (2006). Size asymmetry in intraspecific competition and the density-dependence of inbreeding depression in a natural plant population: a case study in cassava (*Manihot esculenta* Crantz, Euphorbiaceae). *Journal of Evolutionary Biology* 19(1): 85-96.
- Queenborough, S. A., Burslem, D. F. R. P., Garwood, N. C., & Valencia, R. (2007a). Neighborhood and community interactions determine the spatial pattern of tropical tree seedling survival. *Ecology*, *88(9)*, 2248-2258.
- Queenborough, S. A., Burslem, D. F. R. P., Garwood, N. C. & Valencia, R. (2007b). Determinants of biased sex ratios and inter-sex costs of reproduction in dioecious tropical forest trees. *American Journal of Botany* 94(1), 67-78.
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

- Reinhart, K. O., & Clay, K. (2009). Spatial variation in soil-borne disease dynamics of a temperate tree, *Prunus serotina*. *Ecology* 90(11), 2984-2993.
- Riba-Hernández, P., Segura, J. L., Fuchs, E. J., & Moreira, J. (2014). Population and genetic structure of two dioecious timber species *Virola surinamensis* and *Virola koschnyi* (Myristicaceae) in southwestern Costa Rica. *Forest Ecology and Management*, *323*, 168-176.
- Ronsheim, M. L., & Anderson, S. E. (2001). Population-level specificity in the plant-mycorrhizae association alters intraspecific interactions among neighboring plants. *Oecologia* 128, 77-84.
- 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.
- Smith, S. E., & Read, D. J. (2008). Mycorrhizal symbiosis. Third edition. Academic, London, UK.
- Song, X., Zhang, W., Johnson, D. J., Yang, J., Asefa, M., Deng, X., Yang, X. & Cao, M. (2020). Conspecific negative density dependence in rainy season enhanced seedling diversity across habitats in a tropical forest. *Oecologia* 193(4):949-957.
- Stump, S. M. & Comita, L. S. (2018). Interspecific variation in conspecific negative density dependence can make species less likely to coexist. *Ecology Letters* 21: 1541-1551.
- Swamy, V., Terborgh, J., Dexter, K.G., Best, B.D., Alvarez, P. & Cornejo, F. (2011). Are all seeds equal? Spatially explicit comparisons of seed fall and sapling recruitment in a tropical forest. *Ecol. Lett.*, 14, 195–201.
- Suzuki, R. O., Kudoh, H., & Kachi, N. (2003). Spatial and temporal variations in mortality of the biennial plant, *Lysimachi rubida*: effects of intraspecific competition and environmental heterogeneity. *Journal of Ecology* 91: 114-125.
- Uriarte, M., Canham, C. D., Thompson, J. & Zimmerman, J. K. (2004). A neighborhood analysis of tree growth and survival in a hurricane-driven tropical forest. *Ecological Monographs* 74(4): 591-614.
- Windsor, D. M. (1990). Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panamá. *Smithsonian Contributions to the Earth Sciences*, *29*, 1-145.

- Wright, S. J., Muller-Landau, H. C., Calderon, O. & Hernandez, A. (2005). Annual and spatial variation in seedfall and seedling recruitment in a Neotropical forest. *Ecology* 86(4), 848-860.
- Zimmerman, J. K., Wright, S. J., Calderón, O., Pagan, M. A. & Paton, S. (2007). Flowering and fruiting phenologies of seasonal and aseasonal neotropical forests: the role of annual changes in irradiance. *Journal of Tropical Ecology*, *23*, 231-251.
- Zang, L., Xu, H., Li, Y. & Zang, R. (2021). Conspecific negative density dependence of trees varies with plant functional traits and environmental conditions across scales in a 60-ha tropical rainforest dynamics plot. *Biotropica* 53(2): 693-702.
- Zhu, Y., Comita, L.S. & Hubbell, S.P. (2015). Conspecific and phylogenetic density-dependent survival differs across life stages in a tropical forest. *J. Ecol.*, 103, 957–966.
- Zhu, Y., Queenborough, S. A., Condit, R., Hubbell, S. P., Ma, K. P. & Comita, L. S. (2018). Density-dependent survival varies with species life-history strategy in a tropical forest. *Ecology Letters* 21(4): 506-515.