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Research article

Effects of tree species diversity and conspecific seedling density on insect herbivory and pathogen infection on big-leaf mahogany seedlings

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The Janzen–Connell hypothesis (JCH) predicts that attack by specialist enemies on seedlings increases with conspecific seedling density, but studies have rarely experimentally tested for the contingency of such effects on the tree community context (e.g. diversity, composition) or measured responses by different enemies (e.g. herbivores, pathogens). We conducted a field study in a large-scale system evaluating tree species diversity and conspecific density effects on leaf damage on mahogany *Swietenia macrophylla* seedlings. We established quadrats of eight levels of seedling density across mahogany tree monocultures and tree species polycultures including mahogany, and recorded percent leaf damage by insects and percent leaf necrosis by a pathogenic fungus on mahogany seedlings. We found contrasting effects of tree species diversity on insects and pathogens. Whereas tree diversity did not affect leaf damage by insects, it had a significant negative effect on leaf necrosis by pathogens whereby, on average, percent leaf necrosis on mahogany seedlings in polyculture was half of that observed in monoculture. We discuss the potential influences of changes in mahogany tree density versus frequency (relative to other tree species) driving this diversity effect. Unexpectedly, we found no effect of seedling conspecific density on either leaf insect or pathogen damage (i.e. density-independent attack). Likewise, we found no significant tree diversity by seedling density interaction, indicating that plant enemies were consistently unresponsive to variation in seedling density across levels of tree diversity. Overall, this study provides a unique test of the JCH by experimentally evaluating seedling density and tree diversity effects on contrasting plant enemies and, in turn, the controls they exert over plant recruitment.

Keywords: density-dependence, enemy traits, Janzen–Connell effect, leaf-cutter ants, pathogenic fungi



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Introduction

Plant diversity is a pervasive driver of ecosystem function and species interactions across trophic levels (Haddad et al. 2009, Nell et al. 2018). Numerous studies have found that it causes decreases in insect damage on plants, for example in the case of insects with a narrow diet breadth (i.e. specialists), whereas in the case of generalists plant diversity can increase, decrease or have no effect on herbivory rates (Schuldt et al. 2010, Castagneyrol et al. 2013, reviewed by Jactel et al. 2021). These diversity effects operate via 'associational' effects (reviewed by Barbosa et al. 2009, Underwood et al. 2014), whereby heterospecific neighbouring plants influence damage on focal plants via altered cues (Tahvanainen and Root 1972, Hambäck et al. 2000), or via changes in host plant density (e.g. 'resource concentration' effect; Root 1973, Moreira et al. 2016). Accordingly, negative effects of diversity on specialist enemies take place because host plants become increasingly rare with increasing diversity (Abdala-Roberts et al. 2015, Rutten et al. 2021) or due to chemical masking or physical interference by heterospecific neighboring plants (Hambäck et al. 2000, Castagneyrol et al. 2013). In contrast, generalist enemies are often unresponsive to changes in host plant density or frequency of heterospecifics since they are not restricted to feeding on any single plant species (Abdala-Roberts et al. 2015, Jactel et al. 2021). In some cases, however, plant diversity can even increase attack by generalists due to benefits of diet mixing in diverse patches or stands (Unsicker et al. 2008, Schuldt et al. 2010). Collectively, this body of research has yielded a predictive understanding of how plant neighbourhood heterogeneity shapes the outcome of plant–enemy interactions.

A related field of work has shown that plant enemy density-dependent responses to resource abundance are ubiquitous, with an understanding of their drivers shedding key mechanistic insight into the outcome of plant–enemy interactions (Abdala-Roberts et al. 2015). A good example of this is the Janzen–Connell hypothesis (JCH; Janzen 1970, Connell 1971) which predicts that attack rates on focal plants decrease with increasing distance from parental trees, mediated by decreasing density of seeds or seedlings away from high-density adult conspecific neighbourhoods (Wright 2002, Bagchi et al. 2010,; reviewed by Carson et al. 2008, Comita et al. 2014). Such enemy responses are expected to promote plant species coexistence because positive density-dependence (PDD) in enemy attack is stronger on dominant species (Bagchi et al. 2014). At the same time, however, some plant enemies have been shown to exhibit negative density-dependent or even density-independent responses (Otway et al. 2005, Abdala-Roberts and Mooney 2013). Expectedly, and as for plant diversity effects, enemy density-dependence is highly contingent on plant life-history traits (Song and Corlett 2021), type of attacker (pathogens versus herbivores; Bagchi et al. 2014, Jia et al. 2020), as well as attacker traits (e.g. diet breadth; Sedio and Ostling 2013). With respect to attacker traits, for example, these density-dependent dynamics can involve enemies with varying degrees

of dietary specializations, where generalists also exhibit density-dependent responses and potentially drive these dynamics as well (Lewis and Gripenberg 2008). Plant and enemy traits may additionally interact themselves (e.g. stronger pathogen and insect effects on shade-tolerant and intolerant plant species, respectively; Jia et al. 2020) as well as with other factors such as spatial scale (Gripenberg et al. 2014, Xiao et al. 2017). Addressing these sources of variation is therefore key for understanding and uncovering the mechanisms behind variation in plant enemy responses to resource availability.

Despite widespread evidence for plant enemy PDD, three significant limitations persist in this field of research. First, few studies testing the JCH have experimentally manipulated plant density to rigorously test attacker responses to resource availability (Bagchi et al. 2010, Germany et al. 2019). Rather, most work has reported correlations between damage and naturally occurring variation in plant density (reviewed by Freckleton and Lewis 2006, Comita et al. 2014). In this sense, efforts to include multiple levels of plant density are especially needed to robustly assess the shape or function of enemy density-dependence (Kim and Underwood 2015, Smith 2022). Second, studies have rarely experimentally controlled for tree community features (e.g. diversity) in testing for seedling density effects (Germany et al. 2019), even though enemy responses to resource availability are expected to change as a function of tree community characteristics. Indeed, studies testing JCH have largely focused on plant density effects while neglecting associational effects occurring in heterospecific tree neighborhoods (Jia et al. 2022), away from parental conspecifics. Studies that manipulate plant density across different levels of tree diversity or composition are needed to better understand variation in enemy responses to host plant abundance as a function of the tree community context and its underlying mechanisms. For example, adult density of a focal species can vary as a function of tree diversity and has been shown to be an important driver of enemy attack on conspecific seedlings due to canopy-to-understorey enemy spill over (Caballol et al. 2022). Such effects would presumably operate via one or more of the mechanisms mentioned previously, including resource concentration or associational effects. Accordingly, these dynamics set the stage for scarcely studied scale-dependent responses as well as interactions across scales in plant enemy attack. Third, simultaneous assessments of damage by different plant enemies are less common (Gripenberg et al. 2014, Krishnadas et al. 2018), including comparisons of insects versus pathogens which have been shown to vary in the strength or function of their density-dependent responses to host plant availability due to differences in traits such as dispersal ability and diet breadth (Jia et al. 2020). Addressing this knowledge gap can illuminate on consumer traits predictive of density-dependent processes shaping herbivory.

Here, we conducted a field study in a large-scale tree diversity experiment in southeast Mexico (Abdala-Roberts et al. 2015) to test for seedling density and tree diversity effects on seedling leaf damage by insects and pathogens. We established quadrats replicating eight levels of seedling density for

a focal species, broad-leaf mahogany *Swietenia macrophylla*, across two levels of tree diversity, namely mahogany tree monocultures and tree species polycultures including mahogany adults, and recorded leaf damage by generalist insects and a specialist fungus on mahogany seedlings. Specifically, we asked: 1) does tree diversity affect leaf damage on mahogany seedlings, and is such effect contingent on the type of attacker? Since most leaf damage recorded on mahogany seedlings was caused by generalist leaf cutter ants and a specialist fungus, we predicted positive and negative effects of tree diversity on insect leaf damage and pathogens, respectively (Moreira et al. 2016). Such responses could be mediated by changes in the density of adult mahogany trees, associational effects from heterospecific trees, or some other mechanism such as diet mixing (in the case of generalists) resulting in canopy-to-understorey effects on seedling leaf damage. 2) Does seedling density affect leaf damage by insects and pathogens? We tentatively assumed that both types of attackers would exhibit PDD, but that it would be stronger for pathogens based on results in other systems reporting on pathogen density-dependence (Bagchi et al. 2014, Song and Corlett 2021). And 3) does tree diversity influence the strength of PDD attack on mahogany seedlings (i.e. interactive effects of tree diversity by seedling density) and are such effects contingent on the type of enemy? In this case, one possibility is that PDD by the specialist pathogen is weaker in tree species mixtures, as this is where pathogen prevalence and infection rates would be lowest due to low adult host density (as predicted by the JCH; Germany et al. 2019), whereas generalist insect PDD would be stronger in tree polycultures where these plant enemies are expected to be more abundant. Alternatively, generalist insects might behave similarly to pathogens, i.e. weaker PDD in tree species mixtures, if they preferentially feed on mahogany. Overall, this study provides insight into density-dependent dynamics in plant–enemy interactions by experimentally testing seedling density and tree diversity effects on different plant enemies, ultimately improving our understanding of the drivers of plant recruitment and forest regeneration.

Material and methods

Study system

The study was conducted at the UADY Tree Diversity Experiment (<https://treedivnet.ugent.be/ExpUADY.html>), a large-scale system located at the Sitio Experimental Uxmal (20°24'44"N, 89°45'13"W) of INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias), located 70 km southwest of Merida, Yucatan (Mexico). The experiment was established in December 2011, covers an extension of 7.2 ha, and includes 74 21 × 21 m plots, each with a planting density of 64 trees per plot (3 m between plants), and 6 m between plots (Abdala-Roberts et al. 2015). We planted six tropical tree species, namely: *Swietenia macrophylla* (Meliaceae), *Enterolobium cyclocarpum* (Fabaceae), *Tabebuia*

rosea (Bignoniaceae), *Ceiba pentandra* (Malvaceae), *Piscidia piscipula* (Fabaceae) and *Cordia dodecandra* (Boraginaceae), all of which are native to the Yucatan Peninsula. Plots were classified as monocultures of one tree species or polycultures composed of random combinations of four out of the six species. This study focused exclusively on plant–enemy interactions associated to big-leaf mahogany, since this was the only species with enough plot-level replication in monoculture to test for interactive tree diversity by seedling density effects (experimental design details in Abdala-Roberts et al. 2015). In addition, we had prior knowledge on the natural history and plant–enemy interactions associated to this species to build upon with the present study (Abdala-Roberts et al. 2015, 2016).

Big-leaf mahogany, the focal tree species, is a self-compatible, long-lived tree distributed from southern Mexico to Bolivia (Pennington and Sarukhán 2005). During this study, insect leaf damage on mahogany seedlings was caused mainly by generalist leaf cutter ants *Acromyrmex octospinosus*. Although leaf cutter ants do not directly feed on leaves and instead use them to cultivate fungi consumed by their larvae, we expected them to show similar responses to host plant density and tree diversity compared to generalist insect herbivores, particularly for highly mobile insects with high plant tissue consumption rates. Damage by leaf cutter ants was observed on seedlings or saplings of several other plant species (e.g. *Neomillspaughia emarginata*, *Piscidia piscipula*) growing in the understory of the study plots (Abdala-Roberts unpubl.). Additional insects on mahogany seedlings included leafhoppers and beetles, but damage by these was much less common (Abdala-Roberts unpubl.). In addition, experimental seedlings were infected by a fungus (*Pseudocercospora* sp.), which was only observed on mahogany trees and seedlings in the system (Abdala-Roberts unpubl.). Several species of this genus have been reported as specialist pathogens on Meliaceae, including big-leaf mahogany (Braun et al. 2003, Farr and Rossman 2015). The fungus genus was identified taxonomically based on spore morphology from laboratory cultures of sampled leaves (Barnett and Hunter 2006).

Experimental design and response variables

We collected mahogany seeds in March 2021 from four adult trees located in tropical dry forest remnants nearby the town of Bacalar, southern Quintana Roo (Mexico). Seeds were germinated in 1 l polyethylene nursery bags and kept in a greenhouse for two months prior to transplantation. During this time, they were watered three times a week. In late July 2021, we transplanted seedlings at the experimental site in 10 mahogany monoculture plots and 10 polyculture plots containing mahogany trees. Mean tree height ranged from 4 to 12 m (depending on the species) when the study was conducted. Within each plot, seedlings were randomly allocated to 1 × 1 m quadrats with eight different densities, namely: 3, 5, 7, 9, 12, 14, 16 and 18 individuals. These densities are within the natural range of variation in mahogany seedling densities observed at sites where mahogany is moderately to

highly abundant in natural and managed tropical dry forests in southern Yucatan and Quintana Roo (López-Martínez unpubl.). We planted eight seedling quadrats per plot (one per density level), at least 3 m (frequently more) from the plot perimeter to avoid edge effects (Berthelot et al. 2021), for a total of 20 quadrats per density level (i.e. 10 per density by tree diversity combination) and 160 quadrats (n = 1680 seedlings). The total number of seedlings was the same across all plots (84). We watered each seedling once with ca 100 ml on the day of transplantation.

In early November 2021, three months after transplantation, we selected a random subsample of three seedlings at densities of 3–9 individuals, and five seedlings at densities of 12–18 individuals (total n = 640 seedlings). For these seedlings, we visually estimated for all leaves per plant the percent of leaf area removed by insects or with necrosis due to *Pseudocercospora* sp. and obtained a mean value per plant which represented an estimate of whole-plant leaf area lost due to enemy attack. In addition, for each seedling we also measured plant height (27.7 ± 0.23 cm [mean \pm SE]) to control for effects of plant size or vigor affecting leaf damage (Martini et al. 2021). Seedling mortality during the study was negligible (<5% across all seedlings) and therefore did not influence results. A preliminary survey of insect leaf damage conducted one month after transplantation indicated that close to a quarter (mean: $23.81 \pm 3.88\%$; monoculture: $20.83 \pm 2.68\%$; polyculture: $26.79 \pm 4.75\%$) of planted seedlings were attacked by *A. octospinosus* leaf cutter ants which were the main insect damaging mahogany seedlings. Finally, we also measured herbaceous cover adjacent to each seedling quadrat based on visual estimates to control for residual variation in understory plant abundance affecting responses. Cover was estimated visually within a 1-m² quadrat randomly placed north, south, west or east side of each seedling quadrat. Instances of leaf damage by insects and pathogens on the same leaf were rare (<5% of leaves measured).

Statistical analyses

We performed general linear mixed models testing for the effects of tree diversity, mahogany seedling density, and their interaction (all fixed) on percent insect leaf damage and necrosis, using mean values per seedling quadrat (n = 159, one quadrat lost due to mortality during the first month). Density was treated as a continuous predictor and previous analyses indicated that its quadratic term was not significant (insect damage: $t = -1.39$, $p = 0.16$; necrosis: $t = -1.22$, $p = 0.23$, i.e. no statistical evidence of non-linear density-dependence) and therefore removed from the models. Both models included tree plot as a random effect, and we also included seedling height (mean value across seedlings per quadrat) and herbaceous cover as covariates. Insect leaf damage was log-transformed and necrosis was square root-transformed to achieve normality of residuals. A preliminary GLMM indicated no association between percent insect leaf damage and necrosis using the latter as predictor since

insect leaf damage started earlier in the season ($F_{1, 138} = 0.66$, $p = 0.42$). We note that analyses were run at the quadrat level since plant density can be viewed as an emergent property of seedling patches affecting plant–enemy interactions, making the quadrat the appropriate scale for assessing responses. Nonetheless, we also ran analyses at the plant-level (including quadrat nested within tree plot and plot as random effects) and results were very similar to those using quadrat level data (Supporting information).

We ran all statistical analyses in R software ver. 4.2.1 (www.r-project.org). We ran the general linear mixed models using the *lmer* function from the ‘lmerTest’ package (Kuznetsova et al. 2017). We report least-square means and associated standard errors as descriptive statistics, obtained from the above models using the *lsmeans* function from the ‘lsmeans’ package (Lenth 2016). In addition, to visualize associations between leaf damage and seedling density, we obtained model predicted values after accounting for the tree plot effect by calculating the fixed effects, random effect (plot), and residual contributions to each observed data point using the fitted model (i.e. estimated Y values) and then subtracted the plot effect using the ‘broom.mixed’ package in R ver. 4.2 (www.r-project.org). These predicted values therefore directly corresponded to the test of main effects as the mixed models accounted for plot.

Results

We found no significant effect of tree diversity on insect leaf damage (monocultures: $1.42 \pm 0.38\%$; polycultures: $3.40 \pm 0.70\%$) (Table 1, Fig. 1). By contrast, there was a significant negative effect of tree diversity on leaf necrosis caused by pathogens (Table 1, Fig. 1), with polycultures ($3.91 \pm 1.10\%$) showing a ca 60% lower mean value relative to monocultures ($9.66 \pm 1.10\%$). Contrary to expectations, we found no significant effect of conspecific seedling density on either insect (slope estimator = 0.011 ± 0.019 , $t = -0.59$, $p = 0.55$) or pathogen (-0.010 ± 0.020 , $t = -0.50$, $p = 0.62$) leaf damage,

Table 1. Results from general linear mixed models testing for effects of tree species diversity (two levels: monocultures, polycultures), mahogany *Swietenia macrophylla* seedling density (eight levels: 3, 5, 7, 9, 12, 14, 16 and 18 individuals per m²), and their interaction (all fixed factors) on percent insect leaf damage (mainly *Acromyrmex octospinosus* leaf cutter ants) and necrosis by a pathogenic fungus (*Pseudocercospora* sp.). Significant ($p < 0.05$) and marginally significant ($0.05 < p > 0.10$) effects are in bold and italics, respectively. Models included tree plot (random, statistics not included), as well as seedling height and herbaceous cover as covariates.

Source	Insect leaf damage			Pathogen leaf necrosis		
	F	df	p	F	df	p
Diversity	0.39	1, 93	0.533	8.14	1, 83	0.005
Density	0.21	1, 136	0.651	0.62	1, 134	0.434
Diversity \times Density	1.67	1, 136	0.198	0.01	1, 134	0.947
Height	3.69	1, 138	<i>0.057</i>	1.29	1, 142	0.258
Cover	3.28	1, 153	<i>0.072</i>	0.81	1, 142	0.370

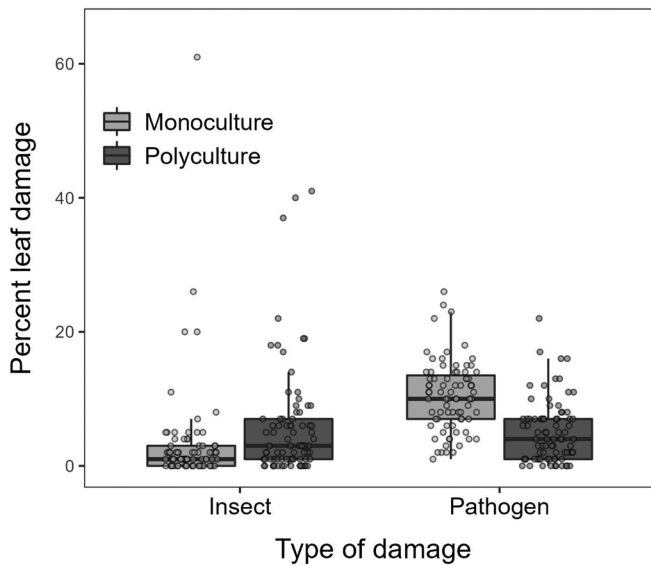


Figure 1. Box plots showing the effect of tree species diversity on percent of leaf area removed by insects and percent of leaf area with necrosis caused by a pathogenic fungus on mahogany *Swietenia macrophylla* seedlings planted at the Universidad Autónoma de Yucatán (UADY) Tree Diversity Experiment. Data points are overlaid in gray, including outliers.

i.e. enemies exhibited density-independent responses at the quadrat level (Table 1, Fig. 2). Further, we found no significant effect of tree diversity by seedling density interaction on either insect or pathogen damage (Table 1), indicating that both types of attackers were consistently irresponsive to variation in seedling density across levels of tree diversity (Fig. 2). We additionally found marginally significant negative effects

of seedling height (slope estimator = -0.038 ± 0.019) and herbaceous cover (-0.015 ± 0.008) on insect leaf damage, but no effect of either covariate on leaf necrosis (Table 1).

Discussion

We found contrasting effects of tree diversity on insects and a pathogenic fungus attacking seedlings of big-leaf mahogany, causing no change in the former but causing a strong reduction in leaf necrosis. As such, this study is one of the few reporting on simultaneous effects of tree species diversity on insects and pathogens (Schuldt et al. 2017). The level of insect leaf damage was low (ca 1–4%) but can still have a substantial effect on seedling survival or growth, particularly when damage occurs early in leaf ontogeny under shaded conditions (Crawley 1985, but see Blundell and Peart 2001). The non-significant diversity effect on insect leaf damage agrees with predictions for generalist insects and contrasts with our previous findings showing negative effects of tree diversity on specialist insects when adult mahogany plants were saplings shortly after establishing the experimental plots (Abdala-Roberts et al. 2015). While some species and groups of polyphagous insects frequently exhibit increased recruitment to high-diversity patches via mechanisms such as diet mixing, reductions in plant defences, or increased plant biomass (Cook-Patton et al. 2011, Brezzi et al. 2017), others, consistent with our findings, have found that leaf damage by generalists is irresponsive to plant diversity (Castagneyrol et al. 2013, Abdala-Roberts et al. 2015; reviewed by Jactel et al. 2021). Thus, in the absence of the above mechanisms, generalists would presumably be insensitive to changes in density

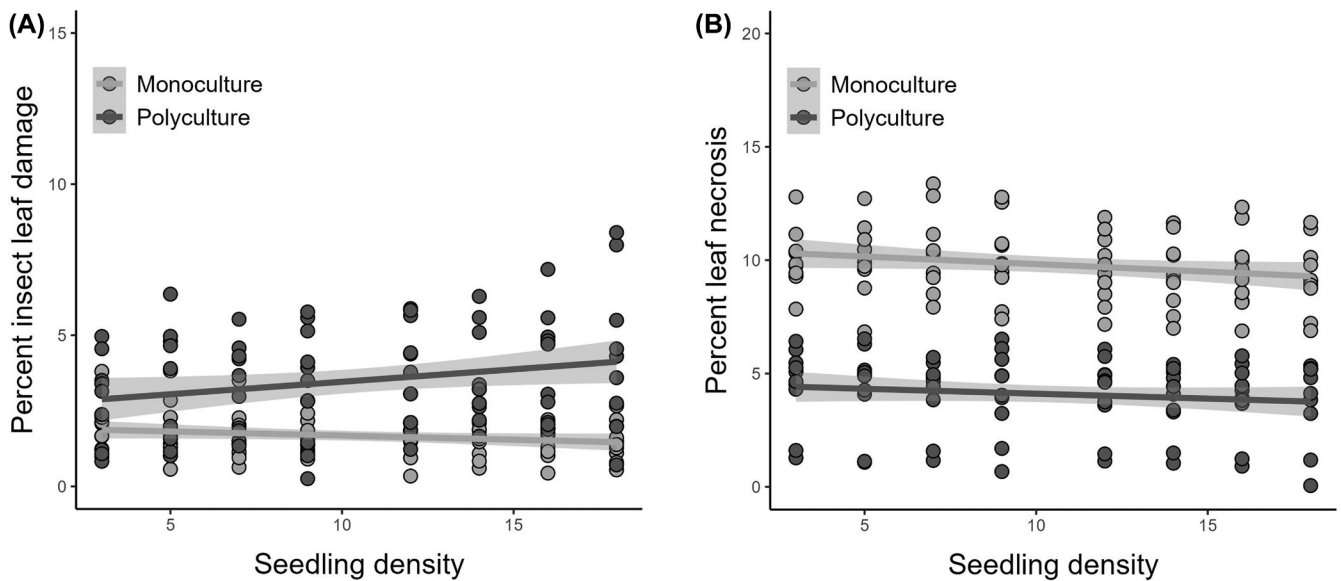


Figure 2. Effects of mahogany *Swietenia macrophylla* seedling density on (A) the percent of leaf area removed by insects and (B) percent of leaf area with necrosis by a pathogenic fungus in mahogany tree monoculture and polyculture plots at the Universidad Autónoma de Yucatán (UADY) Tree Diversity Experiment. Data shown are seedling quadrat-level values after controlling for fixed and random effects in the model. Lines are predicted relationships and their 95% confidence intervals.

or frequency of a focal plant species with diversity as they are not limited to feeding on a particular host and therefore perform similarly regardless of the plant community context (Abdala-Roberts et al. 2015). Additionally, leaf cutter ants *A. octospinosus*, the main insect attacking mahogany seedlings during the study, are highly mobile foragers which may not be influenced by tree diversity effects at the studied plot spatial scale (Bommarco and Banks 2003, Gripenberg et al. 2014). Ttests at larger spatial scales as well as detailed work controlling for spatial distribution of ant nests are needed to achieve a deeper understanding of leaf cutter ant responses to tree population and community variation.

In contrast to insects, and in line with predictions, tree diversity level (polyculture versus monoculture) strongly reduced leaf necrosis by the *Pseudocercospora* fungus, a highly common pathogen on mahogany trees in the system causing substantial leaf necrosis exclusively to mahogany seedlings. These findings imply that tree plot size and study system layout presumably tested for effects at a scale relevant for pathogen dispersal, which was presumably lower compared to highly mobile leaf cutter ants. This result is consistent with previous work showing reductions in attack rates by specialist plant enemies, including both insects (Castagneyrol et al. 2013, Abdala-Roberts et al. 2015, reviewed by Moreira et al. 2016) and fungal plant pathogens (Mitchell et al. 2002, Hantsch et al. 2014, Rutten et al. 2021), with increasing plant diversity. Further, it provides evidence for poorly studied understory-canopy negative feedbacks among conspecifics via pathogens as adult trees were the main source of seedling infection in this study (Caballol et al. 2022). It is important to note, however, that our experimental design testing for tree diversity effects does not separate mahogany tree density (number of individual trees per plot) versus frequency (proportion or abundance of mahogany trees relative to heterospecifics per plot) effects, two important sources of variation which could have contributed to reductions in seedling pathogen infection rates in polyculture. To disentangle these drivers, future work testing for different (replicated) combinations of mahogany density and frequency levels at the plot scale is needed (Kim and Underwood 2015). In addition, we cannot conclude that differential responses by pathogens and ants were dictated by diet breadth (or any other) attacker trait, as this would require having multiple attacker species per trait level. Further long-term studies testing for multiple, replicated combinations of adult conspecific tree densities and heterospecific tree frequencies, as well as measurements of attack by multiple enemy species grouped based on contrasting trait levels (e.g. guilds) are needed to better understand the mechanisms behind observed patterns of attack on mahogany, including canopy-to-understory interactions via plant enemies.

Unexpectedly, we found no evidence of seedling density effects on leaf damage by either pathogens or insects, whereas previous work in natural forests has shown that mahogany seedlings experience density-dependent attack by specialist insects (Norghauer et al. 2006). In our case, most leaf damage in our system was caused by generalist leaf cutter ants and we

therefore speculate that contrasting outcomes likely implicate differences in insect traits, including diet breadth (Sedio and Ostling 2013) and mobility (Bommarco and Banks 2003). For example, specialist insect herbivores often exhibit strong responses to host plant density (Abdala-Roberts and Mooney 2013, Kim and Underwood 2015, reviewed by Moreira et al. 2016) and while generalists have also been shown to exhibit density-dependence (Schuldt et al. 2010, Brezzi et al. 2017), detailed studies on how diet breadth shapes the occurrence and strength of insect and pathogen responses to host plant availability are still largely lacking (Freckleton and Lewis 2006, Song and Corlett 2021). In addition, as for tree diversity effects, high mobility in the case of leaf cutter ants could have precluded the detection of density effects at the quadrat scale (Gripenberg et al. 2014). And finally, it is possible that levels of insect leaf damage were not high enough for density-dependent patterns to emerge (Ctvrtecka et al. 2014). In the case of fungal pathogens, on the other hand, previous work has shown that they tend to exhibit more consistent and stronger (e.g. overcompensating; Bell et al. 2006, Freckleton and Lewis 2006) density-dependent responses than insect herbivores (Bagchi et al. 2010, Jia et al. 2020, reviewed by Song and Corlett 2021). However, as for insects, we found no evidence of density-dependence. As mentioned above, this result could also relate to the spatial scale at which seedling density effects were tested relative to rates of pathogen dispersal. Further work assessing spatial patterns of infection rates for mahogany adults and seedlings across multiple time points over the growing season is needed (Song and Corlett 2021). It is important to also consider that a recent meta-analysis reported that the occurrence and strength of plant enemy density-dependent responses varies substantially and in general terms is lower than previously assumed (Song and Corlett 2021). Further work explicitly testing for enemy (and plant) traits (Jia et al. 2020) known to influence foraging patterns can shed insight on the causes behind lack of or variability in density-dependent responses.

Finally, our findings indicated no evidence of a tree diversity by seedling density interaction on leaf damage by either type of plant enemy, thus rejecting the yet rarely tested influence of tree community context on seedling density implied by the JCH. Here, again, the scale at which seedling density was manipulated may have not been relevant to the scale of insect movement or pathogen dispersal, thus precluding interactive effects across scales. Interestingly, in one of the few available studies experimentally testing for these non-additive effects, Germany et al. (2019) found decreasing (rather than increasing) insect leaf damage with increasing seedling density in tree monospecific stands whereas in heterospecific stands insect leaf damage was density independent. Interestingly, the fungal pathogen was highly responsive to effects of tree diversity at the plot level but not to seedling density at the quadrat level, which presumably precluded an interaction and implies scale-dependency in this species' responses. Such scale-dependency is important to consider for tree species where ontogenetic differences in size (apparency) or defences (adults versus seedlings or juveniles) set the stage for spatial effects

of plant–enemy interactions (Moreira et al. 2017). Evidence thus far for JCH non-additive dynamics between tree community context and seedling density comes from handful of studies and even fewer have involved experimental approaches (Germany et al. 2019). Manipulative studies addressing insect density-dependent responses to changes in tree community features are needed to shed mechanistic insight into bottom–up and top–down controls over plant–enemy interactions and their effects on seedling recruitment and species coexistence. Doing so for multiple plant and herbivore species concurrently is also needed to move closer to understanding density-dependent enemy responses at the community level.

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Author contributions

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Xoaquín Moreira: Conceptualization (equal); Investigation (equal); Methodology (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.xsj3tx9mp> (Abdala-Roberts et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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