LETTER

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Pathogens and insect herbivores drive rainforest plant diversity and composition

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Tropical forests are important reservoirs of biodiversity[1], but the processes that maintain this diversity remain poorly understood[2]. The Janzen–Connell hypothesis[3,4] suggests that specialized natural enemies such as insect herbivores and fungal pathogens maintain high diversity by elevating mortality when plant species occur at high density (negative density dependence; NDD). NDD has been detected widely in tropical forests[5–9], but the prediction that NDD caused by insects and pathogens has a community-wide role in maintaining tropical plant diversity remains untested. We show experimentally that changes in plant diversity and species composition are caused by fungal pathogens and insect herbivores. Effective plant species richness increased across the seed-to-seedling transition, corresponding to large changes in species composition[5]. Treating seeds and young seedlings with fungicides significantly reduced the diversity of the seedling assemblage, consistent with the Janzen–Connell hypothesis. Although suppressing insect herbivores using insecticides did not alter species diversity, it greatly increased seedling recruitment and caused a marked shift in seedling species composition. Overall, seedling recruitment was significantly reduced at high conspecific seed densities and this NDD was greatest for the species that were most abundant as seeds. Suppressing fungi reduced the negative effects of density on recruitment, confirming that the diversity-enhancing effect of fungi is mediated by NDD. Our study provides an overall test of the Janzen–Connell hypothesis and demonstrates the crucial role that insects and pathogens have both in structuring tropical plant communities and in maintaining their remarkable diversity.

Understanding the mechanisms that allow species to coexist in natural ecosystems is one of the most enduring questions in community ecology. The key challenge is to identify how competitive exclusion is prevented, particularly in situations where large numbers of species share similar resource requirements[10]. This question has special relevance to tropical forest plant communities, which can have exceptional species richness[2,9]. The rapid degradation and destruction of tropical forests[11,12] and the large impact this may have on global biodiversity[1], carbon and water cycling and climate feedbacks[13] makes understanding the mechanisms maintaining and structuring their diversity imperative.

There is compelling evidence that natural enemies, including insect herbivores and fungal and oomycete pathogens (hereafter referred to collectively as pathogens), regulate many plant populations in the tropics[7,14,15] and elsewhere[16,17]. Transmission of natural enemies is more effective between plants growing in areas of high conspecific density, reducing plant survival. The Janzen–Connell hypothesis suggests that this negative density dependence (NDD) will promote plant community diversity by preventing dominant species from competitively excluding other species[3,4]. This hypothesis is one of the most widely invoked explanations for species coexistence, and ultimately high diversity, in plant communities.

Although numerous studies have revealed NDD in plant communities[6,8,18], there is considerably less empirical support for the contention that this will translate into enhanced community diversity[2,9]. A key study[5] in Panama documented NDD at the seed-to-seedling transition in a suite of 53 species, and linked this NDD to increased community diversity. However, the causes of this NDD were not identified. Although reduced herbivory by vertebrates can alter the composition of tropical plant communities[19–21], such effects rarely show NDD, and insect herbivores and pathogens are widely regarded as the most likely causes of NDD leading to enhanced plant diversity[2,9,22]. Despite this, studies demonstrating a causal link between insect- and pathogen-mediated NDD and plant community diversity are lacking.

We compared community diversity of seeds and recruiting seedlings in a tropical forest in Belize, Central America, and investigated whether experimentally excluding natural enemies decreased plant diversity, as predicted by the Janzen–Connell hypothesis. The effective number of species (inverse Simpson's dominance index, $1/D$) among seedlings recruiting in unmanipulated (control) plots was significantly higher than among seeds falling in adjacent seedfall traps ($\Delta\log(1/D) = 0.69 \pm$ s.e.m. $= 0.058$, $t_{107} = 11.91$, $P < 0.001$), corresponding to a doubling of the effective number of plant species at the seed-to-seedling transition. To determine whether insect herbivores or pathogens could be contributing to this increase in diversity we compared the diversity of seedlings growing in control plots (sprayed weekly with water) to plots where we suppressed either insects by spraying an insecticide (Engeo), or pathogens by spraying one of two fungicides, Amistar or Ridomil. Each of the pesticide treatments reduced species diversity, but the effects were only statistically significant for the fungicide Amistar (Fig. 1a; $t_{105} = -2.45$, $P = 0.016$), which reduced the effective number of species by approximately 16%. This result clearly implicates pathogenic fungi in promoting seedling diversity.

Two other changes in the plant community at the seed-to-seedling transition were evident: a shift in species abundances, and altered species composition. These trends were also affected by pesticide treatments. Insecticide treatment increased the total number of recruiting seedlings by a factor of 2.7 compared to the control (Fig. 1b; $t_{105} = -7.67$, $P < 0.001$), demonstrating that plant-feeding insects are a major cause of mortality at this life stage. Although Amistar enhanced seedling recruitment, this effect was marginally nonsignificant ($t_{105} = 1.81$, $P = 0.074$). Dissimilarity in species composition between the seeds and seedlings, measured using the abundance-weighted Morisita–Horn index (R_h)[23], was approximately 87% in the control plots (Fig. 1c). Treating seedlings with insecticide dramatically and significantly reduced this dissimilarity ($t_{105} = -7.86$, $P < 0.001$). The fungicides (Amistar and Ridomil) did not reduce the dissimilarity to seeds significantly, but nevertheless the dissimilarity between the species compositions of the fungicide-treated plots and the control plots was about 20% (Extended Data Fig. 1). Overall, our results suggest that insects disproportionately kill certain plant species, reducing their abundances during the transition from seeds to seedlings. Insects thus strongly influence the structure of plant communities

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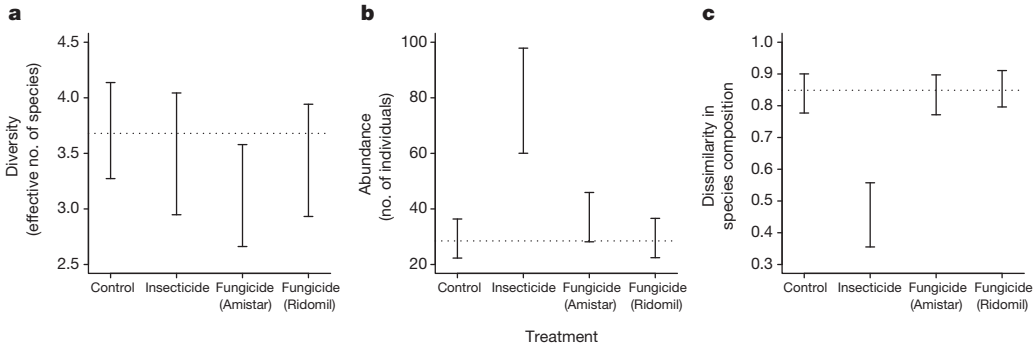

Figure 1 | Suppression of insects and pathogens alters seedling community composition and diversity, respectively. **a**–**c**, Effects of insecticide (Engeo) and two fungicides (Amistar and Ridomil) on the mean effective number of species recruiting as seedlings (**a**); the mean seedling abundance summed across all species (**b**); and the mean abundance-weighted Morisita–Horn dissimilarity in species composition for seedlings under each treatment compared to seeds in adjacent seed traps (**c**). The error bars represent the 95% confidence intervals of the mean across the 36 stations.

in this forest; however, by doing so relatively independently of plant density, their net effect on plant species diversity is small.

For 18 species, sufficient data were available to conduct a formal test for NDD (see Methods). The slope of the relationship between the log number of seeds and the log number of seedlings in the control plots was less than 1 in 13 of the 18 species, and significantly less than 1 for 3 of these (Fig. 2a and Supplementary Table 1), indicating NDD[5]. Furthermore, the mean slope across species was significantly less than 1 in the control treatment ($t_{48} = -2.68$, $P = 0.010$), suggesting that NDD is widespread, as has been found in previous studies of the seed-to-seedling transition in tropical forests[5]. Suppressing fungi using the fungicide Amistar reduced the strength of NDD so that the mean slope was no longer significantly different from 1 (Fig. 2b; $t_{48} = -1.54$, $P = 0.130$). Neither Ridomil nor Engeo reduced the strength of NDD, with the mean slope remaining significantly less than 1 in both treatments (Fig. 2b). Thus, the significant effects of fungal pathogen exclusion on seedling diversity shown in Fig. 1 can be causally linked to a reduction in the magnitude of NDD.

The strength of NDD in control plots was greatest in the species that were most abundant as seeds (Fig. 3a; $t_{16} = -4.33$, $P = 0.001$; Extended Data Table 1). Greater NDD might be detected in these species because their high densities facilitate transmission of insects and pathogens, or because pests and diseases adapt to exploit the most abundant resources. The positive relationship between NDD and seed abundances contrasts with the results of two recent studies investigating NDD in relation to adult abundances[15,24]. These differing results may arise because the rank abundances of species can shift substantially between seeds and adults, and because seed abundance reflects fecundity (which will be inversely correlated with seed size) as well as adult abundance. A third possibility is that abundant seed production is correlated with other traits (for example, lower defence investment or shade tolerance[25]), which are associated in turn with greater susceptibility to density-responsive natural enemies. Regardless of the cause, by reducing the survival of common species disproportionately, NDD may have increased the diversity of recruits more than expected from the average NDD effect. All pesticide treatments weakened the relationship between NDD and abundance markedly (Fig. 3b–d and Extended Data Table 1). By weakening NDD, especially in species that are abundant as seeds, fungicide application may have removed one mechanism for enhancing diversity at the seed-to-seedling transition, leading to the significantly lower seedling diversity observed in the Amistar treatment.

As a final evaluation of the contribution of NDD to enhancing the diversity of recruiting seedlings, we used a simulation approach. Changes in community diversity and composition across the seed-to-seedling transition and following the exclusion of natural enemies could result from either NDD or trade-offs between seed production and allocation to defence against insects and pathogens. To distinguish these two possibilities we used models fitted to the 18 most abundant species to simulate communities in two scenarios (see Methods). In the first scenario (low-density survival), per-capita recruitment was independent of seed density and equal to that expected under each treatment in the absence of conspecific neighbours. In the second scenario (NDD survival), per-capita recruitment was dependent on both pesticide treatment and seed density. Simulations in the low-density survival scenario greatly underestimated the effective number of species in the control plots (Fig. 4a). Total seedling abundance was also overestimated (Fig. 4b) and dissimilarity in species composition underestimated (Fig. 4c) in the control and insecticide treatments in the

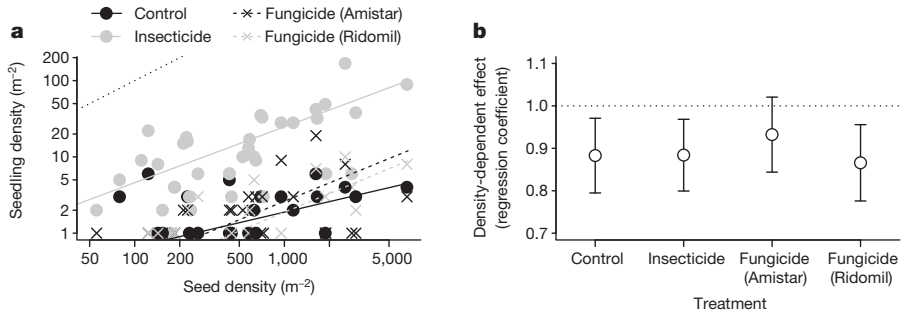

Figure 2 | Recruitment across the seed-to-seedling transition showed NDD in the control, but spraying with the fungicide Amistar removed this NDD. **a**, The relationship between number of recruits and number of seeds for one example species, *Terminalia amazonia*. Without NDD, the expected slope is 1 on a log–log scale (dotted line) and the *y* intercept represents recruitment at low density. The observed slope was lowest (and <1) in the control; treatment with fungicides but not insecticides increased the slope. **b**, The NDD effect is significantly <1 across 18 species in the control treatment, indicating prevalent NDD. Spraying with Amistar, but not other pesticides, removed this effect. Error bars show 95% confidence intervals of the mean.

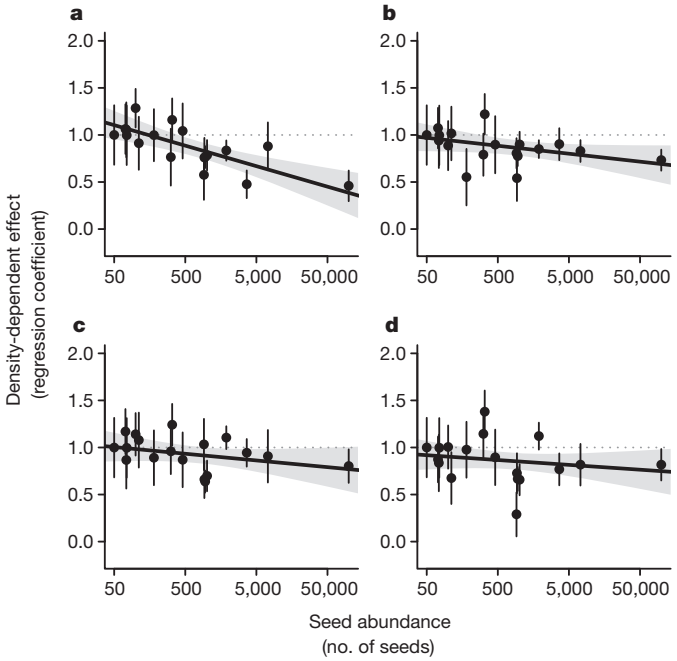

Figure 3 | Negative density dependence is strongest in species that are most abundant as seeds. The relationship between seed abundance and the strength of NDD is shown for the 18 species analysed. **a**–**d**, The relationship for the control plots sprayed with water (**a**), plots sprayed with the insecticide Engeo (**b**), plots sprayed with the fungicide Amistar (**c**), and plots sprayed with an alternative fungicide, Ridomil (**d**). The bold lines are the relationships fitted with a weighted linear mixed-effects model (weights are the inverse of the standard deviations, which are indicated by error bars), with the 95% confidence intervals of the mean. The dotted line shows the null expectation of regression coefficients of 1 in the absence of an effect of seed abundance on the strength of NDD.

absence of NDD. Similar results were obtained using an alternative simulation scenario, where per-capita recruitment reflected that recorded at the mean seed density for each species[5] (Extended Data Fig. 2). Adding NDD to the simulations replicated the observed data better. Overall, these simulations confirm that pathogen-mediated NDD is responsible for increasing the diversity of seedlings.

Although individual components of the Janzen–Connell hypothesis have been tested repeatedly since its formulation more than 40 years ago[3,4], experimental tests of the key overall hypothesis that natural enemies cause NDD and thus promote species coexistence and enhance species diversity are rare. One such study[19] found no evidence that excluding vertebrate herbivores reduced NDD or diversity. However, although vertebrates have occasionally been implicated as drivers of NDD[26–28], the primary causes of NDD are thought to be insects and pathogens[2,9,22]. The results presented here build on existing evidence for widespread NDD in tropical plant communities[5,6,8,18,24] by establishing the cause of NDD, and by linking it to increased plant species diversity, as suggested by the Janzen–Connell hypothesis.

Our experiments highlight that both insect herbivores and pathogens help structure tropical plant communities at the early stages of community assembly and provide support for a pivotal role for natural-enemy-mediated NDD in maintaining species diversity in this tropical forest. Although the magnitude of the NDD we observed was relatively small, this study was conducted over a relatively short timescale (17 months) in a tropical forest of relatively low plant species diversity (approximately 320 tree species have been recorded in the reserve[29]). The effects of NDD will probably accumulate over time, and may be stronger in more species-rich forests. Indeed, similar experiments in other forests are now needed to evaluate the generality of the Janzen–Connell hypothesis as an explanation for variation in species diversity among tropical plant communities.

METHODS SUMMARY

We established 36 sampling stations within a 1-hectare (ha) area in the Chiquibul Forest Reserve, Belize. Each station had three seed traps and four seedling plots. Plots were randomly assigned to four treatments: control (sprayed with water), insecticide (Engeo), or one of two fungicides (Amistar or Ridomil), applied weekly for 17 months. We recorded numbers of seeds from each species collected weekly in each trap. Number and identities of seedlings germinating in each plot were recorded monthly during the peak recruitment period (April to August) and every 2 to 4 months otherwise. We compared the total number of individuals and their diversity (inverse Simpson's dominance index, $1/D$) between seeds and seedlings in the control plot and among pesticide treatments using mixed-effects models. We also compared dissimilarity in species composition between seeds and seedlings (abundance-weighted Morisita–Horn dissimilarity index) among pesticide treatments. In the absence of NDD, a slope of 1 is expected for the relationship between log number of seeds and log number of seedlings[5]. We estimated this slope and the effect of pesticide treatments for 18 species. To determine the average effect of density and the effect of overall species abundance, we modelled the slopes (see Methods) of each species and pesticide treatment combination as a function of log total seed abundance and pesticide treatment, using a weighted mixed-effects model. Finally, to determine whether NDD could generate observed differences in communities among treatments, we used the models of the 18 species to simulate communities, assuming survival to be either density dependent or density independent, based on the establishment probability expected in the absence of conspecific neighbours. We calculated abundance, diversity and dissimilarity based on 1,000 simulations for each scenario and compared the mean and 95% confidence intervals of the observed data to those derived from the simulations. Data (Supplementary Data 1) and code for analyses (Supplementary Notes 1) are provided as Supplementary Information.

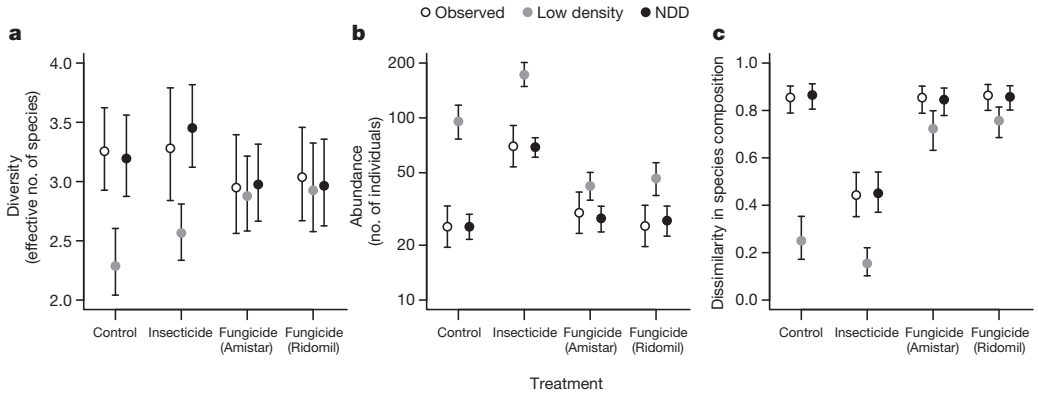

Figure 4 | Including NDD in model simulations reproduces the observed diversity patterns, whereas excluding NDD underestimates diversity in the control and insecticide treatments. Observed diversity (**a**), total abundance (**b**) and dissimilarity in species composition to the seeds (**c**) in each treatment were compared with those simulated either assuming a constant survival for each species equal to its estimated low-density survival (low-density survival) or NDD survival. The error bars are 95% confidence intervals of the mean extracted from models fitted to the data (observed) or the 95% quantile (simulations) from 1,000 simulations in each scenario.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to O.T.L. (owen.lewis@zoo.ox.ac.uk).

METHODS

Field survey. Our field site was close to the Las Cuevas Research Station in southwest Belize (16° 43′ 53′′ N, 88° 59′ 11′′ W) at 450 m elevation within the 170,000-ha Chiquibul Forest Reserve protected area. This site has limestone geology and a relatively intact flora and vertebrate fauna. It experiences a marked dry season, typically from February to May, with annual rainfall approximately 1,500 to 1,800 mm (ref. 30). We established 36 sampling stations on the forest floor, positioned at 20-m intervals on a 120 m × 120 m grid. Each station comprised seven 1-m^2 quadrats, placed as close together as possible while avoiding trees and large rocks. Three of the quadrats at each station were randomly selected as locations for 1-m^2 seed traps made from 1-mm mesh fibreglass netting, suspended 80 cm above the ground using PVC poles. The remaining quadrats were cleared of existing seedlings and assigned at random to one of three enemy exclusion treatments or to a control treatment. Two fungicide treatments were used: Amistar (Syngenta Ltd; active ingredient, azoxystrobin), which has broad-spectrum systemic activity against a range of plant pathogenic fungi, and Ridomil Gold MZ 68WP (Syngenta Ltd; active ingredients, mancozeb and metalxyl), which protects plants from infection by oomycetes and fungi. The insecticide used was Engeo (Syngenta Ltd; active ingredient, thiamethoxam), which provides both systemic and contact protection against a range of insects. Pesticides were applied weekly with a hand mister, following the manufacturers' guidelines (0.005 g of Amistar, 0.25 g of Ridomil Gold or 0.0025 ml of Engeo, in each case dissolved in 50 ml of water). Control plots were sprayed with 50 ml of water at the same time as pesticide applications. Treatments began in July 2007 except for the Engeo application, which began in April 2008. All treatments were applied weekly until September 2009. Only data from April 2008 onwards (during which all treatments were applied) were used in the analyses presented here.

Seeds were collected weekly from the traps; damaged or inviable seeds (partially eaten or immature) were discarded and the remaining seeds were counted and identified to species level, where possible, or as morphospecies. A subset of the seeds from each species and morphospecies were placed on moist tissue paper in seed germination trays. We photographed examples of all seed and seedling morphospecies to match seeds to seedlings in cases where species identification was not possible. This ensured consistent classification throughout the experiment, and facilitated subsequent plant identification. In this way we matched 97% of the individual seeds collected in our study to seedlings.

We censused the seedling plots for new seedlings every month during the peak period of fruiting and recruitment (April to August) and less frequently (every 2 to 4 months) during the rest of the year. At each census, all new seedlings were tagged and identified with species or morphospecies. Unidentified seedlings were photographed. By comparing these photographs to seedlings germinated from collected seeds we were able to match 90% of the observed seedlings to seeds.

To confirm that the significant effects of insecticide treatment were a consequence of reduced attack from insects rather than a direct effect of Engeo on plant survival[31], we set up experiments in May 2010 in which a subset of the focal plant species (*Stemmadenia donnell-smithii* ($n = 60$), *Cordia alliodora* ($n = 80$), *Cryosophila stauracantha* ($n = 70$), *Combretum laxum/fruticosum* ($n = 70$), *Terminalia amazonia* ($n = 100$) and *Forsteronia* sp. ($n = 70$)) were grown from seed at high density under insect-free conditions, and with Engeo treatment. Freshly collected seeds were sown into 60 seed trays (36 cm length × 24 cm width × 5 cm depth) filled with locally collected soil that had been sorted to remove large stones and roots. Each tray was divided into six sections, with seeds of each species sown into one section. Trays were enclosed in a bag made from insect-proof nylon netting to exclude insects. The netting was raised above the surface of the tray to allow seedlings to grow. For the shadehouse experiment, 30 trays were placed in randomly allocated positions on raised benches in a small forest gap, covered with waterproof shade netting. For the field experiment 30 trays were placed on the forest floor in a randomized grid design, spaced by 200 cm. Trays in the shadehouse were watered regularly (approximately every 2 to 3 days). Half of the trays (chosen at random) in both experiments were sprayed weekly with 0.0025 ml m^{-2} of Engeo using a hand mister. The remaining trays were sprayed with an equal volume of water. Germinating and surviving seedlings were censused after 8 weeks (shadehouse experiment) or 7 weeks (field experiment). We analysed the number of seedlings at the end of the experiment as a function of insecticide treatment using generalized linear models for each species, assuming a negative binomial distribution for the errors. No significant ($P < 0.05$) effects of Engeo on survivorship were documented in any species in either experiment (see Extended Data Table 2).

Analysis. We calculated the total number of seeds or seedlings observed in each seedling plot (N) and the reciprocal of the Simpson's dominance index ($1/D$, $D = \sum_{k}^{s} p_k^2$, where p_k is the proportional abundance of species k in a community with s species) as a measure of the effective number of species[32]. We quantified differences in species composition among treatments by calculating the Morisita–Horn index of dissimilarity (R_h)[23]

$$R_{hij} = 1 - \frac{2 \cdot \sum_k^s \left(N_{ik} \cdot N_{jk}\right)}{\left(\frac{\sum_k^s N_{ik}^2}{\left(\sum_k^s N_{ik}\right)^2} + \frac{\sum_k^s N_{jk}^2}{\left(\sum_k^s N_{jk}\right)^2}\right) \cdot \sum_k^s N_{ik} \cdot \sum_k^s N_{jk}} \quad (1)$$

between the seed traps and all the seedling plots, i, at each station, j. We calculated the pairwise dissimilarity between each plot and each trap, and then took the mean for the three traps at each station. Results were qualitatively unchanged using other diversity and dissimilarity metrics (for example, Shannon's diversity index and the Bray–Curtis index of dissimilarity; see Extended Data Tables 3 and 4). All diversity and dissimilarity indices were calculated using the 'vegan' package[33] in R v3.0.1 (ref. 34). We compared these metrics between control plots and seed traps and among pesticide treatments (control, insect exclusion with Engeo, true fungi exclusion with Amistar or oomycete and true fungi exclusion with Ridomil), using linear mixed-effects models (fitted using the 'nlme' package[35] in R v3.0.1 (ref. 34)) with different intercepts for the stations included as a normally distributed random effect. We assumed a Gaussian error distribution for the models of N (log-transformed), $1/D$ (log-transformed) and dissimilarity (logit-transformed). There was evidence of heteroscedasticity in the residuals of the models of $1/D$ and R_h, so this was accounted for by explicitly modelling the variance as a function of pesticide treatment (for $1/D$) or as an exponential function of the expected values (for R_h).

We used these models to test three hypotheses: first, diversity is greater among seedlings than among seeds; second, excluding natural enemies with pesticides decreases diversity; and, third, excluding natural enemies with pesticides alters species composition.

For a subset of species we examined the effects of pesticides, seed density and their interaction on seedling recruitment at the level of individual species. For this analysis, we selected all 18 species that met two criteria: seeds and/or seedlings of these species were recorded at ≥5 stations (the sets of stations with seeds and seedlings did not have to overlap); and mean seed density varied at least threefold among stations. The species that met these criteria are listed in Supplementary Table 1. The relationship between the number of seeds in plot i at station j, $N_{0,ij}$, and the expected number of recruits, $N_{1,ij}$ can be described by the equation[5]

$$N_{1,ij} = \exp(\alpha) N_{0,ij}^{\beta} \quad (2)$$

where $\exp(\alpha)$ is the ratio of seedlings to seeds at low density ($N_{0,ij} = 1$). The parameter β is 1 if survival is independent of conspecific density and less than 1 when this ratio is reduced at high density (that is, NDD). Because we did not measure the seed rain in the seedling plots at each station j directly, $N_{0,ij}$ has to be estimated from the adjacent seed traps at station j instead. Ignoring the error in these estimates of $N_{1,ij}$ biases the estimation of β towards 0 (ref. 36), and therefore overestimates the importance of NDD. To overcome this potential bias, we modelled N_0 and N_1 jointly as:

$$\hat{N}_{0,ij} = \mathrm{NegBin}\left(\lambda_j, \kappa_0\right); \lambda_j \sim \mathrm{lognorm}\left(\bar{\lambda}, \sigma^2\right)$$

$$\hat{N}_{1,ijL} = \mathrm{NegBin}\left(\exp\left(\alpha_L\right) N_{0,ij}^{\beta_L}, \kappa_{1,L}\right) \quad (3)$$

where both $\hat{N}_{0,ij}$ (the number of seeds in plot i at station j) and $\hat{N}_{1,ij}$ (the number of recruits in plot i at station j) were drawn from negative binomial distributions defined by the expected number of individuals and stage ($t = 0, 1$) and treatment (L) specific size or overdispersion parameters, $\kappa_{t,L}$. The expected number of seeds in the plots at station j is λ_j and the λ_j were drawn from a lognormal distribution with mean $\bar{\lambda}$ and variance σ^2. The number of seeds falling in the seedling plots is treated as missing data which need to be imputed from the seed trap data collected at the same station. The parameters α_L and β_L correspond to the low-density survival rate and effect of density on recruitment under treatment L as described by equation (2). This hierarchical model was fitted using the INLA package[37] in R v3.0.1 (ref. 34).

We used estimates of β_L from these models to test two hypotheses for each species: first, survival is negatively density dependent ($\beta_{\mathrm{control}} < 1$); second, natural enemies cause the observed negative density dependence, so that applying pesticides weakens the relationship between seed density and survival ($\beta_{\mathrm{control}} < \beta_{\mathrm{pesticide}}$).

We tested whether the estimates of β across the 18 species were significantly different from 1 in the control treatment and whether they varied among pesticide treatments and with the logarithm of the seed abundance ($N_{0,k}$) of each species, k. This was achieved by fitting a linear mixed-effects model to estimates of β with species included as a random effect. The contribution of each estimate of β to the model was weighted by $\omega_{k,L}$, the inverse of its standard deviation. The model can be described as

$$\hat{\beta}_{kL} = \gamma_0 + \gamma_{1,L} + \gamma_2 \cdot \log(N_{0,k}) + \gamma_{3,L} \cdot \log(N_{0,k}) + b_k + \omega_{k,L}\varepsilon_{kL}$$
$$b_k \sim \mathrm{Norm}\left(0, \sigma_b^2\right); \varepsilon_{kL} \sim \mathrm{Norm}(0, \sigma_\varepsilon^2) \quad (4)$$

where the γ represent the estimated fixed effects parameters, b_k is the random effect for species k and ε_{kL} is the error under treatment L for species k. The parameters $\gamma_{1,k}$ (pesticide effect on mean NDD) and $\gamma_{3,k}$ (pesticide effect on the relationship between overall seed abundance and strength of NDD) are zero for the control treatment and represent the change in these parameters under each pesticide treatment compared to the control.

Finally, we tested whether the estimated effects of NDD and pesticides on recruitment of individual species could account for the observed differences in diversity among pesticide treatments. We used the parameters estimated in the 18 species-specific models to simulate new communities in three scenarios. For each species, the number of seeds in each trap was drawn from a negative binomial distribution with mean λ_j and size κ_0. In the 'NDD survival' scenario, the number of seedlings in plots at station j with treatment L was drawn from a negative binomial distribution with mean = $\exp(\alpha_{1,L})\lambda_j^{\beta_{1,L}}$ and size = $\kappa_{1,L}$. The 'low-density survival' scenario assumed that survival was independent of seed density and was equal to the survival for each species within each pesticide treatment when density was 1 (that is, when seedlings had no conspecific neighbours). The number of seedlings was therefore drawn from a negative binomial distribution with mean = $\exp(\alpha_{1,L})\lambda_j$. We then calculated the effective number of species for the simulated communities at each station and treatment combination and extracted the means for each treatment. This procedure was repeated 1,000 times and the median and 95% quantiles across the simulations were extracted in each scenario. A similar procedure was used to simulate communities expected in a third scenario, consistent with previous studies[5], where seed-to-seedling transition probabilities reflected those recorded at the mean seed density for each species. This was achieved by refitting the model to each species after fixing the values of all the β_L to 1 and using this model for the simulations. We then calculated the mean total abundance, effective number of species and dissimilarity to species composition of the seeds in each treatment using the observed data for the 18 species. We compared these observed data to the simulations in the low-density and NDD scenarios (main text) and the mean-density and NDD scenarios (Extended Data Fig. 2).

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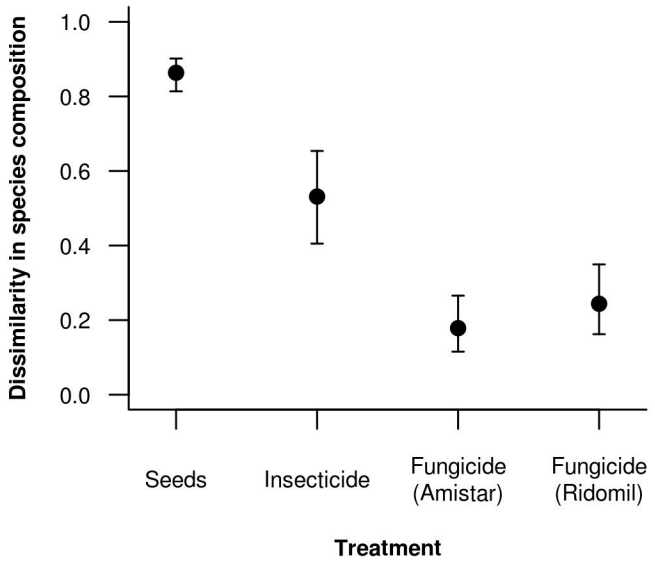
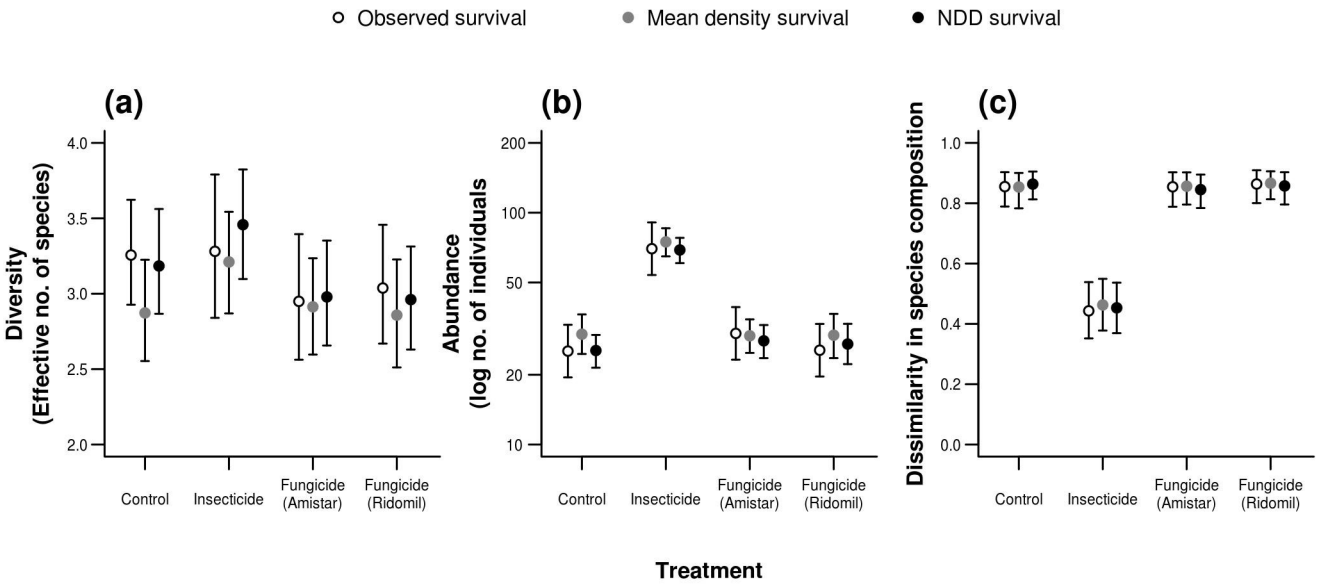


Extended Data Figure 1 | The mean abundance-weighted Morisita–Horn dissimilarity in species composition (and 95% confidence intervals), comparing seedlings recruiting in the control plots with seedlings in the pesticide treatments and with seeds falling into seed traps.



Extended Data Figure 2 | A comparison of the observed seedling communities (observed survival) with those simulated either fixing survival to the mean for each species in each treatment (mean density survival) or allowing survival to be negatively density dependent (NDD survival). The simulated values are means and 95% confidence intervals based on 1,000 simulations for effective number of species, total abundance and community dissimilarity to seeds falling in adjacent traps.

Extended Data Table 1 | Coefficients from the model relating the strength of NDD to treatment, log total seed abundance, and their interaction

Term	Parameter	Std. Error	d. f.	t	P
Intercept (Water) (γ_0)	-0.12	0.044	48	-2.68	0.010
Insecticide effect (Engeo) ($\gamma_{1,\,Engeo}$)	0.00	0.047	48	-0.02	0.982
Fungicide effect (Amistar) ($\gamma_{1,\,Amistar}$)	0.05	0.048	48	1.02	0.312
Fungicide effect (Ridomil) ($\gamma_{1,\,Ridomil}$)	-0.02	0.049	48	-0.34	0.732
log N_0 (standardised) (γ_2)	-0.37	0.086	16	-4.33	0.001
Engeo:log N_0 (standardised) ($\gamma_{3,\,Engeo}$)	0.23	0.085	48	2.65	0.010
Amistar:log N_0 (standardised) ($\gamma_{3,\,Amistar}$)	0.25	0.096	48	2.60	0.012
Ridomil:log N_0 (standardised) ($\gamma_{3,\,Ridomil}$)	0.28	0.095	48	3.00	0.004
Among species variance (σ_b^2)	0.012				
Residual variance (σ_ε^2)	0.469				

The strength of NDD was measured as the coefficient for seed density, β, from equation (3). The log total seed abundance was standardized across species. The model was fitted as a mixed-effects model with the contribution of each value of β weighted by the inverse of its standard deviation.

Extended Data Table 2 | Coefficients from the negative binomial model fitted to the shadehouse and field trials of effects of the insecticide Engeo on seedling survival

		Shadehouse				Field			
		Coefficient	SE	Z	P	Coefficient	SE	Z	P
Stemmadenia	Intercept	-0.57	0.044	-12.858	<0.001	-0.67	0.128	-5.220	<0.001
	EngeoTreatment	0.04	0.062	0.621	0.535	-0.05	0.181	-0.282	0.778
Cordia	Intercept	-0.43	0.086	-5.053	<0.001	-0.35	0.064	-5.495	<0.001
	EngeoTreatment	-0.07	0.121	-0.592	0.554	-0.06	0.090	-0.633	0.527
Cryosophila	Intercept	-0.33	0.036	-8.941	<0.001	-0.61	0.042	-14.366	<0.001
	EngeoTreatment	-0.01	0.051	-0.154	0.877	-0.03	0.060	-0.531	0.595
Combretum	Intercept	-0.67	0.043	-15.452	<0.001	-3.11	0.332	-9.364	<0.001
	EngeoTreatment	0.02	0.061	0.243	0.808	0.83	0.456	1.824	0.068
Terminalia	Intercept	-4.83	0.289	-16.725	<0.001	-6.62	0.743	-8.908	<0.001
	EngeoTreatment	-0.09	0.417	-0.208	0.835	1.10	0.878	1.251	0.211
Forsteronia	Intercept	-0.67	0.043	-15.567	<0.001	-1.12	0.108	-10.360	<0.001
	EngeoTreatment	0.01	0.061	0.152	0.879	0.00	0.152	0.000	1.000

Note that Engeo did not have a significant effect on survival in any of the species tested (shaded rows).

Extended Data Table 3 | Tests of pesticide effects on seedling species diversity using different diversity indices

Treatment	Parameter	Std.Error	d. f.	t	P
Log Inverse Simpson's Diversity Index (1/D)					
Intercept (Water)	1.30	0.059	105	22.04	0.000
Insecticide (Engeo)	-0.06	0.077	105	-0.83	0.410
Fungicide (Amistar)	-0.18	0.072	105	-2.45	0.016
Fungicide (Ridomil)	-0.08	0.072	105	-1.10	0.274
Log Shannon's Diversity Index (H)					
Intercept (Water)	1.56	0.053	105	29.30	0.000
Insecticide (Engeo)	0.03	0.063	105	0.54	0.591
Fungicide (Amistar)	-0.15	0.067	105	-2.29	0.024
Fungicide (Ridomil)	-0.07	0.067	105	-1.05	0.298
Log Fisher's alpha (α)					
Intercept (Waterl)	1.33	0.084	104	15.92	0.000
Insecticide (Engeo)	-0.13	0.086	104	-1.57	0.120
Fungicide (Amistar)	-0.18	0.095	104	-1.88	0.063
Fungicide (Ridomil)	-0.04	0.126	104	-0.33	0.744
Rarefied Species Richness					
Intercept (Waterl)	1.16	0.033	105	35.38	0.000
Insecticide (Engeo)	-0.06	0.037	105	-1.56	0.122
Fungicide (Amistar)	-0.11	0.043	105	-2.55	0.012
Fungicide (Ridomil)	-0.03	0.041	105	-0.77	0.446

The indices were calculated using the vegan v2.0833 package in R v3.0.134. Rarefaction was based on samples of five individuals. The table presents parameters of linear mixed-effects models fitted to these metrics as functions of treatment. The first parameter (intercept) represents mean diversity in the control treatment and the following parameters represent the difference between the control treatment and the three pesticide treatments.

Extended Data Table 4 | Tests of pesticide effects on dissimilarity in species composition, comparing assemblages of seedlings germinating in plots to those of seeds falling in adjacent seed traps

Treatment	Parameter	Std.Error	d. f.	t	P
Logit Morisita-Horn Dissimilarity Index					
Intercept (Water)	1.72	0.240	105	7.18	0.000
Insecticide (Engeo)	-1.91	0.243	105	-7.86	0.000
Fungicide (Amistar)	-0.03	0.270	105	-0.12	0.904
Fungicide (Ridomil)	0.12	0.272	105	0.44	0.664
Binomial Dissimilarity Index					
Intercept (Water)	8.251	0.205	105	40.208	0.000
Insecticide (Engeo)	-0.965	0.183	105	-5.278	0.000
Fungicide (Amistar)	0.070	0.205	105	0.342	0.733
Fungicide (Ridomil)	0.013	0.203	105	0.063	0.950
Logit Bray-Curtis Dissimilarity Index					
Intercept (Water)	3.123	0.175	105	17.896	0.000
Insecticide (Engeo)	-1.412	0.110	105	-12.889	0.000
Fungicide (Amistar)	-0.144	0.110	105	-1.312	0.192
Fungicide (Ridomil)	-0.008	0.110	105	-0.075	0.940
Logit Jaccard Dissimilarity Index					
Intercept (Water)	3.814	0.175	105	21.757	0.000
Insecticide (Engeo)	-1.417	0.110	105	-12.847	0.000
Fungicide (Amistar)	-0.148	0.110	105	-1.344	0.182
Fungicide (Ridomil)	-0.008	0.110	105	-0.075	0.940

Four alternative metrics of dissimilarity were calculated using the vegan 2.08 package in R v3.0.1 (ref. 33). The table presents the fixed-effects parameters of linear mixed-effects models used to describe these metrics as functions of treatment. The intercept represents the mean dissimilarity between seed and control plot assemblages, and the other three parameters indicate the difference between seed–control plot dissimilarity and seed–pesticide plot dissimilarity.

form of CP violation is thought to be responsible for the dominance of matter over antimatter throughout the Universe — a feature responsible for our very existence. Symmetry violation can, indeed, have profound consequences.

Apart from parity violation, electromagnetic and weak interactions are quite similar. Both can be viewed as exchanges of packets (quanta) of energy called bosons. Electromagnetism is mediated by massless photons, whereas heavy, charged W bosons mediate weak interactions. Although some sort of electroweak unification, jointly describing both interactions, seemed natural[4], parity violation caused problems. In 1961, it was shown[5] that unification was possible if, in addition to charged W bosons, another heavy neutral boson, now called the Z boson, also existed. Unfortunately, even then, parity violation made it difficult to accommodate or relate elementary-particle masses. The problem was solved in 1967, when it was demonstrated[6] how the introduction of symmetry breaking through the Higgs mechanism could be used to provide mass. A predicted remnant of that mechanism — the Higgs boson — was detected in 2012 at CERN, Europe's high-energy physics laboratory near Geneva, Switzerland, and François Englert and Peter Higgs were awarded last year's Nobel Prize in Physics for the theoretical work on the Higgs mechanism.

In the early 1970s, support for the existence of the Z boson was observed in neutrino-scattering experiments[7]. But follow-up studies proved inconclusive, in that they did not confirm the parity-violating predictions of electroweak unification. Then an experiment[8,9] called E122, conducted at the SLAC National Accelerator Laboratory in Menlo Park, California, measured a small parity-violating difference between the scattering of right- and left-handed electrons on up and down quarks in a target of deuterium atoms. The up and down quarks are the lightest of the six possible types of quark, and make up all nuclei. This result unequivocally confirmed the parity-violating predictions of electroweak unification. For their work on electroweak unification and its implications, Sheldon Lee Glashow[5], Abdus Salam[10] and Steven Weinberg[6] received the physics Nobel prize in 1979.

During the 35 years since E122 was completed, better sources of right- and left-handed electrons have been developed, experimental techniques have improved and more-intense electron beams have become available. Parity violation has been used for the precise measurement of parameters that describe the electroweak interaction and to investigate nuclear properties. But the parity-violating difference measured in the E122 experiment has not been improved on — until now.

In their study, the Jefferson Lab team decided to redo the SLAC E122 experiment. The researchers worked at lower energy but with much higher intensity and polarization (degree of handedness). As a result, they improved on some aspects of parity-violating differences between the scattering of right- and left-handed electrons on up and down quarks by about a factor of five. With their higher statistics, they were able to untangle the two parity-violating effects: the dominant effect due to electron parity violation, which had already been clearly measured in E122, and a much smaller parity-violating effect attributable to the quarks in the deuterium nuclei, which was beyond the sensitivity of the SLAC experiment.

Why measure such small effects, and so precisely? Perhaps, like mountain-climbing enthusiasts, physicists study them because they are there and represent challenges. However, unlike mountains, in the case of parity-violating effects sometimes smaller is better. Testing the tiny quark parity-violation prediction is a nice example: a deviation from expectations could signal the presence of a new tiny effect. Indeed, the team's measurement probes some types of additional parity-violating effects that could be as much as 30 times weaker than ordinary weak forces. Precision studies also provide access to small nuclear effects that are hard to probe in other ways. An example is the breaking of charge symmetry (the interchange of up and down quarks in deuterium).

Parity-violating polarized electron scattering experiments are expected to continue at the Jefferson Lab, using higher-energy electrons and better particle-detection systems, after upgrades to the facility, now in progress, are completed. One can anticipate better measurements of electroweak parameters, more-refined nuclear-physics studies and improved searches for new interactions.

A great accomplishment can lead to the demise of a scientific endeavour. A good example is the race to put a man on the Moon. That goal started more than 50 years ago and was a spectacular success, but further undertakings ended after the mission was accomplished. Fortunately, electron-scattering studies of parity violation did not suffer that fate. Following the success of E122 at SLAC, the programme changed direction, but improvements in technical expertise and accelerator facilities continued. The Jefferson Lab has taken leadership in polarized-electron scattering initiatives. As long as these initiatives address frontier questions and interesting goals, they should prosper and grow. ■

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ECOLOGY

Plant diversity rooted in pathogens

Ecologists have long pondered how so many species of plant can coexist locally in tropical forests. It seems that fungal pathogens have a central role, by disadvantaging species where they are locally common. SEE LETTER P.85

HELENE C. MULLER-LANDAU

Tropical forests routinely contain more than 200 tree species in a single hectare (Fig. 1). Why don't a few species come to dominate, by chance or by virtue of being better competitors? Multiple hypotheses have been proposed to answer this question, most of which invoke some sort of niche differentiation with respect to resources and/or natural enemies. But despite decades of research, the issue remains unresolved. In this issue, Bagchi *et al.*[1] (page 85) report the results of an elegant field study that clearly implicates natural enemies, specifically fungal pathogens, as crucial to maintaining tropical-plant diversity.

In 1970, ecologists Daniel Janzen[2] and Joseph Connell[3] proposed that natural enemies that target specific host plants maintain high tropical-plant diversity by elevating the mortality of each plant species in areas where it is abundant. Fundamentally, the idea is that host-specialized enemies, including pathogens and insect herbivores, can attack more efficiently and do more damage where their hosts are more plentiful. As a result, each host species fares better when it is

rare and less well as it becomes more common — a phenomenon known as negative density dependence. Many empirical studies have found such negative density dependence in tropical forests[4,5], and the Janzen–Connell hypothesis is the most often cited explanation for these patterns and for high local diversity of plant species in tropical forests. However, niche differences in resource requirements or other factors could also cause negative-density-dependent patterns[6], and few studies have explicitly linked such patterns to particular natural enemies (although see ref. 7 for an exception).

Bagchi *et al.* tested this hypothesis experimentally by using pesticides to remove (or at least reduce) fungal pathogens and, separately, insects at the seedling-establishment stage. Working in a tropical forest in Belize, the authors censused seeds falling into seed traps and seedlings that became established in neighbouring 1-square-metre plots that were treated with a fungicide or with an insecticide, or not treated. In untreated plots, seedling establishment was negatively density dependent and there was a large increase in local species diversity from the seed to the seedling stage, consistent with previous work[4]. Bagchi and colleagues' crucial findings were that fungicide application resulted in the near disappearance of negative density dependence and a drop in seedling species diversity. By contrast, insecticide application merely weakened negative density dependence and led to no change in species diversity, although it did increase the total number of seedlings and caused a dramatic shift in species composition.

Figure 1 | Shades of green. The forest canopy on Barro Colorado Island, Panama, provides visual evidence of how small areas can contain many different tropical tree species. Bagchi and colleagues' findings[1] suggest that fungal pathogens play a crucial part in maintaining this diversity.

This is the first study to explicitly link a particular group of natural enemies to negative density dependence and the maintenance of species diversity in tropical forest plants. It clearly implicates fungal pathogens as the most important drivers of these patterns at the seedling-establishment stage. In the past, there have been more studies of insects than of pathogens as agents of the Janzen–Connell effect — no doubt owing in large part to the greater ease of working with insects. Although insect attack has been found to increase with host-plant density in several tropical plant species[8], the ability of insects to respond to high host density, and thus induce negative density dependence, may ultimately be restricted by their own enemies, such as parasites or predators[9]. Pathogens seem less likely to be similarly checked, which may explain their greater contribution to negative density dependence.

Bagchi and colleagues' results demonstrate that fungal pathogens and insect herbivores influence tropical plant communities in qualitatively different ways. Their distinct roles relate to the two ways in which differences among plant responses to natural enemies can affect species diversity and composition. First, as discussed above, differences in natural enemies can contribute to niche differences that stabilize individual species' abundances and species diversity. Alternatively, or in addition, they can alter differences in competitive ability (fitness) among species[10], thereby modifying species abundances, and potentially which species can successfully compete at all. The large shifts in species composition seen during insecticide treatment suggest that insects have major impacts on fitness differences in this ecosystem. Overall, it seems that fungal pathogens are more important determinants of niche differences and thus species diversity, whereas insects have greater influence on fitness differences and thus species composition.

This work also contributes novel observations on the strength of negative density dependence in different species. Contrasting hypotheses predict either a negative relationship between a species' average abundance and its negative density dependence if greater abundance makes a species more apparent to its enemies, or a positive one if the causality is reversed and lower negative density dependence leads to increased abundance[5]. Previous studies[5,11] that quantified tree abundance over large areas have found that more-abundant species experience less negative density dependence. Bagchi *et al.* find that species that are more abundant as seeds suffer stronger negative density dependence, which at first seems to contradict these earlier findings. However, seed abundance depends not only on tree abundance but also on seed size, which varies widely among tropical tree species. Small-seeded species are likely to be particularly vulnerable to natural enemies, and small seeds are produced in greater numbers, and thus differences in seed size may reconcile Bagchi and colleagues' results with previous work. Future studies should seek to disentangle the roles of species traits and abundances in driving interspecific variation in negative density dependence.

Indeed, this groundbreaking experimental work lays the foundation for a host of studies exploring the roles of natural enemies in structuring tropical-plant diversity. Bagchi *et al.* investigated effects on seedling establishment, a single life stage — integration of such effects over the entire life cycle will ultimately provide a more complete picture. Replication of these experiments across climatic gradients could also test the idea that some climates are more conducive to natural enemies, and that this contributes to greater species diversity of forests in these areas. Furthermore, such comparative studies or others that explicitly manipulate temperature, rainfall or atmospheric carbon dioxide could address how global change affects interactions between plants and natural enemies and thereby illuminate the future of tropical plant diversity. ■

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