

## FOREST ECOLOGY

# Herbivores as drivers of negative density dependence in tropical forest saplings

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Ecological theory predicts that the high local diversity observed in tropical forests is maintained by negative density-dependent interactions within and between closely related plant species. By using long-term data on tree growth and survival for coexisting *Inga* (Fabaceae, Mimosoideae) congeners, we tested two mechanisms thought to underlie negative density dependence (NDD): competition for resources and attack by herbivores. We quantified the similarity of neighbors in terms of key ecological traits that mediate these interactions, as well as the similarity of herbivore communities. We show that phytochemical similarity and shared herbivore communities are associated with decreased growth and survival at the sapling stage, a key bottleneck in the life cycle of tropical trees. None of the traits associated with resource acquisition affect plant performance, indicating that competition between neighbors may not shape local tree diversity. These results suggest that herbivore pressure is the primary mechanism driving NDD at the sapling stage.

**T**ropical forests contain high levels of alpha diversity, with many species coexisting at a single site. For instance, 1 ha of Ecuadorian rainforest contains 655 tree species, more than the number in all of North America (1). Such high levels of alpha diversity are challenging to explain because competition should cause dominant species to increase in abundance until they exclude inferior competitors from a community. Whereas many mechanisms have been proposed that could delay or prevent competitive exclusion, negative density dependence (NDD), a process by which a species's growth rate and survival decline at high conspecific densities, has received considerable attention and support [reviewed in (2–5)]. NDD is widely considered to be responsible for the high local diversity characterizing tropical forests because it prohibits dominance by any one species and allows species to recover from low density (2, 3). Evidence from long-term forest dynamic plots around the world confirms that many species demonstrate NDD (6, 7). However, the mechanisms responsible for NDD remain controversial [(4), but see (8–11)].

Most studies examining the mechanisms underlying NDD have focused on seed predation and seedling mortality (4, 12, 13). Another vulnerable life stage is recruitment from seedling to adult, the sapling stage. This stage can persist for decades because of slow growth in the

low light of the shaded understory. Many species reach a height of only 30 to 40 cm after 20 years of growing in this shaded environment. During this bottleneck, deterministic diversifying processes have long-lasting effects on the composition and relative abundance of species in tropical forests (14). Here, we compare the two most prominent mechanisms hypothesized to drive NDD during the prolonged sapling stage. The first mechanism suggests that conspecifics suffer increased competition for resources with increased conspecific density (2, 3). Although this has received less support (4), it has been difficult to falsify. Another hypothesis is that conspecifics share host-specific pests, such as herbivores or pathogens, reducing growth and survival at higher conspecific density (15, 16). Shaded saplings flush new leaves only a few times per year, during which they lose ~25% of their leaf area to herbivores and only 3 to 4% to pathogens (17). In combination with low light availability, the loss of substantial leaf area to herbivores over decades could be a major contributor to lower growth and increased mortality of saplings. Hence, throughout the prolonged sapling stage, NDD and community assembly could be driven by herbivore pressure and/or competition for shared resources.

In this study, we focus on species of *Inga* (Leguminosae), found on Barro Colorado Island (BCI) in Panama. *Inga* includes ~300 tree species in moist and wet forests throughout the New World and constitutes one of the most abundant genera at a given site, manifesting high levels of coexistence, with more than 40 species observed at a single site (1, 18). Therefore, it represents an excellent candidate group with which to ask how so many closely related species can coexist at small spatial scales.

We follow the approach of several recent studies that compared sapling growth and survival with conspecific density and neighborhood phylogenetic similarity, showing that seedlings and saplings perform better in areas of low phylogenetic similarity (6, 7). These studies show that NDD interactions are not limited to conspecific interactions but that they occur over larger phylogenetic distances (19). This may result because, although closely related species can be ecologically and functionally similar, some of the more phylogenetically distant species may also have similar traits (20–23). In this case, including traits in the analysis should provide a more detailed and precise understanding of the mechanisms underlying NDD. To test these mechanisms, we replace phylogenetic relatedness with similarity in terms of resource acquisition and antiherbivore defense traits. A trait-based analysis has the potential to compare the two proposed mechanisms of NDD because it partitions plant strategy into a number of continuous, often orthogonal, axes of variation (24).

Resource acquisition traits for our nine focal species include wood density, leaf morphology, average tree height, and leaf elemental composition (25). This suite of traits has been shown to capture how species respond to their physical environment (24). We also selected five defensive traits previously shown to influence host selection of insect herbivores feeding on *Inga* (17, 26, 27). These include the timing and synchrony of new leaf production (phenology), the leaf expansion rate and chlorophyll content (developmental strategy), the density of trichomes (hairs), the size of extrafloral nectaries, and the number of ant visitors (ants). Most notably, we quantified the chemical similarity between each *Inga* species and neighboring congeners by leveraging recent advances in mass spectrometry and untargeted metabolomics to compare plant secondary metabolites that are associated with defense against insect herbivores (26, 27). We used an index of structural and compositional chemical similarity, which integrates both the abundance and structural similarity of hundreds of individual secondary metabolites produced in a leaf (25).

In addition to the aforementioned traits, we directly tested the role of attack by insect herbivores by quantifying the degree to which focal individuals shared herbivores with neighboring congeners. We then used generalized linear models (GLMs) to quantify the degree to which neighborhood trait and herbivore similarities influence focal tree growth and survival. Specifically, we asked whether *Inga* saplings have reduced growth and survival when growing near congeneric neighbors that are similar in resource acquisition traits, are similar in defense traits, and share herbivore species.

To answer these questions, we used data from the forest dynamics plot on BCI in which the locations, identities, and sizes of all stems greater than 1 cm in diameter at breast height have been recorded in eight censuses from 1981 to 2015 (27). From these census intervals, we

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calculated a normalized growth rate and tracked survival. Whereas the BCI plot contains a total of 14 *Inga* species, only individuals from nine species of *Inga* were sufficiently abundant to be used in this analysis both in terms of focal individuals and neighboring congeners (table S1).

For each individual focal *Inga* sapling, we quantified neighborhood trait similarity on the basis of the similarity of resource acquisition and defense traits between the focal species and its neighboring congeneric trees within a 10-m radius. To do so, we applied Eq. 1 to all focal tree and congeneric pairs within each neighborhood:

$$\sum_{i=1}^{n_{\text{neigh}}} \frac{\text{basal area}_{\text{neigh}} \times \text{trait similarity}_{\text{focal-neigh}}}{\text{distance}_{\text{focal-neigh}}} \tag{1}$$

where  $n_{\text{neigh}}$  is the number of neighboring congeneric trees. Our model assumes the effect that neighboring trees have on focal tree growth and survival scales with the neighboring trees' size (biomass) and decreases with distance to the focal tree (28). We used a 10-m radius because it has been shown to capture the majority of neighborhood effects (28) (table S2).

To test the influence of neighborhood similarity on growth and survival, we ran a series of GLMs where all nine species were included in the same model after normalizing growth within each species, size class, and census interval grouping (19). We used a null model to correct for variation in species richness among neighborhoods (6, 7, 21, 23, 27).

If pressure from insect herbivores is the primary driver of NDD, we predict that a higher density of plants with similar defense traits and herbivore communities will negatively affect growth and survival. Alternatively, if resource competition drives NDD at the sapling stage, we expect that a higher density of plants with similar resource acquisition traits will decrease growth and survival.

All nine axes of trait space were uncorrelated (fig. S1) and showed little phylogenetic signal (table S3). These findings are congruent with the results of similar, more extensive studies on the evolution of defense traits in *Inga*, which demonstrate that closely related species tend to be divergent in antiherbivore traits (17, 26). The lack of correlation of these traits enables us to independently test the relative roles of defensive traits and resource acquisition traits in mediating NDD interactions between congeneric neighbors.

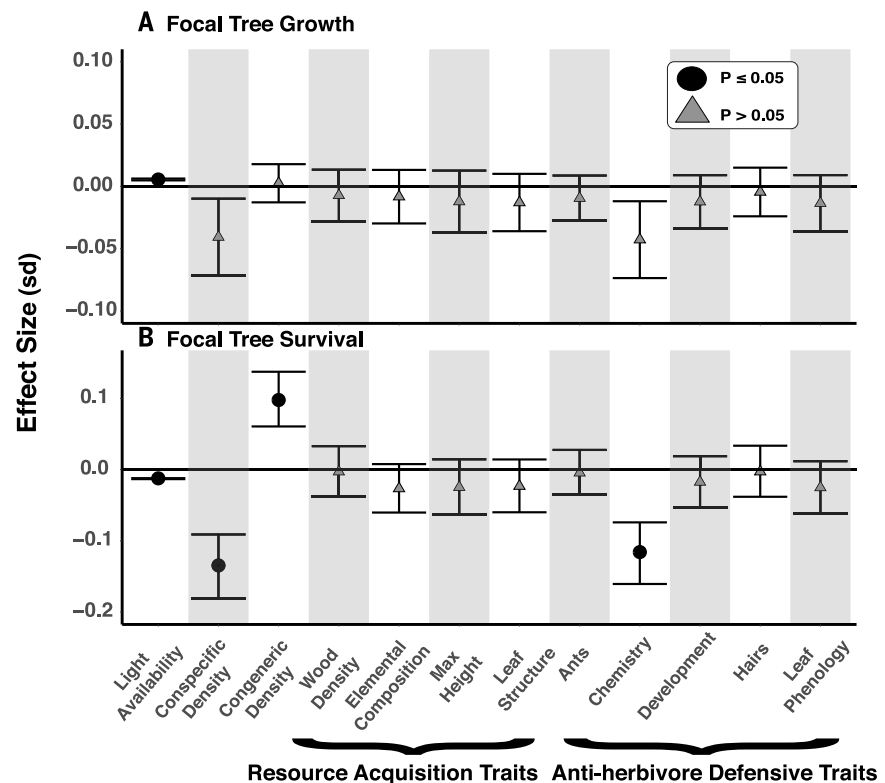
The focus of this analysis is on the effect of plant traits on ecological interactions between closely related individuals that persist in the understory for decades. Thus, we sought to control for variation in abiotic factors. Light is the most limiting factor for growth in the forest understory, which receives as little as 1% of the total sunlight (29). By contrast, tree-fall gaps have higher light, temperature, nitrogen, and

phosphorus (30, 31), leading to higher productivity in gaps. However, as these are abiotic factors and not plant traits under selection, we sought to control for light levels in our analyses in order to focus on the traits involved with NDD. To do so, we curated a list of tree-fall gap-colonizing species, in part on the basis of data from (32) and the experience of P. D. Coley and T. A. Kursar (table S4). As a proxy for light levels, we included the percentage of individuals within a 10-m radius that were gap specialists as a random effect in our GLMs. The density of gap specialists had a strong positive effect on focal tree growth, confirming that light is a limiting factor for growth (Fig. 1 and table S5). By contrast, we found that the survival of focal trees decreased in areas of high density of gap specialists (Fig. 1 and table S5). Although no interaction effects were detected (table S5), when we included the number of gap specialists as a random effect in our model we found that the strength of the effects generally increased, indicating that high light availability can swamp the effect that antagonistic biotic interactions have on growth (table S6).

Neighborhood similarity in traits associated with resource acquisition did not affect focal tree growth or survival (Fig. 1). By contrast, chemical similarity had the largest negative influence on both growth and survival (Fig. 1). In other words, *Inga* trees growing near

chemically similar neighbors grew more slowly and survived less. After *P* values were adjusted for multiple inferences, the negative effect of chemical similarity became nonsignificant for growth but remained highly significant for survival (Fig. 1 and table S6). We have previously reported that defensive chemistry explains most of the variation in herbivore host use for *Inga* (26, 27) and that leaf extracts are toxic to a generalist Lepidoptera insect (17). These results, together with the negative effect of chemical similarity on neighboring *Inga* species, suggest that pressure from insect herbivores is the primary driver of NDD at the sapling stage.

To directly assess the effects of shared herbivores between focal trees and neighboring congeners on growth and survival, we DNA-barcoded the community of Lepidopteran and sawfly herbivores found feeding on the expanding leaves of understory *Inga* saplings by following methods outlined in the supplementary materials. We focused on these herbivore assemblages because they are the most common and cause the most damage to expanding leaves (33–35). Briefly, we visually searched young leaf flushes and collected only larvae that were observed feeding to record host associations of herbivores. Herbivores were observed on 36% of all saplings sampled, and a total of 613 individual herbivores were collected (table S7).



**Fig. 1. Effect of neighborhood similarity.** Neighborhood effects of gap species density, conspecific density, congeneric density, resource acquisition traits, and defense traits on the (A) growth and (B) survival of focal individuals. Estimated coefficients and 95% confidence intervals from generalized linear mixed-effects models are shown.

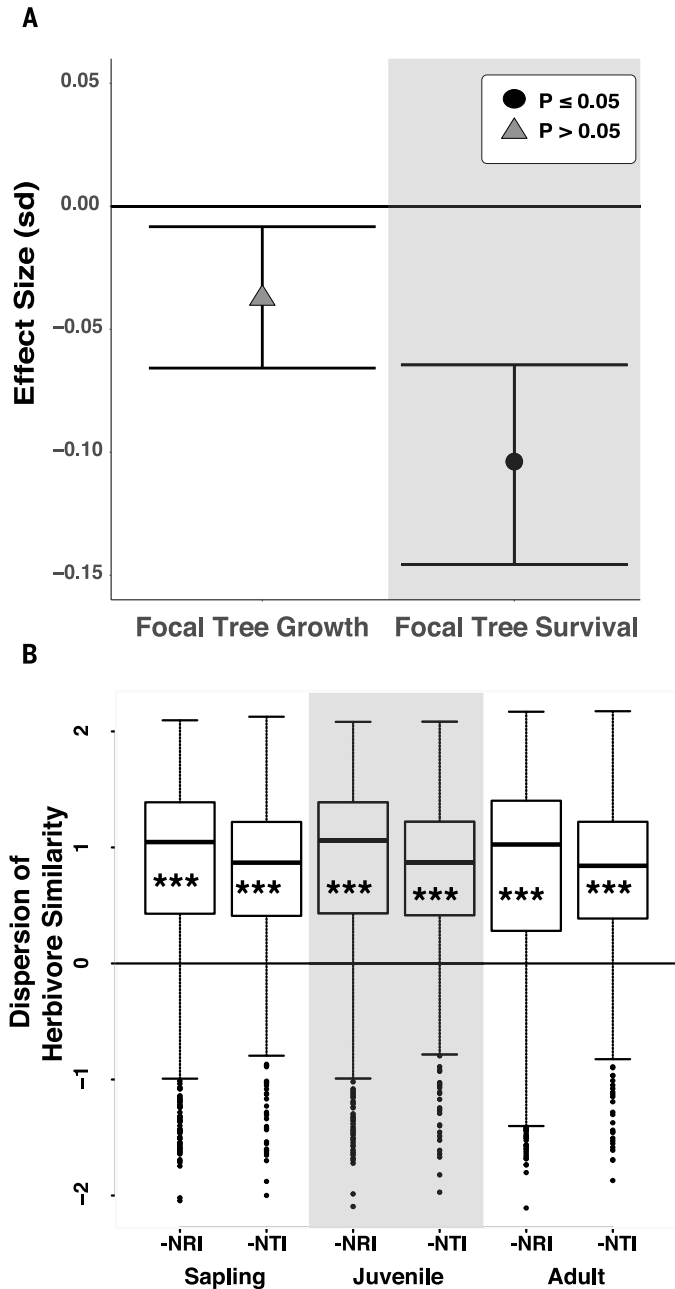
The herbivore community comprised 55 molecular operational taxonomic units (MOTUs) from 24 families (fig. S2). Consistent with global trends (36), we found that the herbivore community associated with *Inga* saplings was markedly specialized. Nearly half ( $n = 31$ ) of the MOTUs were restricted to a single host, whereas only a

single MOTU was shared among all nine *Inga* species (fig. S3). The average herbivore similarity between any two species of *Inga* was only 19% (fig. S4).

We found that similarity in the herbivore community had a negative relationship with both focal tree growth and survival (Fig. 2A). Herbi-

vore similarity (fig. S4) was also overdispersed within the BCI 50-ha plot (Fig. 2B), meaning that congeneric neighbors shared fewer herbivores than would be expected by a random null model of community assembly (37). Such a spatial structure is indicative of deterministic ecological processes driving community assembly and is congruent with observations that antiherbivore defense traits are often overdispersed (22, 23, 38). Moreover, this spatial structure would be the expected outcome given our observation that neighborhood herbivore and chemical similarities result in reduced growth and survival (Fig. 1).

Together, our results suggest that for saplings, a key life-history stage of tropical forest trees, the mechanisms driving NDD and community assembly largely derive from herbivore pressure and not competition for resources. Specifically, as the density of plants with similar defenses increases, the loss of leaf area to herbivores also increases, ultimately causing lower growth and increased mortality of understory saplings. These results for saplings are consistent with studies of seeds and seedlings, demonstrating the critical role natural enemies play in the maintenance of species diversity in tropical rainforests (4, 9, 11–13, 39, 40).



**Fig. 2. Influence of herbivore similarity on focal tree growth and survival and community assembly.** (A) Similarity in herbivore communities between congeneric neighbors decreases the growth and survival of focal trees. Error bars represent the 95% confidence intervals for model coefficients. (B) Herbivores are less similar among neighbors than the null expectation for *Inga* communities in the BCI 50-ha plot. Values given are the additive inverses of the net relatedness index (NRI) and nearest taxon index (NTI) according to (37). Values of  $>0$  indicate overdispersion, and values of  $<0$  indicate underdispersion. The departure from the null expectation (zero) of communities being randomly assembled was evaluated by two-sided  $t$  tests ( $***P < 0.001$ ).

#### REFERENCES AND NOTES

- R. Valencia, R. Condit, R. B. Foster, K. Romoleroux, G. V. Munoz, J.-C. Svenning, E. Magard, M. Bass, E. C. Losos, H. Balslev, in *Forest Diversity and Dynamism: Findings from a Large-Scale Plot Network*, E. C. Losos, E. G. Leigh, Eds. (Univ. of Chicago Press, 2004), pp. 609–620.
- P. Chesson, *Annu. Rev. Ecol. Syst.* **31**, 343–366 (2000).
- J. S. Wright, *Oecologia* **130**, 1–14 (2002).
- J. Terborgh, *Am. Nat.* **179**, 303–314 (2012).
- W. P. Carson, J. T. Anderson, E. G. J. Leigh, S. A. Schnitzer, in *Tropical Forest Community Ecology*, W. P. Carson, S. A. Schnitzer, Eds. (Wiley-Blackwell, 2008), pp. 210–241.
- M. R. Metz, W. P. Sousa, R. Valencia, *Ecology* **91**, 3675–3685 (2010).
- Y. Zhu, L. S. Comita, S. P. Hubbell, K. Ma, *J. Ecol.* **103**, 957–966 (2015).
- R. Bagchi *et al.*, *Ecol. Lett.* **13**, 1262–1269 (2010).
- R. Bagchi *et al.*, *Nature* **506**, 85–88 (2014).
- E. C. Fricke, J. J. Tewksbury, H. S. Rogers, *Ecol. Lett.* **17**, 593–598 (2014).
- J. Zambrano *et al.*, *J. Ecol.* **105**, 616–626 (2017).
- L. S. Comita *et al.*, *J. Ecol.* **102**, 845–856 (2014).
- S. A. Mangan *et al.*, *Nature* **466**, 752–755 (2010).
- P. T. Green, K. E. Harms, J. H. Connell, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 18649–18654 (2014).
- D. H. Janzen, *Am. Nat.* **104**, 501–528 (1970).
- J. H. Connell, in *Dynamics of Populations*, vol. 298, P. J. den Boer, G. R. Gradwell, Eds. (Centre for Agricultural Publishing and Documentation, 1971), pp. 298–312.
- P. D. Coley, M.-J. Endara, T. A. Kursar, *Oecologia* **187**, 361–376 (2018).
- K. G. Dexter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **114**, 2645–2650 (2017).
- C. Wills *et al.*, *PLOS ONE* **11**, e0156913 (2016).
- J. Cavender-Bares, D. D. Ackerly, D. A. Baum, F. A. Bazzaz, *Am. Nat.* **163**, 823–843 (2004).
- N. G. Swenson, B. J. Enquist, *Ecology* **90**, 2161–2170 (2009).
- T. A. Kursar *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 18073–18078 (2009).
- D. Salazar, M. A. Jaramillo, R. J. Marquis, *Ecology* **97**, 3176–3183 (2016).
- N. J. B. Kraft, R. Valencia, D. D. Ackerly, *Science* **322**, 580–582 (2008).
- See supplementary materials and methods.
- M.-J. Endara *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **114**, E7499–E7505 (2017).
- M.-J. Endara *et al.*, *Front. Plant Sci.* **9**, 1237 (2018).
- C. Fortunel *et al.*, *Ecology* **99**, 2272–2283 (2018).

29. R. L. Chazdon, R. W. Pearcy, D. W. Lee, N. Fetcher, in *Tropical Forest Plant Ecophysiology*, S. S. Mulkey, R. L. Chazdon, A. P. Smith, Eds. (Chapman & Hall, 1996), pp. 1–55.
30. P. M. Vitousek, J. S. Denslow, *J. Ecol.* **74**, 1167–1178 (1986).
31. J. S. Denslow, A. M. Ellison, R. E. Sanford, *J. Ecol.* **86**, 597–609 (1998).
32. S. J. Wright *et al.*, *Ecology* **91**, 3664–3674 (2010).
33. D. H. Janzen, *Biotropica* **20**, 120–135 (1988).
34. J. A. Barone, *J. Anim. Ecol.* **67**, 400–409 (1998).
35. V. Novotny *et al.*, *Conserv. Biol.* **18**, 227–237 (2004).
36. M. L. Forrister *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 442–447 (2015).
37. C. O. Webb, *Am. Nat.* **156**, 145–155 (2000).
38. J. Vleminckx *et al.*, *Front. Plant Sci.* **9**, 836 (2018).
39. R. D. Holt, M. B. Bonsall, *Annu. Rev. Ecol. Evol. Syst.* **48**, 447–471 (2017).
40. D. W. Schemske, G. G. Mittelbach, H. V. Cornell, J. M. Sobel, K. Roy, *Annu. Rev. Ecol. Evol. Syst.* **40**, 245–269 (2009).
41. ForestGEO Data Portal; <http://ctfs.si.edu/datarequest/>.
42. D. L. Forrister, Data for Forrister *et al.* 2019, Herbivores as drivers of NDD, Zenodo (2019); doi:10.5281/zenodo.2571716.

43. D. L. Forrister, Data for Forrister *et al.* 2019, Herbivores as drivers of NDD, GitHub (2019); [https://github.com/dlforrister/Forrister\\_et\\_al\\_2019\\_Herbivores\\_as\\_drivers\\_of\\_NDD.git](https://github.com/dlforrister/Forrister_et_al_2019_Herbivores_as_drivers_of_NDD.git).

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performed chemical analysis. D.L.F. performed modeling and wrote the first draft of the manuscript. All authors contributed to revisions and gave final approval for publication.

**Competing interests:** No authors have competing interests.

**Data and materials availability:** The Barro Colorado Forest Census Plot data are archived and openly available for download online from the CTFS and ForestGEO (41). The new DNA sequences generated for this paper have been deposited in the International Barcode of Life (iBOL) under sample numbers IngaHerbiv0001 to IngaHerbiv0280. All R code and trait data used in this analysis have been deposited in Zenodo (42) and are available for download from GitHub (43).

#### SUPPLEMENTARY MATERIALS

[www.sciencemag.org/content/363/6432/1213/suppl/DC1](http://www.sciencemag.org/content/363/6432/1213/suppl/DC1)  
Materials and Methods  
Figs. S1 to S6  
Tables S1 to S8  
References (44–70)

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### Herbivores shape tropical forests

In tropical forests, high local tree diversity is driven by negative density dependence, a process whereby plant performance is inhibited by closely related neighbors. Negative density dependence could be caused by competition for resources among neighbors or result from shared herbivores and pathogens. Using data from forest plots in Panama, Forrister *et al.* compared the contributions of these mechanisms. They found no effect of competition, but strong effects of plant chemistry and shared herbivores on coexisting *Inga* tree species.

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