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Widespread herbivory cost in tropical nitrogen-fixing tree species

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Summary:

While mature and recovering tropical forests constitute a large carbon sink, recent observations suggest this sink is strongly limited by nitrogen[1–3]. Nitrogen-fixing trees, through a symbiotic relationship with bacteria, provide the main natural source of new nitrogen to tropical forests[3,4]. However, fixer abundances are tightly constrained[5-7], highlighting the fundamental unanswered question of what limits new nitrogen entering tropical ecosystems. Here we examine the untested hypothesis that herbivory is responsible for limiting tropical forest symbiotic nitrogen fixation. We evaluate whether fixers experience more herbivory than non-fixers, if herbivory carries a substantial carbon cost, and if high herbivory is due to herbivores targeting fixers' nitrogen-rich leaves[8,9]. We analyzed 1,626 leaves from 350 seedlings of 43 tropical tree species in Panama and find that: (a) while herbivory reduces growth and survival for seedlings, fixers undergo 26% more herbivory than non-fixers; (b) fixers experience 34% greater carbon opportunity costs due to herbivory than non-fixers, exceeding the metabolic cost of fixing nitrogen; but, unexpectedly, (c) high herbivory for fixers is not driven by high leaf nitrogen. Our findings reveal that herbivory may be substantial enough to limit tropical symbiotic nitrogen fixation and could constrain its role in alleviating nitrogen limitation on the tropical carbon sink.

Introduction:

Increasing evidence suggests that nutrients might limit the ability of tropical forests to sustain a long-term carbon sink in mature and secondary forests[1,2,10]. Nitrogen-fixing trees (i.e., trees in the Fabaceae family with the capacity to fix nitrogen; also referred to as fixers) act as a source of new nitrogen to terrestrial ecosystems[4]. Observations of high symbiotic nitrogen fixation rates in tree fall gaps[11,12], in mature forests with high net carbon uptake rates[13] and in some secondary forests recovering from disturbance[4] point to nitrogen limitation as being pervasive in tropical forests[2], with the trait of nitrogen fixation providing growth benefits to tropical trees capable of fixing during periods of nitrogen limitation[4]. Given this substantial benefit of nitrogen fixation to tropical trees, it remains unclear why the trait of fixation is not more widespread: nitrogen fixers represent only ~7%, ~8.5% and 5-15% of basal area in mature Asian and Central African and all ages of Neotropical forests, respectively[5–7,14].

Previous hypotheses to explain what constrains tropical symbiotic nitrogen fixation cite high carbon costs associated with the fixation trait. Breaking the triple-bond of the dinitrogen molecule requires substantial reducing power, leading to a high carbon cost for acquiring nitrogen from fixation relative to taking up inorganic nitrogen from the soil[15], especially if another nutrient like phosphorus or molybdenum limits fixation[16]. Building and maintaining root nodules where the symbiotic bacteria reside presents an additional carbon cost[15]. These constraints may explain the low abundances of temperate nitrogen-fixing tree species, which constitute <1% of temperate trees and cannot avoid fixation-associated costs with their constant fixation rates, regardless of soil nitrogen supply[17,18]. But these constraints cannot account for the low abundances of tropical nitrogen fixers, which employ a facultative fixation strategy that allows them to downregulate fixation as benefits decline, such as when growing in nitrogen-rich soil[4,11]. Accordingly, tropical fixers can avoid fixation-associated costs and should remain competitive even when the costs of fixation would otherwise outweigh the benefits. These previously identified fixation-associated costs are therefore insufficient to explain why the trait has not become more abundant over ecological and evolutionary time. Tropical fixers must face an additional cost associated with fixation that persists regardless of how much they are actively fixing[15-17].

Of the costs previously proposed to be associated with the fixation trait and which are independent of fixation rate[14–18], one has the potential to be substantial: herbivory[16,17,19]. In Neotropical forests, herbivory pressure is high and largely driven by insects that target nitrogen-rich plant tissues to meet their requirements for growth[8,9,20]. Herbivores may therefore be particularly attracted to nitrogen-fixing tree species that contain high leaf nitrogen concentrations relative to non-fixing species, regardless of fixation rates[21]. This pattern could lead to elevated herbivory and a high carbon cost for fixer species, diminishing the benefits of fixation. Such a herbivory cost would reduce fixer growth and competitive ability, ultimately suppressing fixer abundance and fixation rates in tropical forests. If this cost was consistently high across fixer species, then herbivory would lead to negative selection for the fixation trait. If the cost varied substantially across species, then tree species in the fixer functional group may be more heterogeneous in their leaf traits and life strategies than previously thought, consistent with the hypothesis that this functional group includes a diversity of characteristics and strategies[22,23].

A constraint on fixation by herbivory has been proposed in several theoretical frameworks, but empirical support has been limited[16–19,24,25] and mechanistic relationships between tropical fixation, leaf nitrogen and herbivory remain unexamined. Herbivory in the tropics is driven by a complex suite of species-specific leaf and whole-plant traits, and herbivores often only attack plant species with specific co-evolved traits[26]. Furthermore, whilst certain leaf traits attract herbivores, leaf traits for defence can reduce herbivory levels and obscure the role of leaf traits that draw herbivores[24-27]. Fixers have been observed to deploy toxic secondary metabolites, elevated concentrations of indigestible constituents like cellulose, and tough leaves to deter herbivores[25-28]. Therefore, due to this complex nature of tropical herbivory, other fixation-associated traits than leaf nitrogen, such as high leaf area or high leaf cellulose and lignin concentrations, must also be considered when examining what governs herbivory across tree species.

In order to evaluate whether herbivory constrains fixation, we examine three hypotheses: first, fixer tree species undergo higher herbivory than non-fixer species; second, high herbivory constitutes a fixation-associated carbon cost greater than the metabolic cost of fixation in mature forests and substantial enough to constrain fixation; and, third, high herbivory for fixers is driven by high leaf nitrogen concentrations rather than by other leaf and plant traits that are associated with fixation. To test our hypotheses, we quantified standing herbivory on 1,626 mature leaves from 350 seedlings and saplings across 23 species capable of fixation and 20 non-fixer species (including non-fixing Fabaceae species) from mature forest in Panama. On a subset of our trees, we tracked active herbivory rates over three months. We next used our field-based measures to estimate the carbon cost of herbivory for fixers vs. non-fixers and examined how herbivory affected seedling growth. Finally, we combined our herbivory measures with data on leaf nutrient concentrations, physical traits, chemical profiles and growth rates to determine what governs herbivory. We find that fixers experience higher herbivory than non-fixers and that this herbivory represents a major carbon cost that limits fixer growth and reduces seedling survival in the understorey. However, contrary to expectation, we found no support for the idea that herbivory is driven by high leaf nitrogen.

Results and discussion:

We found support for our first hypothesis that fixers undergo higher herbivory than non-fixers. We examined whether fixer seedlings had more leaf area removed than non-fixers when considering all leaves ($\text{Proportion}_{\text{all}}$). Fixer seedlings had a significantly higher proportion of leaf area lost than non-fixers when considering all leaves (9.2% vs 7.6% of total leaf area, respectively; Fig. 1a; Extended Data Table 1). While our recorded leaf damage was likely from insect herbivores, we would expect that mammalian herbivores would also disproportionately prefer the leaves of fixers[8], and therefore lead to even higher herbivory for fixers relative to non-fixers. Our findings are consistent with findings on two species in young tropical forests (Costa, S., Batterman, S., West, F., Hall, J., Breugel, M., Medvigy, D. and Hedin, L., in revision) and herbaceous fixers in temperate grasslands[24] and suggest that, when compared to non-fixers, high herbivory is widespread across fixer species.

We next determined whether this difference in herbivory between the fixer and non-fixer functional groups emerged from a higher rate of leaf attack (incidence of herbivory), a greater amount of leaf area eaten per attacked leaf (summed across damaged leaves on a seedling; $\text{Proportion}_{\text{damaged}}$), or both. Twenty-one percent more fixer leaves were attacked than non-fixer leaves (incidence of herbivory; Fig. 1b; Extended Data Table 1), consistent with the idea that fixers are targeted more frequently by herbivores than non-fixers. Fixers also had a higher proportion of leaf area lost from leaves with herbivory; however, this trend was not significant ($\text{Proportion}_{\text{damaged}}$; 9.3% vs 6.8%; Fig. 1c; Extended Data Table 1). Thus, we conclude that the significantly higher herbivory for the fixer functional group resulted primarily from a higher attack rate, not from greater leaf area loss per attacked leaf. This suggests that fixers may have evolved defence strategies to prevent herbivores from consuming large quantities of leaf area when they are attacked.

We also evaluated whether the high herbivory for fixers was driven by just a few species, or if it was general across species. Negative selection by herbivores would present an evolutionary cost that would constrain the fixation trait across species. Whether this cost is clustered across a few species or is general across most fixers could help explain whether fixer species have either diversified to have distinct strategies or if they function as a unified functional group. To examine variation among species, we predicted mean herbivory with bootstrap estimates of standard error (Fig. 2) for each species using statistical models for the herbivory metrics above that control for variation in leaf size, seedling height and location. We then re-ran our analysis on these species' values. Overall, fixer species had higher predicted herbivory than non-fixer species ($\text{Proportion}_{\text{all}}$; Figs. 1d,2). This pattern emerged from both a higher incidence of herbivory for fixer species (probability of herbivory; Fig. 1e; Extended Data Fig. 1a) and, for leaves that were damaged, a higher amount of leaf area removed from fixer seedlings (which differed from our findings for fixer seedlings, likely due to lower variation at the species scale; $\text{Proportion}_{\text{damaged}}$; Fig. 1c,f; Extended Data Fig. 1b). Despite fixers having higher mean herbivory than non-fixers, there was considerable variation within each group and a large overlap between the two groups in the proportion of leaf area lost to herbivory (range: fixers: 19%-37%; non-fixers: 6%-39%; Fig. 2). Species from the genus *Inga* had higher herbivory than non-*Inga* species (Fig. 2; Extended Data Fig. 1a), but herbivory for non-*Inga* fixer species was still greater than for non-fixers species ($p<0.05$). Variation in herbivory across species may arise from diversification of fixer species as they evolve novel herbivore defence strategies, discussed below. Thus, we conclude that the herbivory trend for fixers vs. non-fixers was not driven by any one species or group.

We next examined our second hypothesis, that high herbivory for fixers constitutes a significant carbon cost greater than the metabolic cost that could constrain the trait of fixation. To test this, we estimated the carbon cost of herbivory as a percentage of net primary productivity (NPP) using the species-level leaf structural carbon concentration, a universal per leaf area photosynthetic rate, the seedling-specific total leaf area lost and assuming a one-year leaf lifespan. These structural and photosynthetic opportunity carbon costs combined diverted ~9% of Net Primary Productivity (NPP) per year for an average fixer seedling (the photosynthetic opportunity cost alone was 8.7% year^{-1}; Figs. 3a,b), compared to 5.9% year^{-1} for non-fixer seedlings. Thus, fixers lost ~3.1% year^{-1} (+/- 1.2% year^{-1}) more of their NPP to herbivory than non-fixer seedlings (range: 6.3-24.1% year^{-1} for fixers, 1.5-27.6% year^{-1} for non-fixers; Extended Data Fig. 1c). This herbivory cost was high relative to the metabolic cost of fixing all leaf nitrogen, which would be minimal if seedlings fixed at low rates in the forest understory and instead took up nitrogen from the soil, as has been previously found[4,11]. Even if fixers replaced 40% of their total leaf nitrogen with fixation each year, as found for seedlings in high understory light levels[29], the metabolic cost would only reach 3% year^{-1} of NPP. This remains less than the herbivory-associated fixation cost. Our carbon cost estimates were insensitive to differences in photosynthetic rates between fixers and non-fixers. While the precise fixation-associated herbivory cost may vary if fixer and non-fixer photosynthetic and respiration rates differed, current evidence demonstrates that tropical fixer and non-fixer photosynthetic rates are comparable[30]. We also consider different leaf lifespans (Extended Data Fig. 2), which regularly exceed one year in shade tolerant forest species[31]. With long-lived leaves, photosynthetic opportunity costs from herbivory will accumulate longer, whilst nitrogen needed for leaf turnover will be low. Thus, our herbivory costs estimated at an annual scale may be conservative (Extended Data Fig. 2). Although tropical fixers can avoid the energetic cost of fixation by downregulating fixation when the cost exceeds the benefit[4,11,32], our research suggests they face herbivory costs even when they gain no benefit from fixation.

Herbivory would therefore divert carbon away from growth and other resource acquisition, decrease the competitive ability of fixers, increase fixer seedling mortality and reduce the likelihood that fixer seedlings reach the canopy and reproduce relative to non-fixers[33]. We found a negative herbivory effect on the growth of all seedlings, with fixers undergoing greater herbivory and therefore suffering greater growth constraints (Fig. 3c; $p<0.05$; Methods). Furthermore, using 586,748 coarse-scale herbivory measurements from our broader dataset of 139,756 seedlings of 223 species, we detected a significant negative effect of high levels of herbivory (>50% leaf area) on seedling survival in 11 out of 13 censuses ($p<=0.05$; Methods). Even low levels of herbivory have been shown to reduce the probability of survival in tropical seedlings: a previous study of 3,000 tree seedlings in Malaysia found that seedlings that lost as little as 1% of leaf area suffered increased seedling mortality the following year[33]. These observations suggest that a cost of 3.1% of NPP – a 34% greater carbon opportunity cost than for non-fixers – would have demographic consequences. Over multiple generations, such negative pressure by herbivory could depress the abundance of fixers and suppress evolution and spread of the fixation trait across tropical tree species. The trait would be maintained and not be completely lost, however, by trees growing in environments favourable to fixation – such as tree fall gaps and secondary forests – where the net benefit of fixation outweighed the cost. Future work would benefit from examining whether these carbon costs due to herbivory are sustained beyond the seedling stage.

Finally, we examined our third hypothesis, that high herbivory for fixers was explained by high leaf nitrogen, or other fixation-associated traits. We identified six traits that differed between fixers and non-fixers: nitrogen, carbon, potassium, cellulose, lignin and leaf area per seedling (Extended Data Table 2, Extended Data Fig. 3). We examined whether any of these traits consistently correlated with measures of herbivory across fixer species, non-fixer species and all species grouped together, which would indicate a relationship between a trait and high fixer herbivory. The only trait to meet these criteria was leaf area, which positively correlated with the incidence of herbivory but not with leaf area lost (Extended Data Tables 2-7), and therefore did not explain higher herbivory for fixers (see Extended Data Table 3). Neither stem length nor variation in leaf area within species explained any additional variation in our models. Importantly, we also found no correlation between leaf nitrogen and herbivory across fixers, non-fixers and the whole species group, including both fixers and non-fixers, in any of our analyses. Thus, our results do not support our third hypothesis that high leaf nitrogen concentrations drive high herbivory for fixers. The only trait that consistently explained all measures of herbivory was the fixation trait itself (which explained up to 24% of variation), suggesting the high herbivory and the trait of fixation are directly linked evolutionarily.

Our inability to identify any one mechanistic driver of high herbivory for fixers is consistent with the growing body of evidence that suggests plant-herbivore relationships are co-evolved and governed by a complex suite of species-specific defence traits[25-28,34,35]. Our observation of higher incidence of herbivory for fixer species indicates that specialized herbivores have evolved to track one common trait across fixer species, such as the trait of generally high leaf nitrogen. However, once an insect herbivore bites a fixer leaf, it eats no more tissue than for non-fixers, consistent with species-specific defence traits making fixer leaves unpalatable[26,28]. Leaf nitrogen offers an example of this inherent complexity: nitrogen in photosynthetic machinery attracts herbivores[8,27], but nitrogen-based defence compounds deter them[34]. Indeed, a previous study found that species-specific differences in leaf chemistry most explained variation in herbivory across four species of fixers and three non-fixers[25]. These multiple roles for leaf traits may obscure relationships between herbivory and traits like high leaf nitrogen across fixer and non-fixer species[26,28,34].

These findings from dozens of tropical mature forest tree species advance earlier evidence of high herbivory on fixers from grassland ecosystems[24] and a handful of tropical tree species in the canopy of secondary forests. They are also consistent with paleoecological evidence that fixer abundance promotes ecosystem level herbivory[35]. Combined, the findings suggest that across ecosystems and biomes fixers bear higher herbivory costs than non-fixers. As these costs reduce growth and survival, they create a filter on the abundance of fixer seedlings, potentially sufficient to constrain nitrogen fixation in tropical forests. The widespread high carbon cost of herbivory for fixers should be incorporated as a constraint on symbiotic nitrogen fixation to improve biogeochemical and climate change models[17-19,36], and provides a plausible ecological and evolutionary explanation for why fixer abundances are capped at 15% across tropical forests[5–7,14]. In sum, herbivory appears to be not only a key factor in governing tropical nitrogen fixation, but also in controlling nitrogen limitation and carbon sequestration by tropical forests impacted by climate change.

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Author contributions:

W.B. and S.A.B. designed the work. W.B. carried out field work. S.J.W., L.S.C, and B.E.S. provided additional data. W.B., L.S.C., S.J.W. and S.A.B. analysed the data. W.B. drafted the article and W.B., S.A.B., S.J.W., L.S.C., and O.L.P. significantly contributed to revisions. All authors provided feedback on the final version of the manuscript.

Competing interests:

The authors declare no competing interests.

Data availability statement:

The datasets generated during and/or analysed during the current study are available in the NERC Environmental Information Data Centre repository at https://doi.org/10.5285/67c95112-edee-435f-9355-9d8bab3a5634.

References:

1. Fernández-Martínez, M. *et al.* Nutrient availability as the key regulator of global forest carbon balance. *Nat. Clim. Chang.* **4**, 471–476 (2014).
2. Wright, S. J. *Plant responses to nutrient addition experiments conducted in tropical forests*. *Ecological Monographs* **89**, (2019).
3. Levy-Varon, J. H. *et al.* Tropical carbon sink accelerated by symbiotic dinitrogen fixation. *Nat. Commun.* **10**, 1–8 (2019).
4. Batterman, S. A. *et al.* Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **502**, 224–227 (2013).
5. Ter Steege, H. *et al.* Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature* **443**, 444–447 (2006).
6. Hedin, L. O., Brookshire, E. N. J., Menge, D. N. L. & Barron, A. R. The Nitrogen Paradox in Tropical Forest Ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **40**, 613–635 (2009).
7. Menge, D. N. L. *et al.* Patterns of nitrogen-fixing tree abundance in forests across Asia and America. *J. Ecol.* **107**, 2598–2610 (2019).

8. Matson Jr, W. J. Herbivory in relation to plant nitrogen content. (1980).

9. Coley, P. D., Bateman, M. L. & Kusar, T. A. The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos* **115**, 219–228 (2006).

10. Wieder, W. R., Cleveland, C. C., Smith, W. K. & Todd-Brown, K. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nat. Geosci.* **8**, 441–444 (2015).

11. Barron, A. R., Purves, D. W. & Hedin, L. O. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. *Oecologia* **165**, 511–520 (2011).

12. McCulloch, L. A. & Porder, S. Light fuels while nitrogen suppresses symbiotic nitrogen fixation hotspots in Neotropical canopy gap seedlings. *New Phytol.* 0–1 (2021) doi:10.1111/nph.17519.

13. Brookshire, E. N. J. *et al.* Symbiotic N fixation is sufficient to support net aboveground biomass accumulation in a humid tropical forest. *Sci. Rep.* **9**, (2019).

14. Gei, M. et al. Legume abundance along successional and rainfall gradients in Neotropical forests. *Nat. Ecol. Evol. 2*, 1104–1111. (2018). https://doi.org/10.1038/s41559-018-0559-6.

15. Vance, C. P. *Carbon and nitrogen metabolism in legume nodules*. (Springer, Dordrecht, 2008).

16. Vitousek, P. M. & Howarth, R. W. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* **13**, 87–115 (1991).

17. Menge, D. N. L., Levin, S. A. & Hedin, L. O. Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. *Proc. Natl. Acad. Sci.* **105**, 1573–1578 (2008).

18. Sheffer, E., Batterman, S. A., Levin, S. A. & Hedin, L. O. Biome-scale nitrogen fixation strategies selected by climatic constraints on nitrogen cycle. *Nat. plants* **1**, 15182 (2015).

19. Vitousek, P. M. & Field, C. B. Ecosystem constraints to symbiotic nitrogen fixers: a simple model and its implications. *Biogeochemistry* **46**, 179–202 (1999).

20. Coley, P. D. & Barone, J. A. Herbivory and plant defenses in tropical forests. *Annu. Rev. Ecol. Syst.* **27**, 305–335 (1996).

21. Fyllas, N. M. *et al.* Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. *Biogeosciences* **6**, 2677–2708 (2009).

22. Batterman, S. A. *et al.* Phosphatase activity and nitrogen fixation reflect species differences, not nutrient trading or nutrient balance, across tropical rainforest trees. *Ecol. Lett.* **21**, 1486–1495 (2018).

23. Menge, D. N. L., Wolf, A. A. & Funk, J. L. Diversity of nitrogen fixation strategies in Mediterranean legumes. *Nat. Plants* **1**, 1–5 (2015).

24. Ritchie, M. E. & Tilman, D. Responses of Legumes to herbivores and nutrients during succession on a nitrogen-poor soil. *Ecol. Soc. Am.* **76**, 2648–2655

(1995).

25. Taylor, B. N. & Ostrowsky, L. R. Nitrogen-fixing and non-fixing trees differ in leaf chemistry and defence but not herbivory in a lowland Costa Rican rain forest. *J. Trop. Ecol.* 1–10 (2019). doi:10.1017/s0266467419000233

26. Endara, M.-J. *et al.* Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. *Proc. Natl. Acad. Sci.* **114**, E7499–E7505 (2017).

27. Kursar, T. A. & Coley, P. D. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochem. Syst. Ecol.* **31**, 929–949 (2003).

28. Kursar, T. A. *et al.* The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proc. Natl. Acad. Sci.* **106**, 18073–18078 (2009).

29. Taylor, B. N. & Menge, D. N. L. Light regulates tropical symbiotic nitrogen fixation more strongly than soil nitrogen. *Nat. Plants* **4**, 655–661 (2018).

30. Adams, M., Turnbull, T., Sprent, J. & Buchmann, N. Legumes are different: Leaf nitrogen, photosynthesis, and water use efficiency. *Proc. Natl. Acad. Sci.* **113**, (2016).

31. Coley, P. D. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* **74**, 531–536 (1988).

32. Batterman, S. A., Wurzburger, N. & Hedin, L. O. Nitrogen and phosphorus interact to control tropical symbiotic N2 fixation: A test in *Inga punctata*. *J. Ecol.* **101**, 1400–1408 (2013).

33. Eichhorn, M. P., Nilus, R., Compton, S. G., Hartley, S. E. & Burslem, D. F. R. P. Herbivory of tropical rain forest tree seedlings correlates with future mortality. *Ecology* **91**, 1092–1101 (2010).

34. Wink, M. Evolution of secondary metabolites in legumes (Fabaceae). *South African J. Bot.* **89**, 164–175 (2013).

35. Currano, E. D. & Jacobs, B. F. Bug-bitten leaves from the early Miocene of Ethiopia elucidate the impacts of plant nutrient concentrations and climate on insect herbivore communities. *Glob. Planet. Change* **207**, 103655 (2021).

36. Wieder, W. R., Cleveland, C. C., Lawrence, D. M. & Bonan, G. B. Effects of model structural uncertainty on carbon cycle projections: Biological nitrogen fixation as a case study. *Environ. Res. Lett.* **10**, (2015).

Figures:

Figure 1. Nitrogen-fixing trees have higher herbivory than non-fixers in a tropical moist forest. Metrics of herbivory from 1,626 leaves, 350 individuals and 43 species for fixers (orange) and non-fixers (grey) in Panama derived at the leaf, seedling and species scale. At the seedling and leaf scale, **a** shows the proportion of leaf area lost from all sampled leaves for each seedling ($\text{Proportion}_{\text{all}}$); **b**, the incidence of herbivory on leaves (i.e., whether or not a leaf had herbivory); and, **c**, the proportion of leaf area lost only on damaged leaves for each seedling ($\text{Proportion}_{\text{damage}}$). At the species scale where each point represents a species, **d**, the predicted proportion of leaf area lost to herbivory across all sampled leaves from each seedling; **e**, the probability of herbivory on individual leaves; and, **f**, the predicted proportion of leaf area lost to herbivory on damaged leaves from each seedling. Predicted values for d, e and f were derived from our models of $\text{Proportion}_{\text{all,}}$ the incidence of herbivory and $\text{Proportion}_{\text{damaged}}$. Lines represent the mean of data at the leaf (**a**), seedling (**c**) and species (**d-f**) scales. **b** shows the mean ± standard error of data at the leaf scale. Asterisks denote statistically significant differences between fixers and non-fixers in mixed effects models (**a**, **b**, **c**) and two-sided non-parametric Wilcoxon rank tests (**d**, **e**, **f**). Data are jittered to show differences, and numbers of biologically independent samples for each category and statistical results are found in Extended Data Table 1.

Figure 2. Species differences in leaf herbivory of nitrogen-fixing and non-fixing trees in a tropical moist forest. Percentage of leaf area lost to herbivory on all sampled leaves per seedling for 23 nitrogen-fixing (orange) and 20 non-fixing (grey) species. Bars represent the predicted mean (± standard error) values derived from the statistical models of $\text{Proportion}_{\text{all}}$ for each species. Fixers had significantly higher predicted leaf area lost than non-fixers, according to a two-sided non-parametric Wilcoxon rank test (p=0.02). The number of biologically independent seedlings sampled for each species can be found in Supplemental Information Table 1.

Figure 3. The cost of herbivory for nitrogen-fixing trees in a tropical moist forest. This includes both the carbon cost estimates associated with fixation and the relationship between herbivory and seedling growth rate for fixer (orange) and non-fixer (grey) seedlings. **a**, The herbivory cost, including the structural carbon and photosynthetic opportunity carbon costs of herbivory as a percentage of annual net primary production (NPP). Box and whisker plots represent the geometric mean, the first and third quantiles, 1.5 times the interquartile range and outliers. **b**, The fixation-associated cost of herbivory for fixers (geometric mean ± standard error) and the metabolic cost of fixing nitrogen (i.e., the metabolism of fixing N_2 to NH_3 and building and maintaining nodules; bar shows mean cost from 0 to 40% light availability and the line shows the maximum fixation cost) across the range of fixation rates observed over a gradient in light availability observed in the understory of mature

tropical forests[39]. The metabolic fixation cost for seedlings in mature forest is likely to be low since the majority of seedlings occur at <16% of light availability where fixers downregulate fixation. **c**, The relationship between herbivory (proportion leaf area lost) for a seedling and seedling stem relative growth rate. Points represent each of the seedlings sampled, and the lines represent model fit and 95% confidence interval. An outlier with a growth rate of >6 mm mm^{-1} year^{-1} was removed to make it easier to observe the relationship between growth rate and herbivory (this data point was included in analyses). The asterisks represent significant differences as determined by two-sided non-parametric Wilcoxon rank tests (**a**, $p < 0.001$; n=351 biologically independent samples) and a significant relationship between stem growth rate and leaf area lost to herbivory in mixed effects models (**c**, $p = 0.04$; n=350 biologically independent samples).

Methods:

Species selection and study site

To compare herbivory across the wide range of fixer and non-fixer species present in biodiverse lowland tropical moist forest, we sampled individuals from 23 fixer species[37] and 20 non-fixer species in the 50-ha plot on Barro Colorado Island (BCI), Panama (latitude: 9.125, longitude: -79.8553), during the wet seasons of 2017 and 2018. Annual rainfall averages ~2600 mm and mean annual temperature is 27° C. Monthly means vary by 1°C[38].

We sampled nearly every fixer species present at the site (23 out of 26) and selected non-fixer species that covered the range of species abundances across the 50-ha plot (see Extended Data Table 1 for number of seedlings sampled per species). We focused our study on seedlings because herbivory acts as a major bottleneck at this life stage[39,40]. All seedlings sampled were included in a long-term census of free-standing, woody seedlings (≥20 cm in stem height and <1 cm stem diameter at 1.3m) in the 50-ha plot that ran from 2001 to 2018[39].

Quantifying herbivory

We quantified herbivory on mature and young leaves separately. For mature leaves, we non-destructively scanned up to six (mean 4.9) randomly selected mature leaves per individual for 184 fixer seedlings and 166 non-fixer seedlings from June to July 2017. To scan leaves, we used a hand-held document scanner (TaoTronics) with a resolution of 1050 Dots per Inch (DPI). We quantified leaf area lost to herbivory using ImageJ (version 1.52j)[41]. For all leaves where the edge was damaged by herbivory, we cloned and used a complete leaf edge to estimate the undamaged leaf area and used these values to calculate the proportion of leaf area lost to herbivory. Additionally, 226 young leaves (one per seedling) were tagged and scanned again in November 2017 to measure herbivory rates, to capture higher herbivory often experienced by young leaves[27] and to determine leaf turnover rates (Extended Data Fig. 3).

We took two approaches to measure herbivory rate on young leaves, which accounted for entire leaflets that were lost and potential leaf growth over the course of measurement. First, we calculated herbivory rate as the difference in leaf area between time point one and time point two, divided by the estimated total leaf area at time point one, which included leaf tissue lost to herbivory. This captured entire leaflets lost to herbivory over the study that otherwise would be underrepresented in the data. Second, for leaves with negative herbivory rates, we recalculated herbivory rate as the difference in percentage of leaf area missing at time point one and time point two. This second method permitted the quantification of herbivory even when leaves grew over the period of observation. Importantly, these two methods give the same value for leaves that did not lose leaflets or grow, and together allowed us to assess our whole dataset.

Calculating carbon costs

We consider two herbivory-associated costs: (1) a structural carbon cost of leaf area lost to herbivory and (2) a photosynthetic opportunity carbon cost since lost leaf area reduces carbon assimilation via photosynthesis over time. We do not consider a potential third additional cost of replacing lost leaf nitrogen due to herbivory. Finally, we calculate the metabolic carbon cost of fixing nitrogen to evaluate the magnitude of the herbivory-associated costs. We considered our 350 seedlings plus one additional seedling that we did not include in our other analyses because we did not have its height measurement, thus n=351 for our herbivory carbon cost estimates. All costs are expressed as a percent of net primary productivity (NPP) per year.

The loss of structural carbon would require the plant to replace that lost carbon when rebuilding leaf tissue, and therefore could come at the expense of allocating carbon to nitrogen fixation. We estimated the structural carbon cost at the leaf scale using species-specific leaf traits (described below). We first generated total leaf mass and leaf mass lost to herbivory by multiplying species-specific leaf mass per area (LMA) by either the total leaf area or leaf area lost to herbivory for each leaf, respectively. We then multiplied the total leaf mass and leaf mass lost to herbivory by species-specific per-mass carbon concentrations to estimate total leaf carbon and the carbon lost to herbivory. We multiply this cost by leaf longevity – here we assume that the leaf lifespan is one year, although the structural carbon cost would not vary with leaf lifespan (Extended Data Fig. 2).

As well as removing structural leaf carbon, herbivory reduces the leaf area available to assimilate carbon by photosynthesis. To estimate this photosynthetic opportunity carbon cost, we used the maximum carbon assimilation rate (A_{max}) observed in field-grown seedlings of a common species in Panama, *Alseis blackiana*[42]. We converted A_{max} values from µmol cm^{-2} s^{-1} to µg cm^{-2} s^{-1} and multiplied this value by the leaf area lost to herbivory per leaf. We assumed 12 hours of photosynthesis per day. We multiply our costs by leaf longevity, here assuming one year lifespan, and express the carbon cost as % NPP $year^{-1}$. This cost would accrue with leaf longevity (Extended Data Fig. 2).

To generate estimates of carbon costs we multiplied the mean structural carbon cost and photosynthetic opportunity cost across sampled leaves (up to 6) for each individual by its total number of leaves. We compared all carbon costs as a percentage of annual net primary production (NPP) per seedling, calculated as the annual photosynthetic capacity for each seedling minus ~47% of carbon lost to respiration each year[43]. Herbivory costs were highly zero inflated (many seedlings had no herbivory), so we took the geometric mean of each as a percentage of annual net primary production cost, which better represents zero inflated distributions than an arithmetic mean. As the geometric mean of any distribution containing zeroes will always be zero, we added 1 to each cost and then subtracted 1 from our average. To isolate the herbivory-associated fixation cost for fixer species, we subtracted the mean herbivory cost for non-fixer species from that of fixer species. Importantly, this method of estimating carbon costs controls for variation in leaf area and measures of photosynthetic rates between fixers and non-fixers.

Finally, we compared these herbivory-associated costs to the metabolic carbon cost of fixing nitrogen[15]. We consider the range of light availability that fixer seedlings have been found to experience in a mature forest understory, and the corresponding fraction of nitrogen that they derive from fixation in those conditions[29]. Fixer seedlings in the understory have been observed to receive up to 16% light, with occasional incidences of 40% full sunlight[29]. At less than 16% light, the condition most seedlings experience, seedlings fix no nitrogen. At 40% light they fix up to 40% of total acquired nitrogen. We estimated maximum nitrogen fixation costs by multiplying seedling total leaf area by species-specific leaf nitrogen concentration, the fraction of nitrogen derived from fixation and leaf longevity. Here we assume leaves last one year, but we also consider how these costs per year vary with leaf longevity (Extended Data Fig. 2). We then convert this total seedling leaf nitrogen to carbon using the common ratio of six grams of carbon per gram of nitrogen fixed[15]. We consider a maximum metabolic fixation cost under mature forest light conditions as the cost of acquiring 40% total leaf nitrogen (to completely turnover all leaves in one year). If plants received all nitrogen from the soil, then this fixation-associated cost would be zero.

Since we do not know the precise lifespans of leaves from our focal trees, we also consider how costs would vary across a range of leaf lifespans (Extended data Fig. 2). We assume that our observed herbivory is the total herbivory the leaf will accumulate over its lifespan. This is a reasonable assumption since the majority of herbivory occurs on young leaves[27]. We multiplied the photosynthetic opportunity cost by a range of leaf lifespans to estimate the accumulated cost as a percent NPP per year. The structural carbon cost remains constant since it would be a one-time cost, regardless of leaf lifespan. We multiplied the metabolic cost by the number of leaf replacements per year (12/leaf longevity) across our range of leaf longevities to get the metabolic cost of fixing nitrogen for leaves over a year. We consider leaves that range from 1 to 35-month lifespan, the range recorded for shade species on Barro Colorado Island[31]. Mean leaf lifespan amongst understory species has been found to be 21.65 months.

Differences in leaf retention between fixer and non-fixer seedlings

We explored the role of leaf retention (turnover rates) in driving higher herbivory measurements for fixers than non-fixers. Theoretically, if non-fixers drop leaves more readily after herbivory it could reduce average herbivory measurements for the non-fixer group, as we did not measure herbivory on fallen leaves. Accordingly, we recorded leaf retention for 226 young leaves over three months in 2017. We found no significant difference in leaf turnover rate between fixers and non-fixers ($p > 0.05$, $R^2 = 0.16$; Extended Data Fig. 3), suggesting that differences in herbivory between the two functional groups are not driven by differences in leaf retention, but are governed by other leaf and species traits.

Seedling growth rates

We tracked the growth of the focal seedlings used to measure herbivory in inventories conducted in 2017 and 2018 (see reference 39 for detailed methods). We calculated the relative growth rate as the natural log of the difference in stem length from 2018 minus 2017.

Seedling survival and herbivory

We examined whether high rates of herbivory resulted in lower survival rates for seedlings in the following year using a broader dataset of 586,748 coarse-scale measurements of herbivory and survival belonging to 139,756 unique seedlings from 223 species over 18 years and 13 censuses. See reference 39 for a detailed description of the data collection. We scored seedlings with more than 50% leaf area missing as having high herbivory, and seedlings with less than 50% as having no or low herbivory.

Species attributes and leaf traits

To determine what might drive differences in herbivory between fixers and non-fixers, we combined our data with species level leaf traits. We tested for differences in leaf nutrient concentrations (nitrogen, carbon, phosphorus, potassium and calcium), physical defence traits (cellulose, hemicellulose, lignin and silicon concentrations), measures of leaf toughness (lamina toughness, vein toughness, lamina density, work to shear and LMA) and chemical similarity (see below) between fixers and non-fixers to determine if any traits could drive patterns of herbivory across our sample species (see Extended Data Table 2 for all traits).

For nutrient and physical defence traits, three leaves were sampled from the highest point of the crown for the six largest and six smallest individuals of each species in the 50-ha plot. Leaves were collected between July 2007 to January 2008 and were sampled across light environments[44]. Since our herbivory data was collected for seedlings under the canopy, we used leaf trait data from shade leaves. Samples were stored on ice until they could be oven dried at 60 °C. All samples were measured within 2-24 hours after sampling[45]. Each value is a mean calculated from three to six individuals for each species in each light level environment.

Phosphorus, potassium, calcium and zinc concentrations were determined using nitric acid digestion (200 mg of dried leaf samples with 2 ml of 70% nitric acid) and leaf nitrogen and carbon concentrations were analysed with an elemental analyser (dry combustion and gas chromatography, with thermal conductivity detection). Cellulose, hemicellulose, silicon and lignin concentrations per unit leaf dry mass were determined using the method followed by Westbrook et al., 2011 (see Appendix A of reference 44). Leaf toughness was measured as resistance to fracture, either per unit dry mass (density corrected fracture toughness), unit volume (fracture toughness (J cm^{-2})) or unit cut length (Work to shear (J cm^{-1})). These measures, alongside lamina density (g cm^{-3}) and Leaf Mass per Area (LMA, g cm^{-2}) (for protocol, see reference 44) were also included as they have been previously found to correlate with increased leaf lifespan and reduced herbivory, but these variables did not differ between fixers and non-fixers, nor did they explain what drove herbivory across species. Thus, we did not include them in our main analyses[44,46,47].

Metrices of chemical similarity were included that allow differences in secondary metabolites to be compared between species and species groups. These metrics distinguish molecular networks of unidentified compounds using methanol extractions of homogenized leaf tissue. Molecules were identified using ultra high-performance liquid chromatography, electrospray ionization and molecular fragmentation, and tandem mass spectrometry of molecular fragments[48]. Networks of these molecules can be constructed that portray the structural similarity of unknown compounds, as molecules with similar structures fragment into many of the same substructures. By then comparing the mass to charge ratio of the fragments of two molecules, one can compare the similarity in the molecule structures. Structural similarity can then be quantified for every pair of compounds as the cosine of the angle between vectors that comprise the mass to charge ratio of their constituent fragments, with a cosine of >0.6 showing a meaningful similarity. All pairwise combinations of said compounds were then used to calculate two variables, first, the nearest neighbour Chemical Structural and Compositional Similarity (nnCSCS), which measures the similarity in leaf secondary metabolites between each species and the most chemically similar species of the 138 species sampled across the 50-ha plot, and second, the mean Chemical Structural and Compositional Similarity (mCSCS), which measures the mean similarity of each focal species across the majority of other species in the 50-ha plot[48].

Statistical analyses

The proportion of leaf area lost across leaves was highly right skewed and contained many zeroes (zero inflated) across both the young and old leaves (as there were many undamaged leaves). To account for this, we used two approaches. First, we analysed individual seedlings with a mixed effects beta regression. This approach allowed us to assess if fixer seedlings lost more leaf area overall by considering the entire data set together, including leaves that did not have area missing (zero values) and leaves with damage. For this method, we summed the amount of leaf area missing across leaves per individual seedling, including leaves with no herbivory damage ($\text{Proportion}_{\text{all}}$). This approach reduced the number of zeroes in the dataset since there were fewer individuals with no herbivory than there were leaves with no herbivory and made a unified statistical approach possible. We ran the mixed effects beta regression model using the glmmTMB package[49] (we also ran a zero-inflated beta regression and compared our model results, see below). A beta distribution is well suited to model variables with upper and lower limits, such as proportions[50]. However, as a beta distribution is only suitable for values above 0 and below 1, and our data contained zeroes, we rescaled our data using the equation:

$$X' = (x(N-1)+0.5)/N$$

Where x is the proportion of leaf damage and N is the total number of individuals (following the methods used in references 50 and 51).

Second, we analysed individual leaves using a hurdle model approach. The first step of the hurdle considered the incidence of herbivory, which is a binary measure of leaves either with herbivory (leaves with any leaf area missing) or no herbivory (no leaf area missing). For the incidence of herbivory, we ran a binary logistic regression using the lme4 package[52] to test if there was a difference in the incidence of herbivory between fixer and non-fixer species. The second step of the hurdle model considered the proportion of leaf area lost only from leaves with area missing ($\text{Proportion}_{\text{damaged}}$), which was a continuous measure. For $\text{Proportion}_{\text{damaged}}$, again, we summed the leaf area lost per seedling to reduce the number of zeroes in the data set and then ran a mixed effects model with normal distribution and square root transformed the proportion of leaf area missing to meet assumptions of normality. This model evaluated whether there was a difference in the leaf area lost on leaves attacked by herbivores between fixers and non-fixers. Together, this hurdle approach allowed us to assess whether more fixer leaves were attacked and/or whether more fixer leaf area was eaten per attack than for non-fixers, allowing us to ultimately understand the mechanism by which herbivory affected fixer and non-fixer seedlings.

In all our models, our beta regression model and our hurdle models, we included the trait of fixation as a fixed effect to test for differences in herbivory between the two groups (see Model 1 below). We expected herbivory to vary with both seedling and leaf size within species. Larger seedlings are likely to have more leaves and therefore be easier for herbivores to locate. Leaf area has been previously shown to drive herbivory in tropical seedlings, as larger leaves may be easier to locate, lay eggs on or may expand through vulnerable early stages of development more slowly[53]. Leaf area was standardized (centred on zero and divided by one standard deviation) within species to account for species level variation in leaf size. Stem length was scaled across all seedlings (centred on zero and divided by one standard deviation). All models also included species identity as a random effect to account for species specific variation in herbivore damage. The 20 m^2 plot in which the seedling was found was likewise included as a random effect to account for spatial autocorrelation in modelling Incidence and $\text{Proportion}_{\text{all}}$, but not for $\text{Proportion}_{\text{damaged}}$ as this led to overfitting with the smaller dataset. In addition, when modelling incidence of herbivory, individual seedling identity was included as a random effect to account for taking repeated measures from the same individuals (since we sampled up to 6 leaves per seedling). To test the consistency of our approach we ran a zero-inflated beta regression model using the R package brms for Bayesian modelling and compared the results to those from our hurdle and transformed beta regression models[54]. In this model we specified zero inflation in our response variable, $\text{Proportion}_{\text{all}}$, and included fixation status, leaf area standardized within species and seedling stem length as explanatory variables. Priors were selected as between 0-1 for both the standard and zero-inflated components of the model. We specified 4 Markov chains each with 5,000 iterations. We found similar results across all of our models, and so our zero-inflated beta regression is not discussed in further detail here (but see Supplementary Information Table 3).

We also used a simple linear mixed effects model to test whether fixer and non-fixer seedling stem growth rate was affected by measures of herbivory after one year, including fixation and initial seedling leaf area and stem length as fixed effects to control for variation in seedling size, whilst controlling for plot and species as random effects. Growth rate was log transformed to meet assumptions of normality. We used pseudo R^2 values to determine how much of the observed variance in herbivory measures was explained by all our models.

We used binomial mixed effects models to test whether seedlings that had high herbivory (>50% leaf area missing) had lower survival rates than seedlings with low herbivory (<50% leaf area missing). This was done across the full dataset of 586,748 measurements from 139,756 unique seedlings. We included height as a fixed effect and height nested within species as a random effect and ran the model for each census. We report the number of censuses when there was a significant relationship between herbivory and survival.

To determine if leaf traits governed the difference in herbivory between fixer and non-fixer species, we identified leaf traits that differed between the fixer and non-fixer groups (using Wilcoxon rank tests). We then restructured the models described above to include the variables that differed to generate new models, by replacing the trait of fixation as an explanatory variable with each of the identified leaf traits. For example, when testing the role of leaf nitrogen, we ran a model using species leaf nitrogen concentration, standardized leaf area and stem length as fixed effects and species and plot identity as random effects. We then ran these models for all species, or just fixer and non-fixer species alone. For the $\text{Proportion}_{\text{damaged}}$ we ran simple linear regressions, excluding random effects, to avoid overfitting with the reduced dataset.

Model 1: Variable specification for our model testing for a difference in $\text{Proportion}_{\text{all}}$ between fixer and non-fixer species (fitted using a beta regression, see above).

$\text{Proportion}_{\text{all}}$ ~ Fixation + Standardized leaf area + Stem length + (1 | species) + (1Iplot)

Model 2: Variable specification for our model examining the role of a given leaf trait found to differ between fixer and non-fixer species (here leaf nitrogen concentration) in driving $\text{Proportion}_{\text{all}}$ across species.

$\text{Proportion}_{\text{all}}$ ~ Leaf Nitrogen Concentration + Standardized leaf area + Stem length + (1 | species) + (1Iplot)

Model 3: Variable specification for the beta regression used to predict species level $\text{Proportion}_{\text{all}}$. Species is now specified as a fixed effect and fixation is removed from the model (see below).

$\text{Proportion}_{\text{all}}$ ~ Species + Standardized leaf area + Stem length + (1Iplot)

We also used our models to predict the probability of herbivory and mean leaf area lost to herbivory across fixer and non-fixer species to account for variation due to seedling size, leaf area, species identity and location in the plot. We restructured all models so that species identity was included as a fixed effect and fixation (or any trait other than standardized leaf area and stem length) was omitted (compare variable specification in Model 2 and 3 above). These restructured models were used to generate predicted levels of each of our three measures of herbivory. First, we used our model of the incidence of herbivory across seedlings to predict the probability of herbivory per species. Species for which we had less than 10 leaves were excluded from our probability model to prevent singularity. Second, we used our two restructured models of the proportion of leaf area lost ($\text{Proportion}_{\text{all,}}$ $\text{Proportion}_{\text{damaged)}}$ to predict the average leaf are lost to herbivory per species (over damaged leaves and all leaves, using the predict() function from the bootpredictlme4 function for the binary logistic regression model and the linear mixed effects model and the predict.GLMM() function from the glmmTMB package for the beta regression model[49,55]). As $\text{Proportion}_{\text{all}}$ was square root transformed to meet assumptions of normality, species level predictions were back transformed for presentation. We ran Wilcoxon Rank tests to determine if there was a difference in the average predicted value between fixer species and non-fixer species for each measure of herbivory. All statistical analyses were carried out in R version 3.5.1[56] using R Studio (version 1.1.463).

The datasets generated during and/or analysed during the current study are available in the NERC Environmental Information Data Centre repository at https://doi.org/10.5285/67c95112-edee-435f-9355-9d8bab3a5634.[57]

Methods references:

37. Sprent, J. I. *Legume nodulation: a global perspective*. (John Wiley & Sons, 2009).
38. Leigh, E. G. *Tropical forest ecology: a view from Barro Colorado Island*. (Oxford University Press, 1999).
39. Comita, L. S., Muller-Landau, H. C., Aguilar, S. & Hubbell, S. P. Asymmetric density dependence shapes species abundances in a tropical tree community. *Science.* **329**, 330–332 (2010).
40. Queenborough, S. A., Metz, M. R., Valencia, R. & Wright, S. J. Demographic consequences of chromatic leaf defence in tropical tree communities: do red young leaves increase growth and survival? *Ann. Bot.* **112**, 677–684 (2013).
41. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671 (2012).
42. Pasquini, S. C. & Santiago, L. S. Nutrients limit photosynthesis in seedlings of a lowland tropical forest tree species. *Oecologia* **168**, 311–319 (2012).
43. Collalti, A. & Prentice, I. C. Is NPP proportional to GPP? Waring's hypothesis 20 years on. *Tree Physiol.* **39**, 1473–1483 (2019).
44. Westbrook, J. W. *et al.* What makes a leaf tough? Patterns of correlated evolution between leaf toughness traits and demographic rates among 197 shade-tolerant woody species in a Neotropical forest. *Am. Nat.* **177**, 800–811 (2011).
45. Wright, S. J. *et al.* Functional traits and the growth–mortality trade-off in tropical trees. *Ecology* **91**, 3664–3674 (2010).
46. Kitajima, K. *et al.* How cellulose-based leaf toughness and lamina density contribute

to long leaf lifespans of shade-tolerant species. *New Phytol.* **195**, 640–652 (2012).

47. Kitajima, K., Wright, S. J. & Westbrook, J. W. Leaf cellulose density as the key determinant of inter- and intra-specific variation in leaf fracture toughness in a species-rich tropical forest. *Interface focus*, ***6***, (2016)

48. Sedio, B. E., Echeverri, J. C. R., Boya, C. A. & Wright, S. J. Sources of variation in foliar secondary chemistry in a tropical forest tree community. *Ecology* **98**, 616–623 (2017).

49. Brooks, M. E. *et al.* glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400 (2017).

50. Smithson, M. & Verkuilen, J. A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. *Psychol. Methods* **11**, 54–71 (2006).

51. Murphy, S. J., Xu, K. & Comita, L. S. Tree seedling richness, but not neighborhood composition, influences insect herbivory in a temperate deciduous forest community. *Ecol. Evol.* **6**, 6310–6319 (2016).

52. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *arXiv Prepr. arXiv1406.5823* (2014).

53. Moles, A. T. & Westoby, M. Do small leaves expand faster than large leaves, and do shorter expansion times reduce herbivore damage? *Oikos* **90**, 517–524 (2000).

54. Bürkner, P. C. brms: An R package for Bayesian multilevel models using Stan. *J. Stat. Softw.* **80**, (2017).

55. Duursma, R. bootpredictlme4. Predict method for lme4 with bootstrap. R package version 0.1. (2020).

56. R Core Development Team. A Language and Environment for Statistical Computing. *R Fountp//www.R-project.org/.dation Stat. Comput.* **2**, https://www.R-project.org (2018).

57. Barker, W.B.; Comita, L.S.; Wright, S.J.; Phillips, O.L.; Sedio, B.E.; Batterman, S.A. (2022). Herbivory rates, species traits and leaf traits across symbiotic nitrogen-fixing and non-fixing species from a Panamanian tropical forest, 2007-2019. NERC EDS Environmental Information Data Centre. https://doi.org/10.5285/67c95112-edee-435f-9355-9d8bab3a5634

Extended data legends:

Figures:

Extended Data Figure 1.

Title: **The difference in herbivory and the carbon cost of herbivory for nitrogen fixer and non-fixer species.**

Legend: **a**, the distribution of the predicted probability of herbivory on leaves of 17 fixer species and 19 non-fixer species. **b**, the distribution of the predicted proportion of leaf area lost to herbivory on attacked leaves of each seedling for 23 fixer species and 20 non-fixer species. **c**, the distribution of the geometric mean of the herbivory carbon cost as a fraction of net primary production (NPP) across species (17 fixer species, 18 non-fixer species) for fixers (orange) and non-fixers (grey). Fixers are represented in orange and non-fixers in grey. Bars in **a** and **b** represent predicted mean values (± standard error of mean) derived from our modelling of Incidence of herbivory and Proportion$_{\text{damaged}}$. Asterisks denote statistically significant differences (p=0.02 for **a**, p=0.04 for **b**, p=0.04 for **c**) between fixers and non-fixers from two-sided non-parametric Wilcoxon rank tests. Numbers above each bar in panel c represent the number of seedlings sampled per species. Note that the number of leaves (**a**) and seedlings (**b)** sampled for each species can be found in the Supplementary Information Table 1.

Extended Data Figure 2.

Title: The herbivory versus metabolic costs of fixation across leaf lifespan.

Legend: How the fixation-associated herbivory costs and metabolic cost of fixing nitrogen vary over leaf lifespan. Costs shown as a percentage of annual NPP per year, using the mean herbivory and leaf area for fixers and non-fixers up until the maximum leaf lifespan for shade species recorded in the 50ha plot on Barro Colorado Island (BCI). The photosynthetic opportunity cost was calculated as the accruing photosynthesis forgone until the end of the leaf lifespan (dark blue line). The structural carbon cost remained constant since the cost per year would not vary with leaf lifespan (red line). The metabolic cost represents the percentage of NPP required to replace either 40% of leaf nitrogen (at 40% light, orange line) or 0% (at 16% light, light blue line) paying six grams of carbon per gram nitrogen over one year, depending on leaf lifespan. The mean leaf lifespan for shade species in the BCI 50ha plot is 21.65 months (green line). These values differ from Fig. 3b since they are at the leaf level, use mean values as parameter estimates and consider variation in leaf lifespan.

Extended Data Figure 3.

Title: **Leaf traits that are potential drivers of herbivory in mature leaves, and herbivory measurements on young leaves.**

Legend: Showing both the leaf traits that varied between fixers and non-fixers in mature leaves, and metrics of herbivory and leaf retention on young leaves. For mature leaves, the difference in **a**, leaf area, **b**, leaf nitrogen concentration, **c**, leaf cellulose concentration, **d**, leaf carbon concentration, **e**, leaf lignin concentration and, **f**, leaf potassium concentration. All differences in leaf variables for mature leaves are significant as determined by two-sided Wilcoxon rank test on n=184 fixer and n=166 non-fixer species. N = 43 (a), 37 (b), 38 (c), 37 (d), 38 (e) and 37 (f) biologically independent samples. For young leaves, **g**, the incidence of herbivory; **h**, the proportion of leaf area lost to herbivory per day for damaged leaves ($\text{Proportion}_{\text{damaged}}$) on each seedling; **i**, the proportion of leaf area lost to herbivory per day on all leaves ($\text{Proportion}_{\text{all}}$) of each seedling; and, **j**, the proportion of sampled leaves that still remained after three months (i.e. leaves that have not been dropped by the plant). Nitrogen fixers are represented in orange and non-fixers in grey. For the measures of herbivory on young leaves there were no difference between fixers and non-fixers, as determined by two-sided non-parametric Wilcoxon rank test (n= 226 (119 fixers, 107 non-fixers)). Points represent seedlings with the lines representing means (± standard error) across seedlings; bars represent mean (± standard error).

Tables:

Extended Data Table 1:

Title: **Fixers undergo greater herbivory than non-fixers.**

Legend: The effect of the trait of fixation, leaf area and stem length in driving the proportion of leaf area lost for all leaves ($\text{Porportion}_{\text{all}}$), the incidence of herbivory and the proportion of leaf area lost to herbivory for damaged leaves ($\text{Proportion}_{\text{damaged}}$) on mature and young leaves, according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. Leaf area was standardized within species. Seedling, species and plot identity were included as random effects in the incidence of herbivory model, and species and plot identity were included as random effects for both proportion models.

Extended Data Table 2:

Title: **No relationship between leaf nitrogen concentration and herbivory.**

Legend: The effect of leaf nitrogen concentration, leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. For each response variables, all three fixed effects were included, only changing the data set between models. Leaf area was standardized within species. Seedling (for the incidence model), species and plot identity were included as random effects.

Extended Data Table 3:

Title: **Leaf area drives some measures of herbivory.**

Legend: The effect of leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. In this focal model, leaf area is not standardized within species. For each response variable, both fixed effects were included, only changing the data set between models. Seedling (for the incidence model), species and plot identity were included as random effects.

Extended Data Table 4:

Title: **No relationship between leaf carbon concentration and herbivory.**

Legend: The effect of leaf carbon concentration, leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. For each response variable, all three fixed effects were included, only changing the data set between models. Leaf area was standardized within species. Seedling (for the incidence model), species and plot identity were included as random effects.

Extended Data Table 5:

Title: **No relationship between leaf potassium concentration and herbivory.**

Legend: The effect of leaf potassium concentration, leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. For each response variable, all three fixed effects were included, only changing the data set between models. Leaf area was standardized within species. Seedling (for the incidence model), species and plot identity were included as random effects.

Extended Data Table 6:

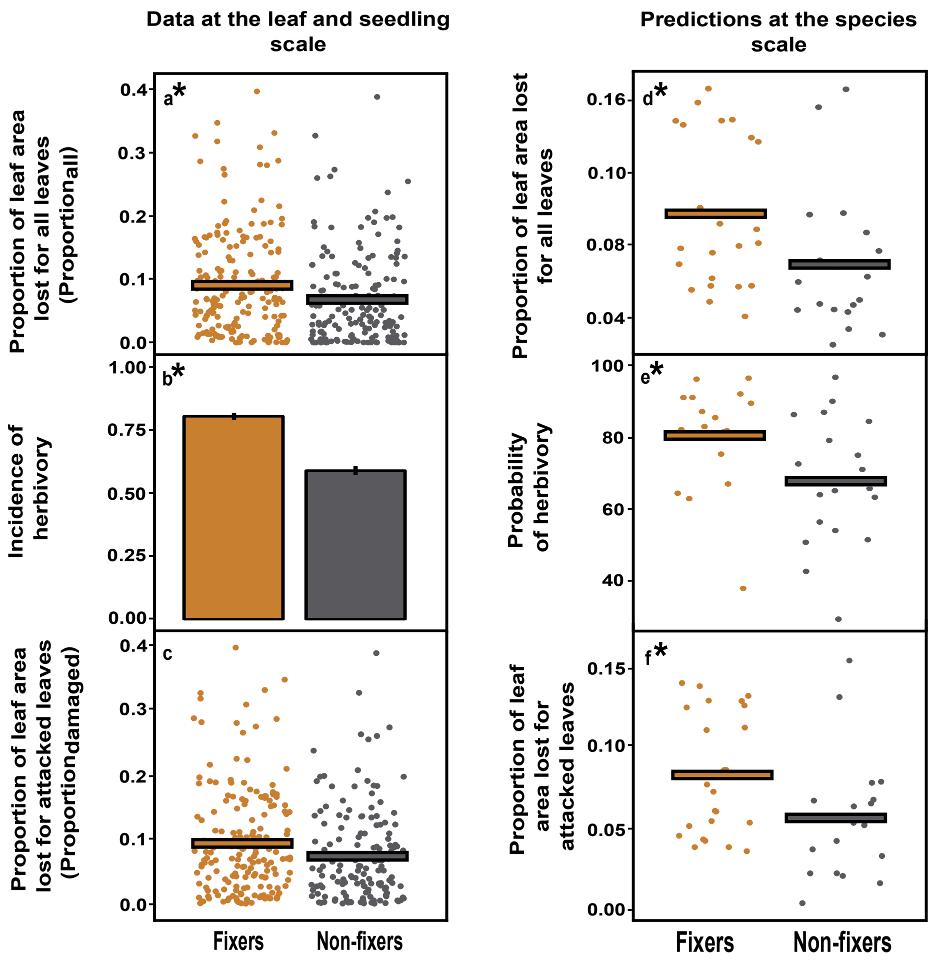
Title: **No relationship between leaf cellulose concentration and herbivory.**

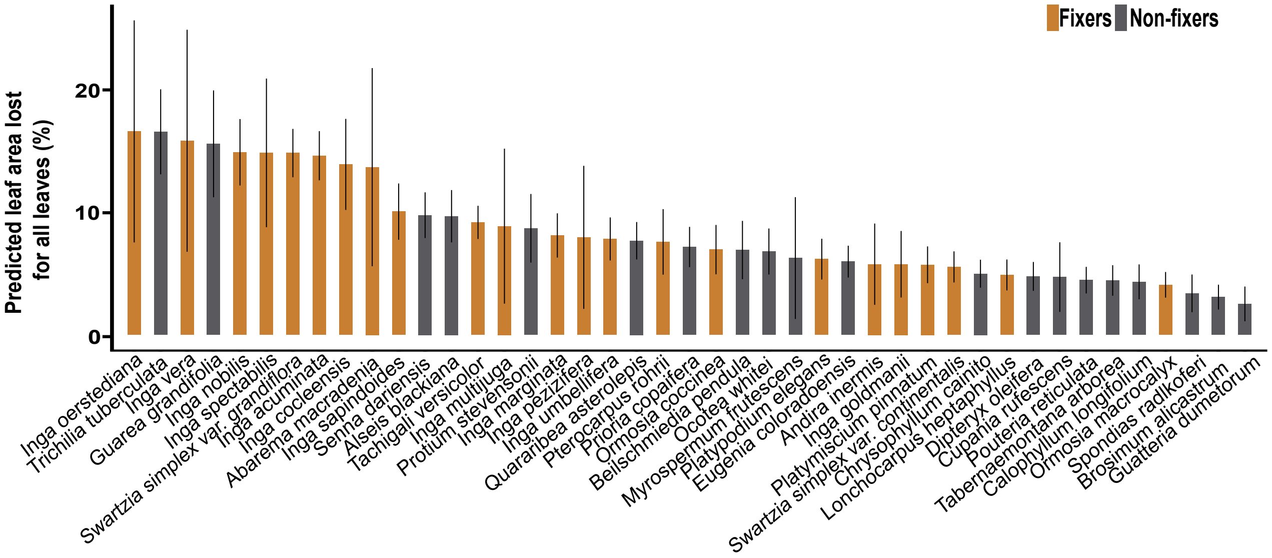
Legend: The effect of leaf cellulose concentration, leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. For each response variable, all three fixed effects were included, only changing the data set between models. Leaf area was standardized within species. Seedling (for the incidence model), species and plot identity were included as random effects.

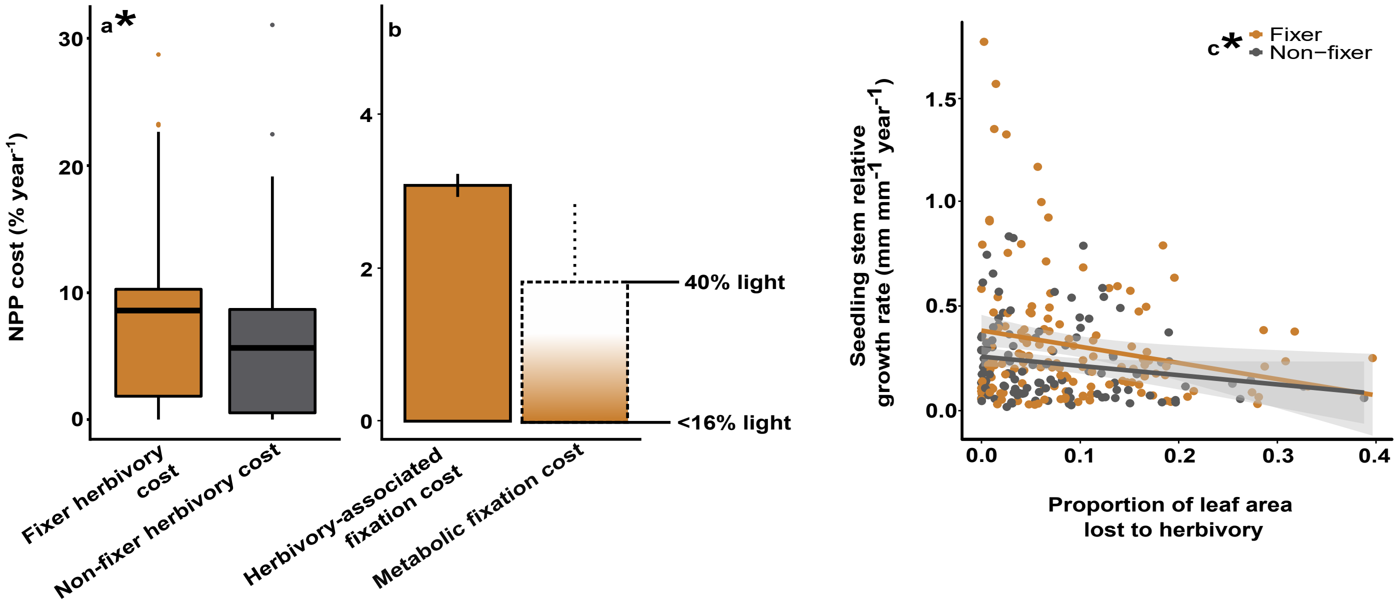
Extended Data Table 7:

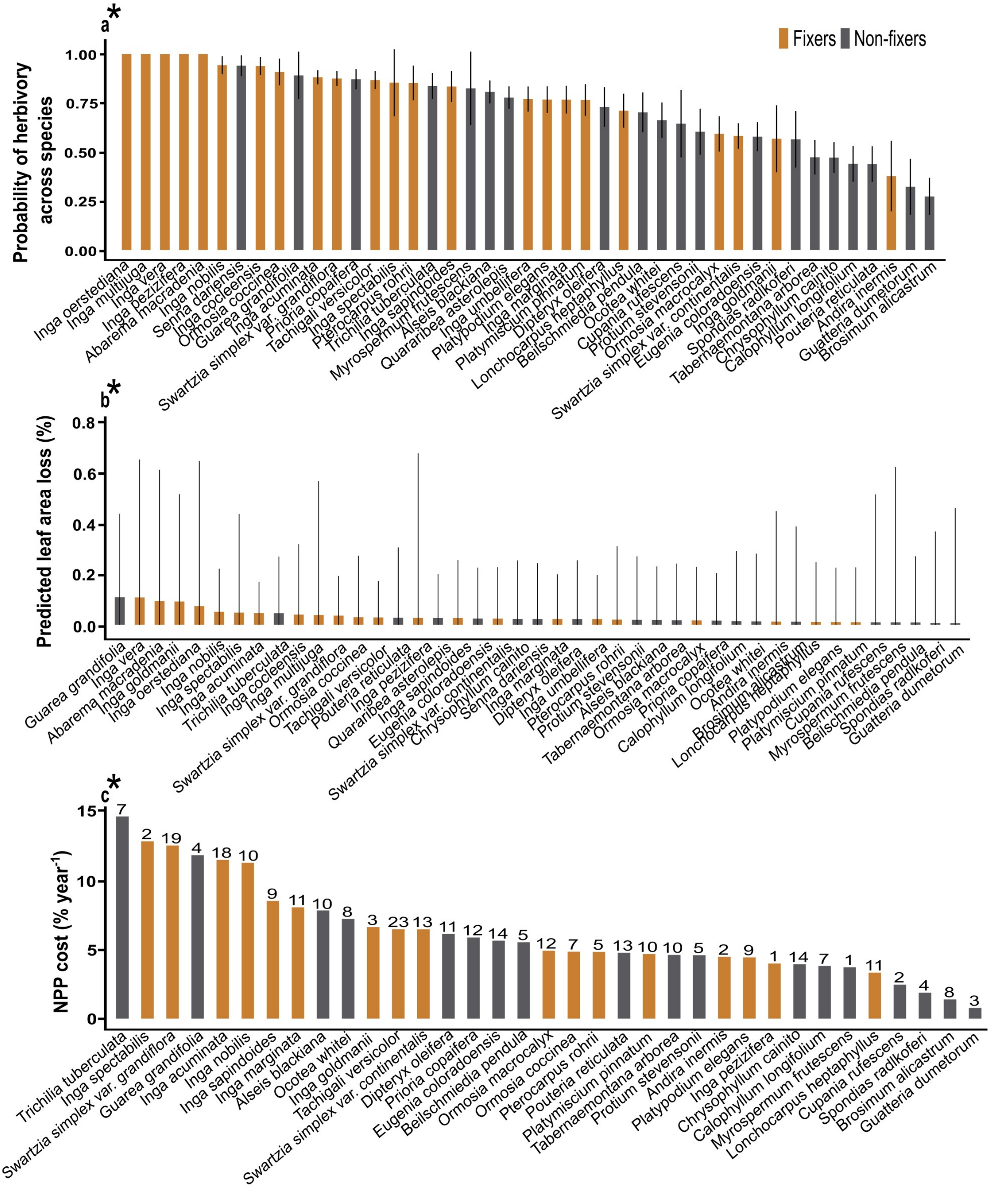
Title: **No relationship between leaf lignin concentration and herbivory.**

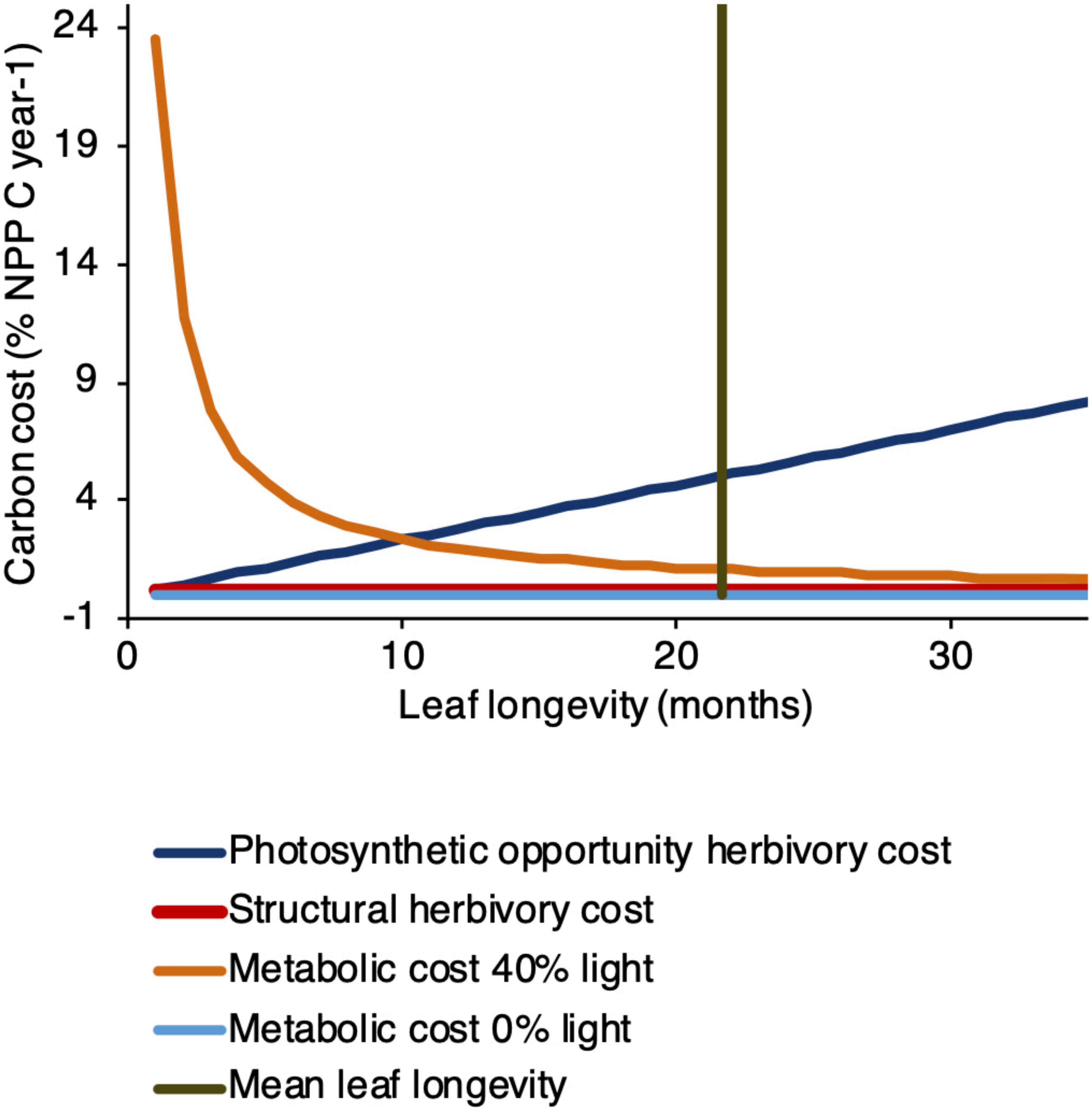
Legend: The effect of leaf lignin concentration, leaf area and stem length in driving the $\text{proportion}_{\text{all}}$ of leaf area lost to herbivory, the incidence of herbivory and the $\text{proportion}_{\text{damaged}}$ of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our hurdle model approach. These models were two sided, and adjustment for multiple comparisons was not required. For each response variable, all three fixed effects were included, only changing the data set between models. Leaf area was standardized within species. Seedling (for the incidence model), species and plot identity were included as random effects.

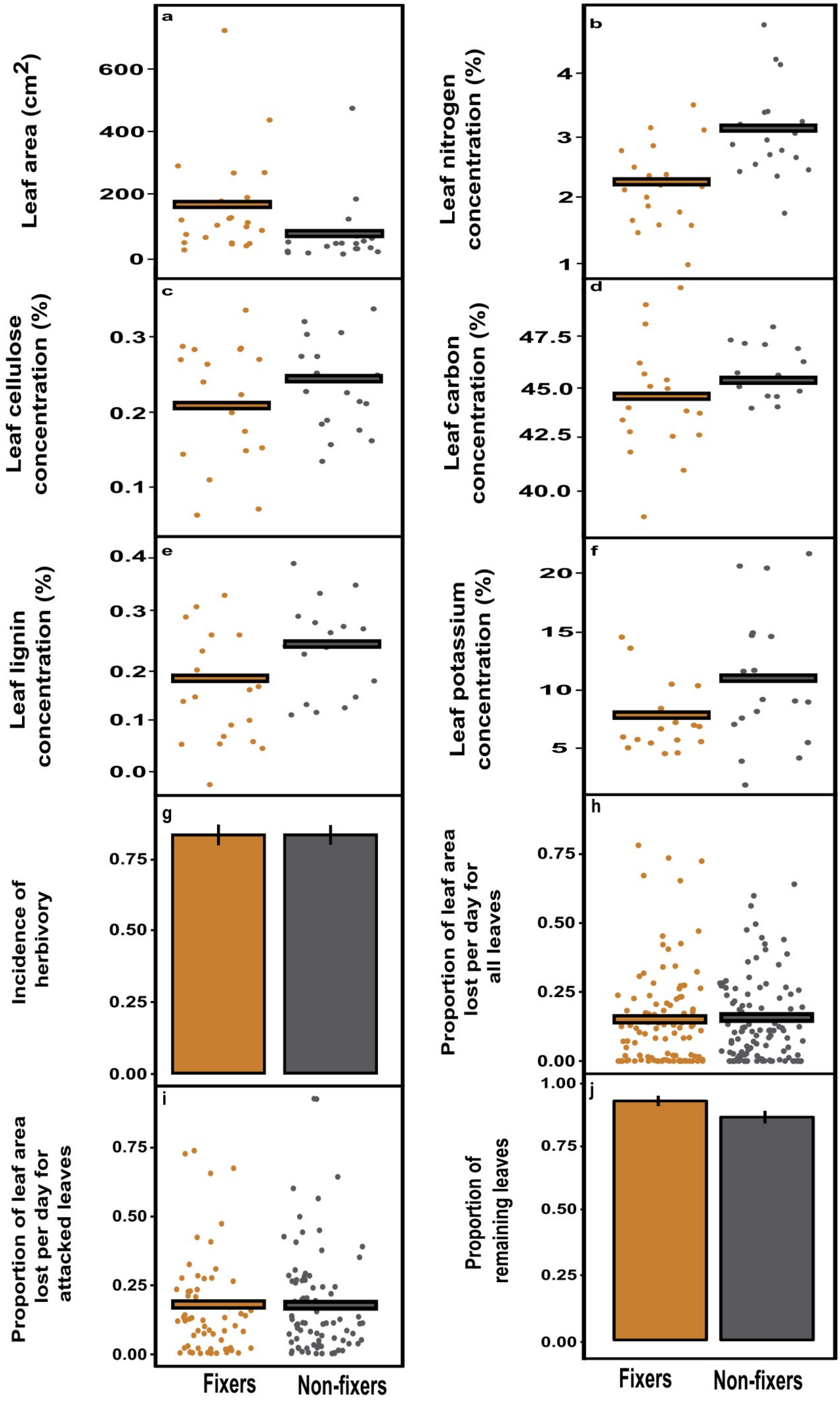

Data at the leaf and seedling scale
Predictions at the species scale
Proportion of leaf area lost for all leaves (Proportion$_{all}$)
Incidence of herbivory
Proportion of leaf area lost for attacked leaves (Proportion$_{damaged}$)
Proportion of leaf area lost for all leaves
Probability of herbivory
Proportion of leaf area lost for attacked leaves
a*
b*
c
d*
e*
f*
0.0
0.1
0.2
0.3
0.4
0.00
0.25
0.50
0.75
1.00
0.04
0.08
0.10
0.16
40
60
80
100
0.05
0.15
Fixers
Non-fixers


Fixers
Non-fixers
Predicted leaf area lost for all leaves (%)
20
10
0
Inga oerstediana
Trichilia tuberculata
Inga vera
Guarea grandifolia
Inga nobilis
Inga spectabilis
Swartzia simplex var. grandiflora
Inga acuminata
Inga cocleensis
Abarema macradenia
Inga sapindoides
Senna dariensis
Alseis blackiana
Tachigali versicolor
Inga multijuga
Protium stevensonii
Inga marginata
Inga pezizifera
Inga umbellifera
Quararibea asterolepis
Pterocarpus rohrii
Prioria copaifera
Ormosia coccinea
Beilschmiedia pendula
Ocotea whitei
Myrospermum frutescens
Platypodium elegans
Eugenia coloradoensis
Andira inermis
Inga goldmanii
Platymiscium pinnatum
Swartzia simplex var. continentalis
Chrysophyllum cainito
Lonchocarpus heptaphyllus
Dipteryx oleifera
Cupania rufescens
Pouteria reticulata
Tabernaemontana arborea
Calophyllum longifolium
Ormosia macrocalyx
Spondias radlkoferi
Brosimum alicastrum
Guatteria dumetorum


a*
NPP cost (% year^{-1})
0
10
20
30
Fixer herbivory cost
Non-fixer herbivory cost
b
0
2
4
40% light
<16% light
Herbivory-associated fixation cost
Metabolic fixation cost
c*
Fixer
Non−fixer
Seedling stem relative growth rate (mm mm^{-1} year^{-1})
0.0
0.5
1.0
1.5
0.0
0.1
0.2
0.3
0.4
Proportion of leaf area lost to herbivory


a*
Fixers
Non-fixers
Probability of herbivory across species
1.00
0.75
0.50
0.25
0.00
Inga oerstediana
Inga multijuga
Inga vera
Inga pezizifera
Abarema macradenia
Inga nobilis
Senna dariensis
Inga cocleensis
Ormosia coccinea
Guarea grandifolia
Inga acuminata
Swartzia simplex var. grandiflora
Prioria copaifera
Tachigali versicolor
Inga spectabilis
Pterocarpus rohrii
Trichilia tuberculata
Inga sapindoides
Myrospermum frutescens
Alseis blackiana
Quararibea asterolepis
Inga umbellifera
Platypodium elegans
Inga marginata
Platymiscium pinnatum
Dipteryx oleifera
Lonchocarpus heptaphyllus
Beilschmiedia pendula
Ocotea whitei
Cupania rufescens
Protium stevensonii
Ormosia macrocalyx
Swartzia simplex var. continentalis
Eugenia coloradoensis
Inga goldmanii
Spondias radlkoferi
Tabernaemontana arborea
Chrysophyllum cainito
Calophyllum longifolium
Pouteria reticulata
Andira inermis
Guatteria dumetorum
Brosimum alicastrum
b*
Predicted leaf area loss (%)
0.8
0.6
0.4
0.2
0.0
Guarea grandifolia
Inga vera
Abarema macradenia
Inga goldmanii
Inga oerstediana
Inga nobilis
Inga spectabilis
Inga acuminata
Trichilia tuberculata
Inga cocleensis
Inga multijuga
Swartzia simplex var. grandiflora
Ormosia coccinea
Tachigali versicolor
Pouteria reticulata
Inga pezizifera
Quararibea asterolepis
Inga sapindoides
Eugenia coloradoensis
Swartzia simplex var. continentalis
Chrysophyllum cainito
Senna dariensis
Inga marginata
Dipteryx oleifera
Inga umbellifera
Pterocarpus rohrii
Protium stevensonii
Alseis blackiana
Tabernaemontana arborea
Ormosia macrocalyx
Prioria copaifera
Calophyllum longifolium
Ocotea whitei
Andira inermis
Brosimum alicastrum
Lonchocarpus heptaphyllus
Platypodium elegans
Platymiscium pinnatum
Cupania rufescens
Myrospermum frutescens
Beilschmiedia pendula
Spondias radlkoferi
Guatteria dumetorum
c*
NPP cost (% year^{-1})
15
10
5
0
7
2
19
4
18
10
9
11
10
8
3
23
13
11
12
14
5
12
7
5
13
10
10
5
2
9
1
14
7
1
11
2
4
8
3
Trichilia tuberculata
Inga spectabilis
Swartzia simplex var. grandiflora
Guarea grandifolia
Inga acuminata
Inga nobilis
Inga sapindoides
Inga marginata
Alseis blackiana
Ocotea whitei
Inga goldmanii
Tachigali versicolor
Swartzia simplex var. continentalis
Dipteryx oleifera
Prioria copaifera
Eugenia coloradoensis
Beilschmiedia pendula
Ormosia macrocalyx
Ormosia coccinea
Pterocarpus rohrii
Pouteria reticulata
Platymiscium pinnatum
Tabernaemontana arborea
Protium stevensonii
Andira inermis
Platypodium elegans
Inga pezizifera
Chrysophyllum cainito
Calophyllum longifolium
Myrospermum frutescens
Lonchocarpus heptaphyllus
Cupania rufescens
Spondias radlkoferi
Brosimum alicastrum
Guatteria dumetorum


Carbon cost (% NPP C year-1)
24
19
14
9
4
-1
0
10
20
30
Leaf longevity (months)
Photosynthetic opportunity herbivory cost
Structural herbivory cost
Metabolic cost 40% light
Metabolic cost 0% light
Mean leaf longevity


a
Leaf area (cm^2)
0
200
400
600
b
Leaf nitrogen concentration (%)
1
2
3
4
c
Leaf cellulose concentration (%)
0.1
0.2
0.3
d
Leaf carbon concentration (%)
40.0
42.5
45.0
47.5
e
Leaf lignin concentration (%)
0.0
0.1
0.2
0.3
0.4
f
Leaf potassium concentration (%)
5
10
15
20
g
Incidence of herbivory
0.00
0.25
0.50
0.75
h
Proportion of leaf area lost per day for all leaves
0.00
0.25
0.50
0.75
i
Proportion of leaf area lost per day for attacked leaves
0.00
0.25
0.50
0.75
j
Proportion of remaining leaves
0.00
0.25
0.50
0.75
1.00
Fixers
Non-fixers
Fixers
Non-fixers