1   **<u>Widespread herbivory cost in tropical nitrogen-fixing tree species</u>**

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11   **Summary:**

12   **While mature and recovering tropical forests constitute a large carbon sink,**
13   **recent observations suggest this sink is strongly limited by nitrogen[1–3].**
14   **Nitrogen-fixing trees, through a symbiotic relationship with bacteria, provide**
15   **the main natural source of new nitrogen to tropical forests[3,4]. However, fixer**
16   **abundances are tightly constrained[5-7], highlighting the fundamental**
17   **unanswered question of what limits new nitrogen entering tropical**
18   **ecosystems. Here we examine the untested hypothesis that herbivory is**
19   **responsible for limiting tropical forest symbiotic nitrogen fixation. We evaluate**
20   **whether fixers experience more herbivory than non-fixers, if herbivory carries**
21   **a substantial carbon cost, and if high herbivory is due to herbivores targeting**
22   **fixers' nitrogen-rich leaves[8,9]. We analyzed 1,626 leaves from 350 seedlings of**
23   **43 tropical tree species in Panama and find that: (a) while herbivory reduces**
24   **growth and survival for seedlings, fixers undergo 26% more herbivory than**
25   **non-fixers; (b) fixers experience 34% greater carbon opportunity costs due to**
26   **herbivory than non-fixers, exceeding the metabolic cost of fixing nitrogen; but,**
27   **unexpectedly, (c) high herbivory for fixers is not driven by high leaf nitrogen.**
28   **Our findings reveal that herbivory may be substantial enough to limit tropical**
29   **symbiotic nitrogen fixation and could constrain its role in alleviating nitrogen**
30   **limitation on the tropical carbon sink.**
31
32   **Introduction:**

33   Increasing evidence suggests that nutrients might limit the ability of tropical forests to
34   sustain a long-term carbon sink in mature and secondary forests[1,2,10]. Nitrogen-fixing
35   trees (i.e., trees in the Fabaceae family with the capacity to fix nitrogen; also referred
36   to as fixers) act as a source of new nitrogen to terrestrial ecosystems[4]. Observations
37   of high symbiotic nitrogen fixation rates in tree fall gaps[11,12], in mature forests with
38   high net carbon uptake rates[13] and in some secondary forests recovering from
39   disturbance[4] point to nitrogen limitation as being pervasive in tropical forests[2], with

40   the trait of nitrogen fixation providing growth benefits to tropical trees capable of

41   fixing during periods of nitrogen limitation[4]. Given this substantial benefit of nitrogen

42   fixation to tropical trees, it remains unclear why the trait of fixation is not more

43   widespread: nitrogen fixers represent only ~7%, ~8.5% and 5-15% of basal area in

44   mature Asian and Central African and all ages of Neotropical forests, respectively[5–

45   7,14].

46   Previous hypotheses to explain what constrains tropical symbiotic nitrogen fixation

47   cite high carbon costs associated with the fixation trait. Breaking the triple-bond of

48   the dinitrogen molecule requires substantial reducing power, leading to a high

49   carbon cost for acquiring nitrogen from fixation relative to taking up inorganic

50   nitrogen from the soil[15], especially if another nutrient like phosphorus or molybdenum

51   limits fixation[16]. Building and maintaining root nodules where the symbiotic bacteria

52   reside presents an additional carbon cost[15]. These constraints may explain the low

53   abundances of temperate nitrogen-fixing tree species, which constitute <1% of

54   temperate trees and cannot avoid fixation-associated costs with their constant

55   fixation rates, regardless of soil nitrogen supply[17,18]. But these constraints cannot

56   account for the low abundances of tropical nitrogen fixers, which employ a facultative

57   fixation strategy that allows them to downregulate fixation as benefits decline, such

58   as when growing in nitrogen-rich soil[4,11]. Accordingly, tropical fixers can avoid

59   fixation-associated costs and should remain competitive even when the costs of

60   fixation would otherwise outweigh the benefits. These previously identified fixation-

61   associated costs are therefore insufficient to explain why the trait has not become

62   more abundant over ecological and evolutionary time. Tropical fixers must face an

63   additional cost associated with fixation that persists regardless of how much they are

64   actively fixing[15-17].

65   Of the costs previously proposed to be associated with the fixation trait and which

66   are independent of fixation rate[14–18], one has the potential to be substantial:

67   herbivory[16,17,19]. In Neotropical forests, herbivory pressure is high and largely driven

68   by insects that target nitrogen-rich plant tissues to meet their requirements for

69   growth[8,9,20]. Herbivores may therefore be particularly attracted to nitrogen-fixing tree

70   species that contain high leaf nitrogen concentrations relative to non-fixing species,

71   regardless of fixation rates[21]. This pattern could lead to elevated herbivory and a

72   high carbon cost for fixer species, diminishing the benefits of fixation. Such a

73   herbivory cost would reduce fixer growth and competitive ability, ultimately

74   suppressing fixer abundance and fixation rates in tropical forests. If this cost was

75   consistently high across fixer species, then herbivory would lead to negative

76   selection for the fixation trait. If the cost varied substantially across species, then tree

77   species in the fixer functional group may be more heterogeneous in their leaf traits

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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 (Proportion$_{all}$). 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; $Proportion_{damaged}$), 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 ($Proportion_{damaged}$; 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 ($Proportion_{all}$; 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; $Proportion_{damaged}$; 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

155 between the two groups in the proportion of leaf area lost to herbivory (range: fixers:
156 19%-37%; non-fixers: 6%-39%; Fig. 2). Species from the genus *Inga* had higher
157 herbivory than non-*Inga* species (Fig. 2; Extended Data Fig. 1a), but herbivory for
158 non-*Inga* fixer species was still greater than for non-fixers species ($p<0.05$). Variation
159 in herbivory across species may arise from diversification of fixer species as they
160 evolve novel herbivore defence strategies, discussed below. Thus, we conclude that
161 the herbivory trend for fixers vs. non-fixers was not driven by any one species or
162 group.
163
164 We next examined our second hypothesis, that high herbivory for fixers constitutes a
165 significant carbon cost greater than the metabolic cost that could constrain the trait of
166 fixation. To test this, we estimated the carbon cost of herbivory as a percentage of
167 net primary productivity (NPP) using the species-level leaf structural carbon
168 concentration, a universal per leaf area photosynthetic rate, the seedling-specific
169 total leaf area lost and assuming a one-year leaf lifespan. These structural and
170 photosynthetic opportunity carbon costs combined diverted ~9% of Net Primary
171 Productivity (NPP) per year for an average fixer seedling (the photosynthetic
172 opportunity cost alone was 8.7% $year^{-1}$; Figs. 3a,b), compared to 5.9% $year^{-1}$ for
173 non-fixer seedlings. Thus, fixers lost ~3.1% $year^{-1}$ (+/- 1.2% $year^{-1}$) more of their
174 NPP to herbivory than non-fixer seedlings (range: 6.3-24.1% $year^{-1}$ for fixers, 1.5-
175 27.6% $year^{-1}$ for non-fixers; Extended Data Fig. 1c). This herbivory cost was high
176 relative to the metabolic cost of fixing all leaf nitrogen, which would be minimal if
177 seedlings fixed at low rates in the forest understory and instead took up nitrogen
178 from the soil, as has been previously found[4,11]. Even if fixers replaced 40% of their
179 total leaf nitrogen with fixation each year, as found for seedlings in high understory
180 light levels[29], the metabolic cost would only reach 3% $year^{-1}$ of NPP. This remains
181 less than the herbivory-associated fixation cost. Our carbon cost estimates were
182 insensitive to differences in photosynthetic rates between fixers and non-fixers.
183 While the precise fixation-associated herbivory cost may vary if fixer and non-fixer
184 photosynthetic and respiration rates differed, current evidence demonstrates that
185 tropical fixer and non-fixer photosynthetic rates are comparable[30]. We also consider
186 different leaf lifespans (Extended Data Fig. 2), which regularly exceed one year in
187 shade tolerant forest species[31]. With long-lived leaves, photosynthetic opportunity
188 costs from herbivory will accumulate longer, whilst nitrogen needed for leaf turnover
189 will be low. Thus, our herbivory costs estimated at an annual scale may be
190 conservative (Extended Data Fig. 2). Although tropical fixers can avoid the energetic
191 cost of fixation by downregulating fixation when the cost exceeds the benefit[4,11,32],
192 our research suggests they face herbivory costs even when they gain no benefit
193 from fixation.

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

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

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

249   These findings from dozens of tropical mature forest tree species advance earlier
250   evidence of high herbivory on fixers from grassland ecosystems[24] and a handful of
251   tropical tree species in the canopy of secondary forests. They are also consistent
252   with paleoecological evidence that fixer abundance promotes ecosystem level
253   herbivory[35]. Combined, the findings suggest that across ecosystems and biomes
254   fixers bear higher herbivory costs than non-fixers. As these costs reduce growth and
255   survival, they create a filter on the abundance of fixer seedlings, potentially sufficient
256   to constrain nitrogen fixation in tropical forests. The widespread high carbon cost of
257   herbivory for fixers should be incorporated as a constraint on symbiotic nitrogen
258   fixation to improve biogeochemical and climate change models[17-19,36], and provides a
259   plausible ecological and evolutionary explanation for why fixer abundances are
260   capped at 15% across tropical forests[5-7,14]. In sum, herbivory appears to be not only
261   a key factor in governing tropical nitrogen fixation, but also in controlling nitrogen
262   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.

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380 **Figures:**

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382 **Figure 1. Nitrogen-fixing trees have higher herbivory than non-fixers in a**
383 **tropical moist forest.** Metrics of herbivory from 1,626 leaves, 350 individuals and
384 43 species for fixers (orange) and non-fixers (grey) in Panama derived at the leaf,
385 seedling and species scale. At the seedling and leaf scale, **a** shows the proportion of
386 leaf area lost from all sampled leaves for each seedling (Proportion$_{all}$); **b**, the
387 incidence of herbivory on leaves (i.e., whether or not a leaf had herbivory); and, **c**,
388 the proportion of leaf area lost only on damaged leaves for each seedling
389 (Proportion$_{damage}$). At the species scale where each point represents a species, **d**,
390 the predicted proportion of leaf area lost to herbivory across all sampled leaves from
391 each seedling; **e**, the probability of herbivory on individual leaves; and, **f**, the
392 predicted proportion of leaf area lost to herbivory on damaged leaves from each
393 seedling. Predicted values for d, e and f were derived from our models of
394 Proportion$_{all,}$ the incidence of herbivory and Proportion$_{damaged}$. Lines represent the
395 mean of data at the leaf (**a**), seedling (**c**) and species (**d-f**) scales. **b** shows the mean
396 ± standard error of data at the leaf scale. Asterisks denote statistically significant
397 differences between fixers and non-fixers in mixed effects models (**a**, **b**, **c**) and two-
398 sided non-parametric Wilcoxon rank tests (**d**, **e**, **f**). Data are jittered to show
399 differences, and numbers of biologically independent samples for each category and
400 statistical results are found in Extended Data Table 1.

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

409 **Figure 3. The cost of herbivory for nitrogen-fixing trees in a tropical moist**
410 **forest.** This includes both the carbon cost estimates associated with fixation and the
411 relationship between herbivory and seedling growth rate for fixer (orange) and non-
412 fixer (grey) seedlings. **a**, The herbivory cost, including the structural carbon and
413 photosynthetic opportunity carbon costs of herbivory as a percentage of annual net
414 primary production (NPP). Box and whisker plots represent the geometric mean, the
415 first and third quantiles, 1.5 times the interquartile range and outliers. **b**, The fixation-
416 associated cost of herbivory for fixers (geometric mean ± standard error) and the
417 metabolic cost of fixing nitrogen (i.e., the metabolism of fixing N$_2$ to NH$_3$ and building
418 and maintaining nodules; bar shows mean cost from 0 to 40% light availability and
419 the line shows the maximum fixation cost) across the range of fixation rates
420 observed over a gradient in light availability observed in the understory of mature

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421 tropical forests[39]. The metabolic fixation cost for seedlings in mature forest is likely to
422 be low since the majority of seedlings occur at <16% of light availability where fixers
423 downregulate fixation. **c**, The relationship between herbivory (proportion leaf area
424 lost) for a seedling and seedling stem relative growth rate. Points represent each of
425 the seedlings sampled, and the lines represent model fit and 95% confidence
426 interval. An outlier with a growth rate of >6 mm mm^{-1} year^{-1} was removed to make it
427 easier to observe the relationship between growth rate and herbivory (this data point
428 was included in analyses). The asterisks represent significant differences as
429 determined by two-sided non-parametric Wilcoxon rank tests (**a**, $p < 0.001$; n=351
430 biologically independent samples) and a significant relationship between stem
431 growth rate and leaf area lost to herbivory in mixed effects models (**c**, $p = 0.04$;
432 n=350 biologically independent samples).
433

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

13

471   leaves grew over the period of observation. Importantly, these two methods give the
472   same value for leaves that did not lose leaflets or grow, and together allowed us to
473   assess our whole dataset.

474   *Calculating carbon costs*

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

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

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

504   To generate estimates of carbon costs we multiplied the mean structural carbon cost
505   and photosynthetic opportunity cost across sampled leaves (up to 6) for each
506   individual by its total number of leaves. We compared all carbon costs as a
507   percentage of annual net primary production (NPP) per seedling, calculated as the
508   annual photosynthetic capacity for each seedling minus ~47% of carbon lost to

14

509 respiration each year[43]. Herbivory costs were highly zero inflated (many seedlings
510 had no herbivory), so we took the geometric mean of each as a percentage of
511 annual net primary production cost, which better represents zero inflated
512 distributions than an arithmetic mean. As the geometric mean of any distribution
513 containing zeroes will always be zero, we added 1 to each cost and then subtracted
514 1 from our average. To isolate the herbivory-associated fixation cost for fixer
515 species, we subtracted the mean herbivory cost for non-fixer species from that of
516 fixer species. Importantly, this method of estimating carbon costs controls for
517 variation in leaf area and measures of photosynthetic rates between fixers and non-
518 fixers.

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

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

15

549 *Differences in leaf retention between fixer and non-fixer seedlings*

550 We explored the role of leaf retention (turnover rates) in driving higher herbivory
551 measurements for fixers than non-fixers. Theoretically, if non-fixers drop leaves more
552 readily after herbivory it could reduce average herbivory measurements for the non-
553 fixer group, as we did not measure herbivory on fallen leaves. Accordingly, we
554 recorded leaf retention for 226 young leaves over three months in 2017. We found
555 no significant difference in leaf turnover rate between fixers and non-fixers ($p > 0.05$,
556 $R^2 = 0.16$; Extended Data Fig. 3), suggesting that differences in herbivory between
557 the two functional groups are not driven by differences in leaf retention, but are
558 governed by other leaf and species traits.

559

560 *Seedling growth rates*

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

565 *Seedling survival and herbivory*

566 We examined whether high rates of herbivory resulted in lower survival rates for
567 seedlings in the following year using a broader dataset of 586,748 coarse-scale
568 measurements of herbivory and survival belonging to 139,756 unique seedlings from
569 223 species over 18 years and 13 censuses. See reference 39 for a detailed
570 description of the data collection. We scored seedlings with more than 50% leaf area
571 missing as having high herbivory, and seedlings with less than 50% as having no or
572 low herbivory.

573

574 *Species attributes and leaf traits*

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

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

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

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

17

623 (mCSCS), which measures the mean similarity of each focal species across the
624 majority of other species in the 50-ha plot[48].

625 *Statistical analyses*

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

642 $$X' = (x(N-1)+0.5)/N$$

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

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

18

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

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

695

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

19

703

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

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

717 $Proportion_{all}$ ~ Fixation + Standardized leaf area + Stem length + (1 | species) +
718 (1|plot)

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

722 $Proportion_{all}$ ~ Leaf Nitrogen Concentration + Standardized leaf area + Stem length +
723 (1 | species) + (1|plot)

724 Model 3: Variable specification for the beta regression used to predict species level
725 $Proportion_{all}$. Species is now specified as a fixed effect and fixation is removed from
726 the model (see below).

727 $Proportion_{all}$ ~ Species + Standardized leaf area + Stem length + (1|plot)

728 We also used our models to predict the probability of herbivory and mean leaf area
729 lost to herbivory across fixer and non-fixer species to account for variation due to
730 seedling size, leaf area, species identity and location in the plot. We restructured all
731 models so that species identity was included as a fixed effect and fixation (or any
732 trait other than standardized leaf area and stem length) was omitted (compare
733 variable specification in Model 2 and 3 above). These restructured models were
734 used to generate predicted levels of each of our three measures of herbivory. First,
735 we used our model of the incidence of herbivory across seedlings to predict the
736 probability of herbivory per species. Species for which we had less than 10 leaves
737 were excluded from our probability model to prevent singularity. Second, we used

738   our two restructured models of the proportion of leaf area lost ($Proportion_{all}$,

739    $Proportion_{damaged}$) to predict the average leaf are lost to herbivory per species (over

740   damaged leaves and all leaves, using the predict() function from the bootpredictlme4

741   function for the binary logistic regression model and the linear mixed effects model

742   and the predict.GLMM() function from the glmmTMB package for the beta regression

743   model[49,55]). As $Proportion_{all}$ was square root transformed to meet assumptions of

744   normality, species level predictions were back transformed for presentation. We ran

745   Wilcoxon Rank tests to determine if there was a difference in the average predicted

746   value between fixer species and non-fixer species for each measure of herbivory. All

747   statistical analyses were carried out in R version 3.5.1[56] using R Studio (version

748   1.1.463).

749   The datasets generated during and/or analysed during the current study are

750   available in the NERC Environmental Information Data Centre repository at

751   https://doi.org/10.5285/67c95112-edee-435f-9355-9d8bab3a5634.[57]

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810

811 **Extended data legends:**

812 **Figures:**

813 **Extended Data Figure 1.**

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

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

830 **Extended Data Figure 2.**

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

832 Legend: How the fixation-associated herbivory costs and metabolic cost of fixing
833 nitrogen vary over leaf lifespan. Costs shown as a percentage of annual NPP per
834 year, using the mean herbivory and leaf area for fixers and non-fixers up until the
835 maximum leaf lifespan for shade species recorded in the 50ha plot on Barro
836 Colorado Island (BCI). The photosynthetic opportunity cost was calculated as the
837 accruing photosynthesis forgone until the end of the leaf lifespan (dark blue line).
838 The structural carbon cost remained constant since the cost per year would not vary
839 with leaf lifespan (red line). The metabolic cost represents the percentage of NPP
840 required to replace either 40% of leaf nitrogen (at 40% light, orange line) or 0% (at
841 16% light, light blue line) paying six grams of carbon per gram nitrogen over one
842 year, depending on leaf lifespan. The mean leaf lifespan for shade species in the
843 BCI 50ha plot is 21.65 months (green line). These values differ from Fig. 3b since
844 they are at the leaf level, use mean values as parameter estimates and consider
845 variation in leaf lifespan.

846

23

847 **Extended Data Figure 3.**

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

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

868 **Tables:**

869 **Extended Data Table 1:**

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

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

879 **Extended Data Table 2:**

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

881 Legend: The effect of leaf nitrogen concentration, leaf area and stem length in driving the
882 proportion$_{all}$ of leaf area lost to herbivory, the incidence of herbivory and the proportion$_{damaged}$
883 of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species
884 alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed

24

885   effects beta regression and our hurdle model approach. These models were two sided, and

886   adjustment for multiple comparisons was not required. For each response variables, all three

887   fixed effects were included, only changing the data set between models. Leaf area was

888   standardized within species. Seedling (for the incidence model), species and plot identity

889   were included as random effects.

890   **Extended Data Table 3:**

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

892   Legend: The effect of leaf area and stem length in driving the $proportion_{all}$ of leaf area lost to

893   herbivory, the incidence of herbivory and the $proportion_{damaged}$ of leaf area lost to herbivory,

894   across all species, fixer species alone and non-fixer species alone (number of observations:

895   350 (184 fixers, 166 non-fixers)), according to our mixed effects beta regression and our

896   hurdle model approach. These models were two sided, and adjustment for multiple

897   comparisons was not required. In this focal model, leaf area is not standardized within

898   species. For each response variable, both fixed effects were included, only changing the

899   data set between models. Seedling (for the incidence model), species and plot identity were

900   included as random effects.

901   **Extended Data Table 4:**

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

903   Legend: The effect of leaf carbon concentration, leaf area and stem length in driving the

904    $proportion_{all}$ of leaf area lost to herbivory, the incidence of herbivory and the $proportion_{damaged}$

905   of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species

906   alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed

907   effects beta regression and our hurdle model approach. These models were two sided, and

908   adjustment for multiple comparisons was not required. For each response variable, all three

909   fixed effects were included, only changing the data set between models. Leaf area was

910   standardized within species. Seedling (for the incidence model), species and plot identity

911   were included as random effects.

912   **Extended Data Table 5:**

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

914   Legend: The effect of leaf potassium concentration, leaf area and stem length in driving the

915    $proportion_{all}$ of leaf area lost to herbivory, the incidence of herbivory and the $proportion_{damaged}$

916   of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species

917   alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed

918   effects beta regression and our hurdle model approach. These models were two sided, and

919   adjustment for multiple comparisons was not required. For each response variable, all three

920   fixed effects were included, only changing the data set between models. Leaf area was

921   standardized within species. Seedling (for the incidence model), species and plot identity

922   were included as random effects.

923

924 **Extended Data Table 6:**

925 Title: **No relationship between leaf cellulose concentration and herbivory.**
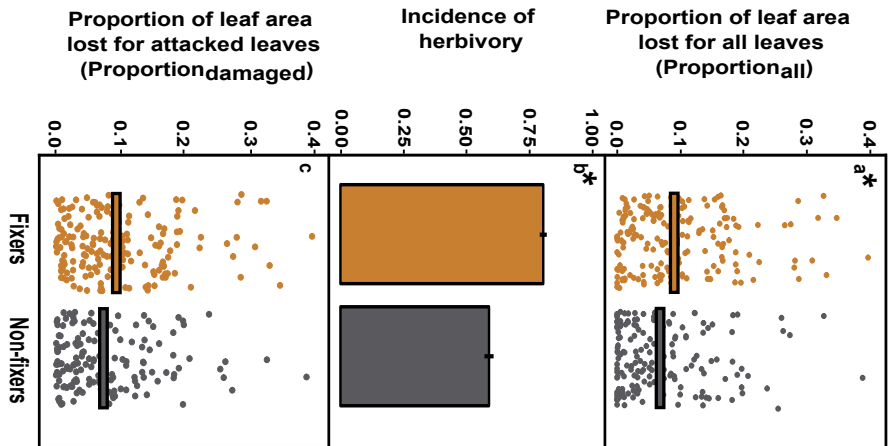
926 Legend: The effect of leaf cellulose concentration, leaf area and stem length in driving the
927 proportion$_{all}$ of leaf area lost to herbivory, the incidence of herbivory and the proportion$_{damaged}$
928 of leaf area lost to herbivory, across all species, fixer species alone and non-fixer species
929 alone (number of observations: 350 (184 fixers, 166 non-fixers)), according to our mixed
930 effects beta regression and our hurdle model approach. These models were two sided, and
931 adjustment for multiple comparisons was not required. For each response variable, all three
932 fixed effects were included, only changing the data set between models. Leaf area was
933 standardized within species. Seedling (for the incidence model), species and plot identity
934 were included as random effects.
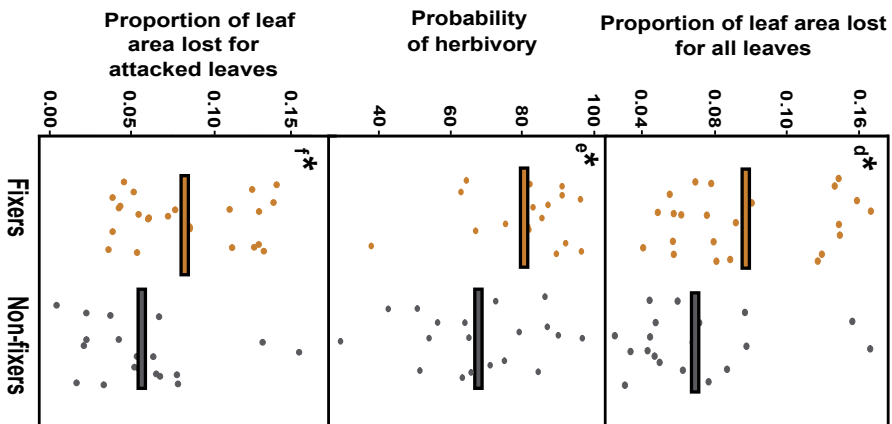
935 **Extended Data Table 7:**

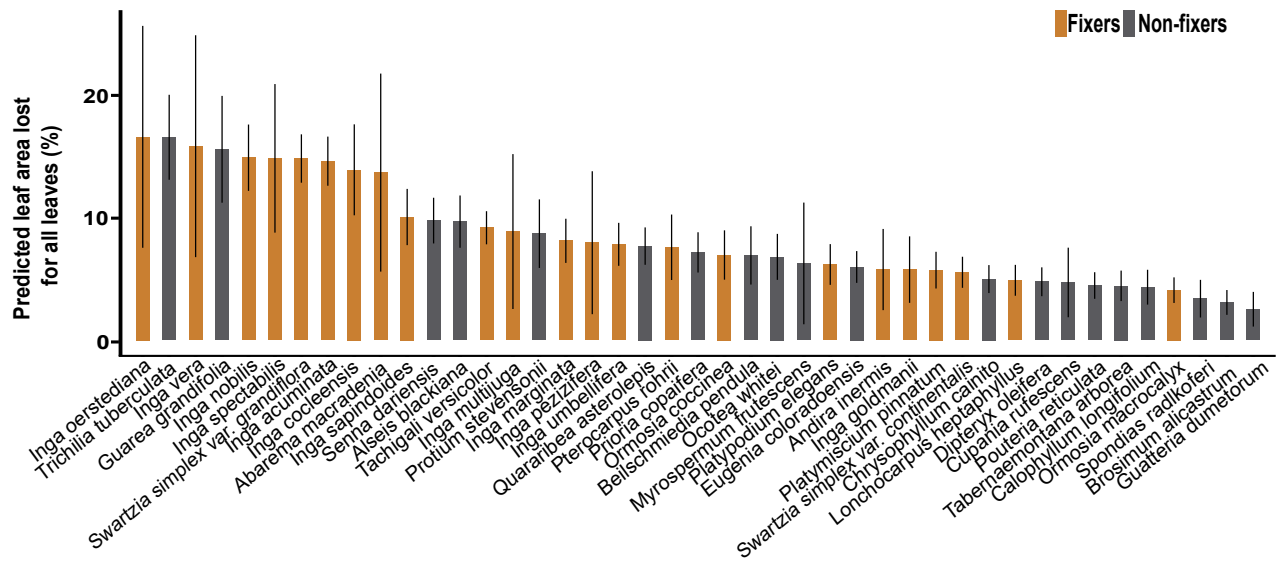936 Title: **No relationship between leaf lignin concentration and herbivory.**

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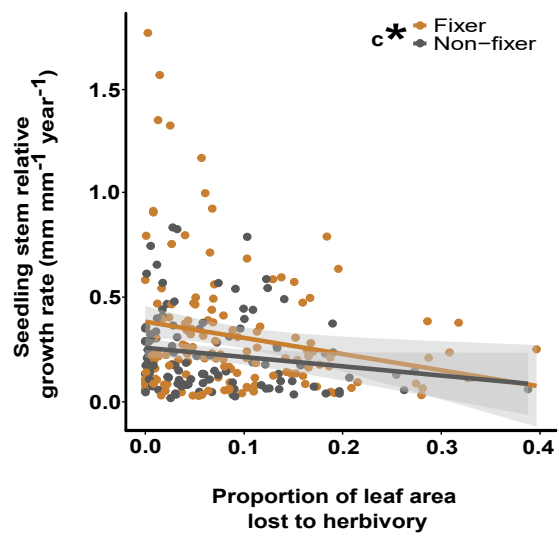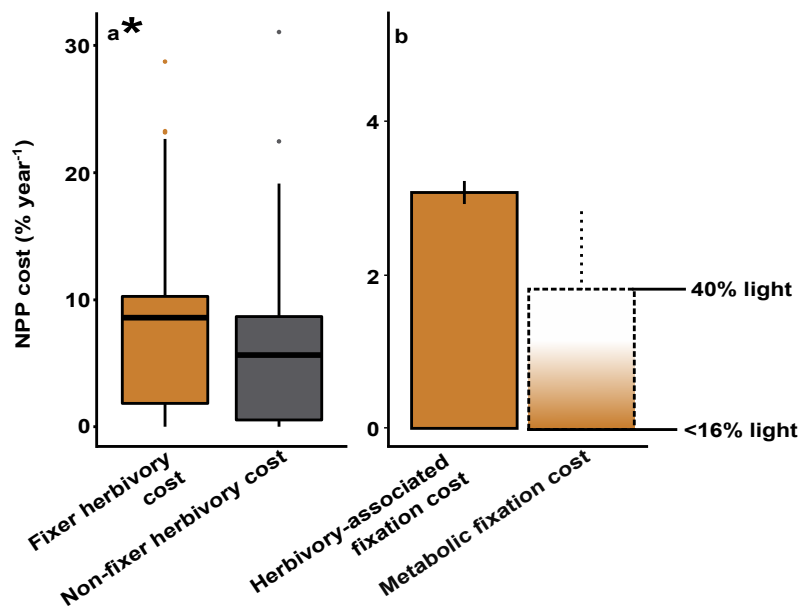945 were included as random effects.
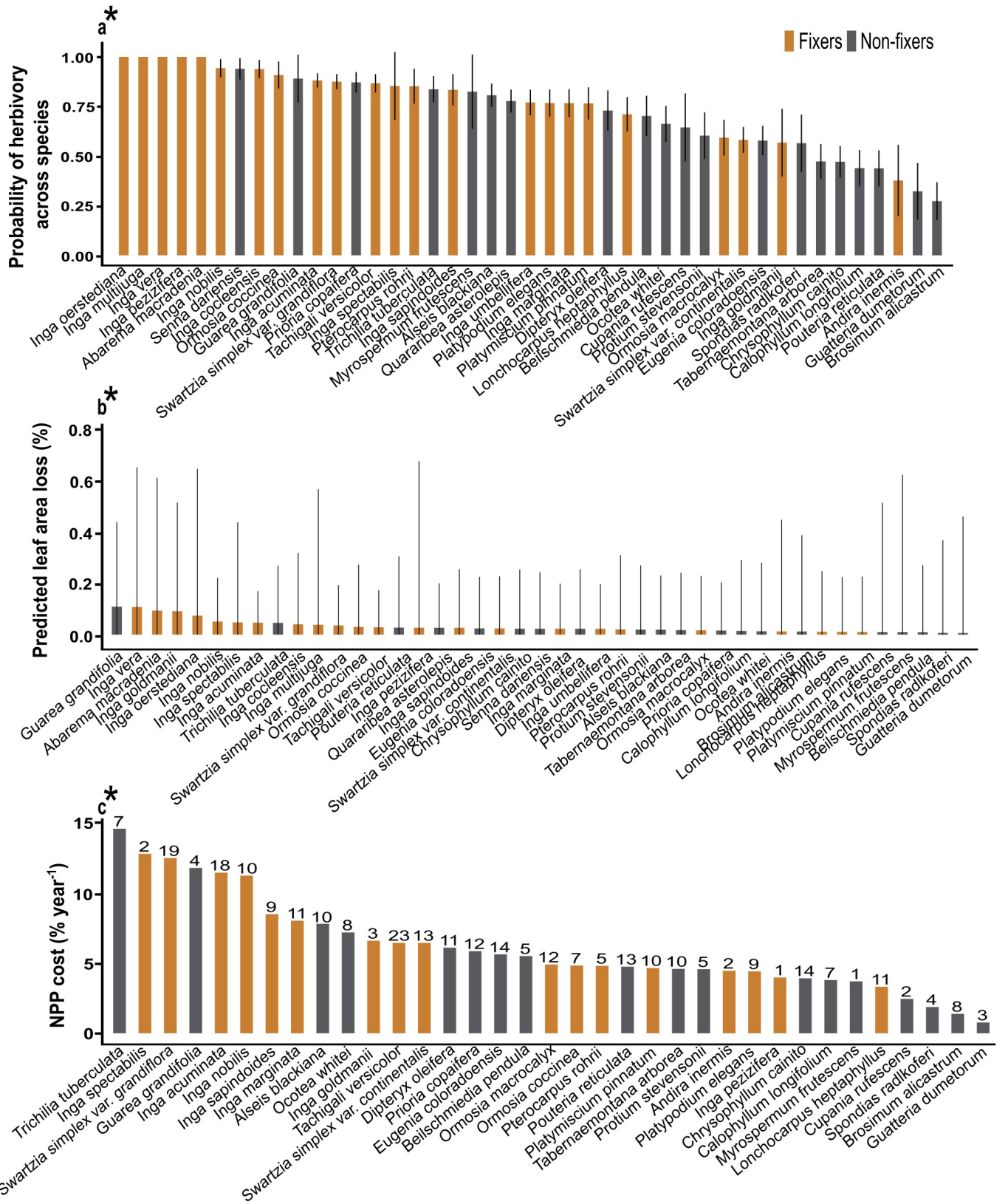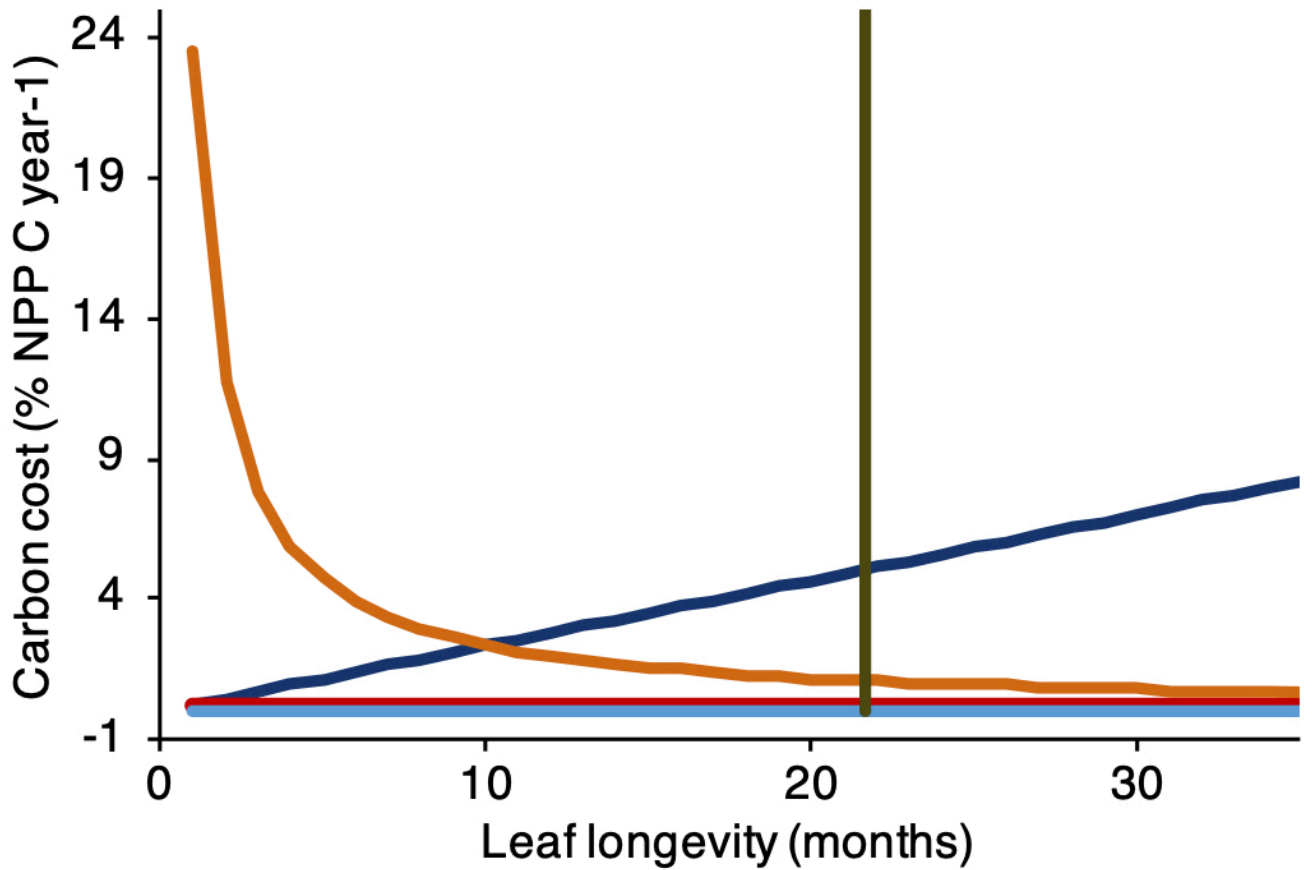
Data at the leaf and seedling scale

Proportion of leaf area lost for attacked leaves (Proportion$_{damaged}$)

c

Incidence of herbivory

b*

Proportion of leaf area lost for all leaves (Proportion$_{all}$)

a*

Fixers   Non-fixers

Predictions at the species scale

Proportion of leaf area lost for attacked leaves

f*

Probability of herbivory

e*

Proportion of leaf area lost for all leaves

d*

Fixers   Non-fixers

Carbon cost (% NPP C year-1) vs Leaf longevity (months)

Legend:
- Photosynthetic opportunity herbivory cost
- Structural herbivory cost
- Metabolic cost 40% light
- Metabolic cost 0% light
- Mean leaf longevity