

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26

PROF. DUNCAN NICHOLAS LUBCHENCO MENGE (Orcid ID : 0000-0003-4736-9844)

MS. WENYING LIAO (Orcid ID : 0000-0001-8777-1817)

Article type : Articles

### **Why are nitrogen-fixing trees rare at higher compared to lower latitudes?**

Duncan N. L. Menge<sup>1,5</sup>, Sarah A. Batterman<sup>2,3,4</sup>, Lars O. Hedin<sup>2</sup>, Wenyong Liao<sup>1,2</sup>, Stephen W. Pacala<sup>2</sup>, and Benton N. Taylor<sup>1</sup>

<sup>1</sup>Department of Ecology, Evolution, and Environmental Biology, Columbia University

<sup>2</sup>Department of Ecology and Evolutionary Biology, Princeton University

<sup>3</sup>School of Geography and Priestley International Centre for Climate, University of Leeds

<sup>4</sup>Smithsonian Tropical Research Institute, Ancon, Panama

<sup>5</sup>Corresponding Author. Email: [dm2972@columbia.edu](mailto:dm2972@columbia.edu)

Running head: N-fixing trees across latitude

Article in *Ecology*

Manuscript received 20 June 2017; revised 8 September 2017; accepted 18 September 2017.

Corresponding Editor: Serita D. Frey

#### **Abstract**

Symbiotic nitrogen (N) fixation provides a dominant source of new N to the terrestrial biosphere, yet in many cases the abundance of N-fixing trees appears paradoxical. N-fixing trees, which should be favored when N is limiting, are rare in higher-latitude forests where N limitation is

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/ecy.2034](https://doi.org/10.1002/ecy.2034)

This article is protected by copyright. All rights reserved

27 common, but are abundant in lower-latitude forests where N limitation is rare. Here, we develop  
28 a graphical and mathematical model to resolve the paradox. We use the model to demonstrate  
29 that N fixation is not necessarily cost-effective under all degrees of N limitation, as intuition  
30 suggests. Rather, N fixation is only cost-effective when N limitation is sufficiently severe. This  
31 general finding, specific versions of which have also emerged from other models, would explain  
32 sustained moderate N limitation because N-fixing trees would either turn N fixation off or be  
33 outcompeted under moderate N limitation. From this finding, four general hypothesis classes  
34 emerge to resolve the apparent paradox of N limitation and N-fixing tree abundance. The first  
35 hypothesis is that N limitation is less common at higher latitudes. This hypothesis contradicts  
36 prevailing evidence, so is unlikely, but the following three hypotheses all seem likely. The  
37 second hypothesis, which is new, is that even if N limitation is more common at higher latitudes,  
38 *more severe* N limitation might be more common at lower latitudes because of the capacity for  
39 higher N demand. Third, N fixation might be cost-effective under milder N limitation at lower  
40 latitudes but only under more severe N limitation at higher latitudes. This third hypothesis class  
41 generalizes previous hypotheses and suggests new specific hypotheses. For example, greater  
42 tradeoffs between N fixation and N use efficiency, soil N uptake, or plant turnover at higher  
43 compared to lower latitudes would make N fixation cost-effective only under more severe N  
44 limitation at higher latitudes. Fourth, N-fixing trees might adjust N fixation more at lower than at  
45 higher latitudes. This framework provides new hypotheses to explain the latitudinal abundance  
46 distribution of N-fixing trees, and also provides a new way to visualize them. Therefore, it can  
47 help explain the seemingly paradoxical persistence of N limitation in many higher latitude  
48 forests.

49 Keywords: Nitrogen, nitrogen fixation, legume, latitude, tree, ecosystem, theory, limitation,  
50 facultative, obligate

## 51 **Introduction**

52 Biological nitrogen (N) fixation is the largest natural N input to the terrestrial biosphere  
53 (Vitousek et al. 2013), and unlike other N inputs, has the capacity to respond to biotic N demand  
54 (Vitousek et al. 2002). This capacity is exceptionally high for symbioses between N-fixing  
55 bacteria and angiosperms (“rhizobial” legume species and “actinorhizal” species in other  
56 families), which can fix  $>100 \text{ kg N ha}^{-1} \text{ y}^{-1}$  (Binkley et al. 1994, Ruess et al. 2009). However, at  
57 the ecosystem scale, N-fixers (we call the plants “N-fixers” or “N-fixing plants” regardless of

58 whether they are actively engaged in N-fixing symbioses) can only fix N at high rates if they are  
59 relatively abundant, which they often are not.

60 The abundance distribution of N-fixing trees across latitude in the Americas is  
61 particularly intriguing. Forests at higher latitudes are more frequently N limited (i.e., N demand  
62 exceeds N supply) than those at lower latitudes (Vitousek & Sanford 1986, Vitousek & Howarth  
63 1991, Hedin et al. 2009, Brookshire et al. 2012). Given that N-fixing trees can access a vast N  
64 pool that other plants cannot (atmospheric N<sub>2</sub>), it seems reasonable that they should have a  
65 competitive advantage in N-limited habitats, and therefore be more abundant at higher latitudes.  
66 However, according to systematic government forest inventories and plot-level data from many  
67 millions of trees, N-fixing trees are 10-fold *less* abundant at higher (>35°N) than lower latitudes  
68 in the Americas (<35°N; ter Steege et al. 2006, Menge et al. 2010, 2014, 2017).

69 High N-fixing tree abundance does not necessarily indicate high rates of symbiotic N  
70 fixation (SNF), which remain poorly quantified. Global models (e.g., Houlton et al. 2008,  
71 Wieder et al. 2015, Ri & Prentice 2017) typically suggest that SNF rates are high (tens of kg N  
72 ha<sup>-1</sup> yr<sup>-1</sup>) at lower latitudes. However, these models are typically parameterized based either on  
73 an early data synthesis (Cleveland et al. 1999) or no N fixation data at all (Wieder et al. 2015).  
74 The early data synthesis (Cleveland et al. 1999) included very few measurements of SNF at  
75 lower latitudes, and more recent studies suggest that many tropical forests with abundant N-  
76 fixing trees have low to moderate rates of SNF (e.g., Barron et al. 2011, Batterman et al. 2013,  
77 Sullivan et al. 2014). Regardless of SNF rates, however, the pattern of N-fixing tree abundance  
78 in the Americas is exceptionally strong, and although abundance itself does not indicate SNF, it  
79 does control the *capacity* for SNF. The capacity for SNF—not the current rates—will help  
80 determine how forests respond to changing environmental conditions. One quarter of  
81 anthropogenic CO<sub>2</sub> emissions are currently absorbed by forests (Ciais et al. 2013), but the extent  
82 to which this will continue may depend on N availability (Hungate et al. 2003, Thornton et al.  
83 2007, Sokolov et al. 2008, Gerber et al. 2010, Wårlind et al. 2014). Therefore, vastly different  
84 capacities for SNF at higher vs. lower latitudes could help determine future carbon storage  
85 (Batterman et al. 2013).

86 Why are N-fixing trees rare at higher compared to lower latitudes? Hans Jenny wrote,  
87 “The question yet to be answered is whether the frequency of leguminous trees in the tropical  
88 forests studied and the related high nitrogen gains are conditioned by equatorial climate or by the

89 history of plant evolution” (Jenny 1950). The plant evolutionary history argument was  
90 crystallized by Crews (1999). Noting that woody legumes are much more speciose in the tropics,  
91 he suggested that something unrelated to N might constrain legume trees to lower latitudes.  
92 However, trait evolution rates suggest that over 2,500 species of higher-latitude woody N-fixing  
93 legumes would be extant if SNF were widely adaptive at higher latitudes (Menge & Crews  
94 2016). Furthermore, legumes (rhizobial symbioses) are not the only N-fixing trees. When  
95 actinorhizal and rhizobial trees are considered together, N-fixing trees comprise only a slightly  
96 lower fraction of taxonomic diversity at higher compared to lower latitudes in the Americas  
97 (Menge et al. 2017). Overall, plant evolutionary history is likely not the explanation.

98 If plant evolutionary history is not the explanation, then there must be one or more  
99 ecological explanations. Even though N-fixing trees are not necessarily fixing N all the time,  
100 their capacity for SNF is their distinguishing ecological feature, so we focus on explanations that  
101 favor SNF itself. The reasoning behind our focus draws on opposing ecological forces. On one  
102 hand, SNF must be advantageous in some environments, otherwise N-fixing plants would be  
103 outcompeted. On the other hand, there must be some constraints or costs of having the capacity  
104 to fix N, otherwise perfectly “facultative” N fixers—those that adjust SNF to balance their  
105 benefits and costs exactly—would outcompete all non-fixing plants (Menge et al. 2009a).

106 Among the ecological mechanisms that could drive the latitudinal pattern of N-fixing tree  
107 abundance, climate (Jenny’s other proposed driver) has often been invoked. N-fixing trees are  
108 more abundant in hotter (Liao et al. 2017) and more arid (Pellegrini et al. 2016, Liao et al. 2017)  
109 ecosystems, but the mechanisms underlying these patterns are not well established. One  
110 previously proposed possibility is that a direct temperature constraint on the process of N  
111 fixation confines N-fixing trees to lower latitudes (Houlton et al. 2008). However, there are  
112 reasons to question a direct temperature constraint. For instance, peak growing season  
113 temperatures, unlike mean annual temperatures, are similar across a range of latitudes.  
114 Additionally, although some nitrogenase enzymes are particularly sensitive to low temperatures  
115 (Ceuterick et al. 1978), nitrogenases from bacteria adapted to higher latitudes are less so (Prévost  
116 et al. 1987). At the organismal level, bacteria and plants have adapted to arctic conditions well  
117 enough to fix N at rates similar to their temperate counterparts (Bordeleau & Prévost 1994). We  
118 speculate that adaptation to colder temperatures, the success of herbaceous N-fixing legumes  
119 (Bordeleau & Prévost 1994, Sprent 2009) and actinorhizal N-fixing plants (Liao et al. 2017) at

120 higher latitudes, the high SNF rates in higher-latitude plants (Binkley et al. 1994, Ruess et al.  
121 2009), and the small temperature differences across latitude during peak growing season suggest  
122 a need to look beyond a direct temperature constraint.

123 Temperature could also constrain SNF indirectly. According to theory, N-fixers that can  
124 adjust SNF rapidly are more competitive than those with substantial time lags (Menge et al.  
125 2009a). Temperature, which influences biological kinetics, likely influences how quickly N-  
126 fixers can adjust SNF, so plants that live at higher latitudes could have unavoidably longer time  
127 lags, particularly at the beginning of the growing season when temperatures are still low.  
128 Significant time lags, particularly in ecosystems with short growing seasons, might select for an  
129 “obligate” SNF strategy that maintains a constant rate of SNF, rather than a facultative SNF  
130 strategy that adjusts to N limitation (Menge et al. 2009a). Theory suggests that obligate N-fixers  
131 are rare at the landscape scale because they are only successful in early successional habitats,  
132 whereas facultative N-fixers are more abundant because they persist throughout succession  
133 (Menge et al. 2009a, 2014). Therefore, temperature and growing season constraints on  
134 facultative SNF could explain the rarity of N-fixing trees at higher latitudes.

135 A second indirect climate-related mechanism also favors obligate N-fixers at higher  
136 latitudes. Sheffer et al. (2015) observed that colder temperatures lead to higher soil C:N,  
137 corresponding to lower rates of decomposition and slower release of bioavailable N in soils. If  
138 colder climates cause higher-latitude forests to have larger N deficits and recover biomass more  
139 slowly, then N limitation lasts longer, favoring the evolution of an obligate SNF strategy.  
140 Tropical forests also experience N limitation, but the condition appears limited to transient  
141 periods of rapid biomass accretion that follow disturbances (Davidson et al. 2004, 2007, Barron  
142 et al. 2011, Batterman et al. 2013). The combination of transient N limitation and rapid growth  
143 favors facultative SNF at lower latitudes (Sheffer et al. 2015).

144 Myriad other ecological mechanisms have been proposed to limit N-fixer abundance,  
145 including preferential herbivory on N-fixers (Vitousek & Howarth 1991, Ritchie & Tilman 1995,  
146 Hulme 1996, Vitousek & Field 1999, Knops et al. 2000, Menge et al. 2008, Kurokawa et al.  
147 2010), greater demand for soil nutrients that the symbionts need to fix N (e.g., phosphorus (P) or  
148 molybdenum (Mo); Vitousek & Howarth 1991, Vitousek & Field 1999, Uliassi & Ruess 2002),  
149 greater energy demand to pay the symbionts (Vitousek & Howarth 1991, Vitousek & Field 1999,  
150 Rastetter et al. 2001), and lower N use efficiency (Menge et al. 2008). Studies addressing these

151 mechanisms have focused on why SNF is rare in N-limited ecosystems, in an effort to  
152 understand the paradox of sustained N limitation (Vitousek & Howarth 1991), but have not  
153 addressed how these mechanisms influence the latitudinal abundance distribution of N-fixing  
154 trees. Understanding the rarity of SNF in N-limited ecosystems is integral to the latitudinal issue,  
155 but only addresses the higher latitude end of the spectrum. Moreover, these studies focus on the  
156 process of SNF, rather than the abundance of trees capable of SNF. A full explanation for the  
157 latitudinal abundance pattern needs to address N-fixing tree abundance, and why N-fixing trees  
158 are abundant at lower latitudes as well as rare at higher latitudes.

159 Here, we introduce a graphical framework to understand the abundance of N-fixing trees  
160 across latitude. This framework starts by providing a general explanation for sustained N  
161 limitation to net primary productivity (synonymous with plant N demand exceeding soil N  
162 supply). Our framework then reveals four classes of hypotheses to explain the latitudinal  
163 abundance pattern of N-fixing trees. Two of these hypotheses are new, one generalizes a  
164 previously proposed mechanism and extends other previously proposed mechanisms to a  
165 latitudinal context, and the fourth is one we have previously developed and include here for  
166 completeness. The first hypothesis proposes that, contrary to current understanding, N limitation  
167 is more common at lower latitudes (*N limitation frequency hypothesis*). The second new  
168 hypothesis proposes that *more severe* N limitation is more common at lower latitudes, even if  
169 some degree of N limitation is more common at higher latitudes (*N limitation severity*  
170 *hypothesis*). We define N limitation severity as the degree of imbalance between plant N demand  
171 and soil N supply, so “more severe” and “more moderate” indicate directions along an N  
172 limitation axis. A third possibility is that SNF is cost-effective under more moderate N limitation  
173 at lower latitudes, whereas it is only cost-effective under more severe N limitation at higher  
174 latitudes (*N fixation benefit-cost hypothesis*). The N fixation benefit-cost hypothesis generalizes  
175 specific mechanisms (e.g., Houlton et al. 2008) and extends previously proposed mechanisms  
176 (e.g., preferential herbivory might limit N-fixers; Vitousek & Field 1999, Menge et al. 2008) to a  
177 latitudinal context (e.g., preferential herbivory might change across latitude). A fourth  
178 possibility, which we developed previously (Menge et al. 2009a, 2014, Sheffer et al. 2015), is  
179 that the regulation of SNF changes with latitude (*Differential regulation hypothesis*). These  
180 hypotheses are not mutually exclusive, and each could be driven by multiple specific  
181 mechanisms.

## Methods

### Theoretical model

182  
183  
184 Our graphical theory is more general than one specific model, but we use a mathematical  
185 model to show how specific plant traits (in the mathematical model) determine the values of the  
186 graphical components. The mathematical model we use is simple by design, following a long  
187 tradition in theoretical ecology, not because we eschew the importance of other factors, but  
188 because including other factors would obfuscate our understanding. Because of its simplicity, our  
189 model might miss some of the specific details that more complex models would capture, but it  
190 can also give more general insights. Our results emerge from the model shown here, but they  
191 could also emerge from other models that include more realistic features. The theory we use  
192 builds on Menge et al. (2008, 2009a, 2009b), and tracks how plant populations,  $B_i$  (kg C ha<sup>-1</sup>), a  
193 soil pool of plant-unavailable N,  $D$  (kg N ha<sup>-1</sup>), and a soil pool of plant-available N,  $A$  (kg N ha<sup>-1</sup>),  
194 change over time:

$$195 \frac{dB_i}{dt} = B_i \left( \min \left[ \omega(F_i)(\nu(F_i)A + F_i), \frac{g(F_i)}{1+\gamma(F_i)\sum_j B_j} \right] - \mu(F_i) \right) \quad (1)$$

$$196 \frac{dD}{dt} = \sum_j \frac{\mu(F_j)B_j}{\omega(F_j)} - mD - \phi D \quad (2)$$

$$197 \frac{dA}{dt} = I + mD - kA - \sum_j \frac{B_j}{\omega(F_j)} \left( \min \left[ \omega(F_j)(\nu(F_j)A + F_j), \frac{g(F_j)}{1+\gamma(F_j)\sum_k B_k} \right] - \omega(F_j)F_j \right) \quad (3)$$

198 The subscripts  $i, j$ , and  $k$  refer to different plant types. In this model (Fig. 1a) plant growth can be  
199 limited by N or another density-dependent factor such as light, P, or another resource.

200 Plant traits can vary with SNF, between non-fixing and N-fixing species regardless of  
201 fixation rate, or both. For simplicity we only consider trait variation with SNF in the main text,  
202 so parameter values are the same for non-fixers and for N-fixing species that are not fixing N  
203 (e.g.,  $\omega_{non} = \omega_{fix}(0) \equiv \omega_0$ ). This feature makes the graphical presentation of our results  
204 simpler because both non-fixing N-fixers and non-fixers have the same N limitation threshold.  
205 A version of the model with species-level variation, where non-fixing and N-fixing species differ  
206 independently of SNF rates, is in Appendix S1.

207 All plants can take N from the plant-available soil pool via uptake,  $\nu$  (ha kg C<sup>-1</sup> yr<sup>-1</sup>), and  
208 N-fixers can also acquire N via fixation,  $F$  (kg N kg C<sup>-1</sup> yr<sup>-1</sup>). Newly acquired N is converted to  
209 new biomass C via N use efficiency,  $\omega$  (kg C kg N<sup>-1</sup>). When not N limited, the plant grows at a  
210 maximum per capita rate  $g$  (yr<sup>-1</sup>), dampened by its susceptibility to competition,  $\gamma$  (ha kg C<sup>-1</sup>) and

211 its competitors' biomass  $C$ ,  $\Sigma B$ . Plant biomass turns over to the soil ( $\mu$ ;  $\text{yr}^{-1}$ ). Plant-unavailable N  
212 is converted to plant-available N ( $m$ ,  $\text{yr}^{-1}$ ), and lost ( $\phi$ ,  $\text{yr}^{-1}$ ). N comes into the plant-available soil  
213 N pool from external inputs such as N deposition ( $I$ ,  $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) and is lost (e.g., leaching or  
214 gas loss) at the rate  $k$  ( $\text{yr}^{-1}$ ). All parameters are strictly positive except for fixation ( $F$ ), which can  
215 be 0.

#### 216 *Previously proposed tradeoffs between SNF and plant traits*

217 Because a given amount of root tissue can be used for either SNF or N uptake, there is  
218 probably a tradeoff between SNF and soil N uptake (Rastetter et al. 2001, Menge et al. 2008,  
219 Sheffer et al. 2015; Fig. 1b):  $\frac{dv(F)}{dF} \equiv v' < 0$ . N-fixing plants have higher average tissue N  
220 concentrations than non-fixing plants (Fyllas et al. 2009, Nasto et al. 2014, Adams et al. 2016),  
221 which is driven in part by symbiotic bacteria, regardless of plant N demand (Wolf et al. 2017).

222 Therefore, we assume that nutrient use efficiency decreases with SNF:  $\frac{d\omega(F)}{dF} \equiv \omega' < 0$ . As  
223 described in the introduction, N-fixers might suffer greater rates of herbivory-driven turnover  
224 than non-fixers because of their high N content  $\frac{d\mu(F)}{dF} \equiv \mu' > 0$ . On the contrary, N-fixing trees  
225 might use extra N to increase herbivore defenses (Vitousek & Field 1999, Menge et al. 2008,  
226 Menge & Chazdon 2016), which could balance or reverse the relationship between SNF and  
227 turnover:  $\mu' \leq 0$ .

228 A number of mechanisms connect SNF to energy, P, or other non-N nutrients (Vitousek  
229 & Howarth 1991, Vitousek & Field 1999, Rastetter et al. 2001, Houlton et al. 2008). Our model  
230 specifies that some other resource limits plant growth if N does not, so competition for other  
231 resources affects the non-N-limited maximum growth rate,  $g$ , or competition,  $\gamma$ . Under  
232 conditions when both the non-fixer and the N-fixer are not N limited, greater demand for energy,  
233 P, Mo, or another resource would mean that N-fixers would experience a lower maximum  
234 growth rate or greater competition than non-fixers:  $\frac{dg(F)}{dF} \equiv g' < 0$ ,  $\frac{d\gamma(F)}{dF} \equiv \gamma' > 0$ . On the  
235 contrary, if N-fixers use their higher N content to increase photosynthetic rates (Field & Mooney  
236 1986) or water use efficiency (Adams et al. 2016), or are better able than non-fixers to access P  
237 via phosphatase enzymes (Houlton et al. 2008), they could have higher maximum growth rates or  
238 a competitive advantage when both non-fixers and N-fixers are not N limited:  $g' > 0$ ,  $\gamma' < 0$ .

#### 239 *Previously proposed latitudinal trends in plant traits and tradeoffs between SNF and plant traits*

240 If temperature constrains SNF directly (Houlton et al. 2008), then plants at higher  
241 latitudes ( $L$ ) need to spend more carbon to get the same amount of N. Our model can incorporate  
242 this in two ways. First, turnover rates of N-fixers might increase more with SNF, or decrease less  
243 with SNF, at higher than at lower latitudes (Fig. 1c):  $\frac{d\mu(F)}{dL} > 0$ . Second, the temperature effect  
244 might require a greater investment in nodules to achieve a similar SNF rate, which would  
245 decrease the carbon available for soil N uptake via roots or mycorrhizae. In this case, the N  
246 uptake rates of N-fixers decrease more with SNF at higher than at lower latitudes (Fig. 1d):  
247  $\frac{d\nu(F)}{dL} < 0$ . A higher turnover cost of SNF at higher latitudes ( $\frac{d\mu(F)}{dL} > 0$ ) could also stem from N-  
248 fixers being more palatable to herbivores than non-fixers at higher latitudes, but less palatable  
249 than non-fixers at lower latitudes (Fig. 1c).

250 The idea that SNF confers a greater phosphatase advantage (Houlton et al. 2008) at lower  
251 latitudes, where P is more limiting than at higher latitudes, would mean that effects of SNF on  
252 the non-N-limited growth parameters change across latitude. If P acquisition enhances the plant's  
253 maximum growth rate more at lower latitudes (Fig. 1e),  $\frac{dg(F)}{dL} < 0$ , whereas if P acquisition  
254 reduces competition with neighboring plants more at lower latitudes (Fig. 1c),  $\frac{dy(F)}{dL} > 0$ . The  
255 final previously proposed mechanism involves the degree to which N-fixing plants regulate SNF  
256 in response to soil N supply vs. N demand. In this scenario,  $F$  is constant at high latitudes but  
257 variable at low latitudes.

258 The trends in this section represent what has been proposed previously. However, our  
259 analytical results do not depend on these assumptions, and one could evaluate the effect of other  
260 cases using our equations in Appendix S1.

261 *Analysis: A framework to classify mechanisms that can maintain N limitation*

262 We show our graphical results in the main text as a function of our N limitation index,  
263 which is the difference between soil N supply flux ( $S = I + mD$ ) and N demand at a snapshot in  
264 time (Appendix S1). Because this approach focuses on the difference between N demand and N  
265 supply, the absolute values of each do not influence the presentation. However, because it is of  
266 interest to examine changes in N demand and N supply independently, we also show our  
267 graphical results as a function of soil N supply in Appendix S2.

268 Our approach requires three graphical components. The first is the “co-limitation  
269 threshold,” the N supply level ( $S_{co}$ ) that divides N limitation from non-N limitation. The co-  
270 limitation threshold is equivalent to N demand. The second component is the “N fixation benefit-  
271 cost threshold,” the level of N supply ( $S_{crit}$ ) at which the benefit of SNF equals the cost. The  
272 benefits vs. costs of SNF are, respectively, the new biomass gained from newly fixed N vs. the  
273 new biomass lost due to the indirect effects of SNF on the other plant parameters. The N fixation  
274 benefit-cost threshold divides the region where N fixation is cost-effective from the region where  
275 it is not. In the main text we give the benefit-cost threshold results for perfectly facultative SNF.  
276 In Appendix S1 we also present results for obligate SNF and with an explicit cost of being  
277 facultative. To find the facultative SNF benefit-cost threshold, we evaluate how a small amount  
278 of fixation influences the relative plant population growth rate. A positive effect indicates a net  
279 SNF benefit, and a negative effect indicates a net SNF cost, so the threshold is where this  
280 quantity equals 0:  $\frac{\partial \frac{dB}{dt}}{\partial F} |_{F=0} = 0$ . The third and final graphical component is the distribution of  
281 habitats across a gradient of N limitation.

282 The pattern we want to explain concerns the relative abundance of N-fixing trees, which  
283 in our model is  $\frac{B_{fix}}{\Sigma B}$ . However, we focus our analysis on three key quantities—the co-limitation  
284 threshold, the N fixation benefit-cost threshold, and the distribution of habitats—rather than  
285 relative abundance itself, for three reasons. First, these three quantities are the key determinants  
286 of the difference in relative growth rate between N-fixing trees and non-fixing trees, so they give  
287 a clear window into relative abundance, even if there is not a one-to-one correspondence. We are  
288 interested in qualitative patterns in this work (fewer N-fixing trees, not more, in an environment  
289 that is more N limited), not specific numbers. Second, studying these three quantities requires  
290 fewer assumptions than examining relative abundance itself. Modeling relative abundance  
291 requires not only a description of how the ecosystem changes (Eqns. 1-3), but also a description  
292 of the starting values and length of time since the ecosystem was at those starting values. The  
293 forest ecosystems we are modeling typically range up to a couple hundred years old (Menge et  
294 al. 2014), whereas this sort of ecosystem model takes thousands of years to approach equilibrium  
295 (Menge et al. 2009b). Explicitly modeling a mosaic of succession would be cumbersome and  
296 would not add qualitative understanding. Third, these three quantities facilitate the graphical  
297 framework that will clarify our hypotheses and the underlying mechanisms.

298 Once we have the graphical framework, we examine how the three components—the co-  
 299 limitation threshold, the N fixation benefit-cost threshold, and the distribution of habitats—might  
 300 vary across latitude. Because our approach uses a simple model, and focuses on graphical and  
 301 analytical (but not numerical simulation) techniques, there is no need for direct parameterization  
 302 of our model or for numerical sensitivity analyses. Our model reveals hypotheses that could  
 303 explain the latitudinal distribution of N-fixing trees, and how underlying plant and ecosystem  
 304 traits influence these hypotheses. It does not attempt to assign quantitative probabilities to them,  
 305 but in the discussion we draw on relevant literature to debate the relative likelihood of the  
 306 different hypotheses.

## 307 Results

### 308 *Sustained N limitation: Graphical theory*

309 Sustained N limitation seems paradoxical because intuition says that SNF should be  
 310 advantageous when N limits production, and should therefore alleviate N limitation (Vitousek &  
 311 Howarth 1991). Graphically, we can show this intuitive statement as a distribution of habitats  
 312 along an N limitation gradient (Fig. 2a; see Appendix S2: Fig. S1a for an N supply gradient). If  
 313 SNF is cost-effective whenever N limits production, then N-fixers fix N in habitats to the left of  
 314 the dashed line. After their newly fixed N is incorporated into the soil, N supply would increase,  
 315 shifting the habitat distribution to the right. In reality many forests are N limited, as in Fig. 2a,  
 316 but have no SNF, unlike Fig. 2a, which is why sustained N limitation seems paradoxical. A key  
 317 assumption underlying this seeming paradox is that SNF is cost-effective whenever soil N supply  
 318 alone is insufficient to meet N-fixers' demand. As shown below (and elsewhere, e.g., Vitousek &  
 319 Field 1999, Menge et al. 2008), this does not have to be true. Fig. 2b, Appendix S2: Fig. S1b  
 320 show a scenario where most habitats are N limited but SNF is only cost-effective in habitats  
 321 where N limitation is sufficiently severe. Next we derive the conditions under which the scenario  
 322 in Fig. 2b occurs.

### 323 *Sustained N limitation: Mathematical results*

324 The co-limitation threshold ( $S_{co}$ ) is the soil N supply at which plants are co-limited:

$$325 S_{co} = \frac{\left(\frac{g(F)}{1+\nu(F)\Sigma B} - \omega(F)F\right)(k+\Sigma B\nu(F))}{\omega(F)\nu(F)} \quad (4)$$

326 The N fixation benefit-cost threshold ( $S_{crit}$ ) for a facultative N-fixer is:

$$327 S_{crit} = \frac{(\mu'_0 - \omega_0)(k + \Sigma B\nu_0)^2}{\omega'_0\nu_0(k + \Sigma B\nu_0 + B\nu_0) + \omega_0\nu'_0(k + \Sigma B\nu_0 - B\nu_0)} \quad (5)$$

328 The “’” indicates a derivative with respect to  $F$ . The “0” subscripts indicate “evaluated at  $F = 0$ .”

329 The key point of Eqns. 4-5 is that the N fixation benefit-cost threshold ( $S_{crit}$ ) does not  
330 have to be the same N supply level as the co-limitation threshold ( $S_{co}$ ). To see this, note that  
331 many parameters appear in only one equation. Therefore, the intuitive scenario ( $S_{crit} = S_{co}$ ; Fig.  
332 2a) is possible, but highly unlikely. By contrast, a range of N limitation where SNF is not cost-  
333 effective ( $S_{crit} < S_{co}$ ; Fig. 2b) is likely, depending on the values of the plant and ecosystem traits  
334 that determine the co-limitation and N fixation benefit-cost thresholds (Appendix S1: Table S1).  
335 Stronger tradeoffs between SNF and N use efficiency ( $\omega$ ), soil N uptake ( $\nu$ ), or turnover ( $\mu$ )  
336 lower the N fixation benefit-cost threshold, and therefore facilitate N limitation.

### 337 *Comparing across latitude*

338 We now use this graphical framework to ask: Why are N-fixing trees rare at higher  
339 compared to lower latitudes?

340 *N limitation frequency hypothesis: N limitation is more common at lower latitudes*

341 The first explanation is that N limitation is more common at lower latitudes (Fig. 2c,  
342 Appendix S2: Fig. S1c), counter to our current understanding.

343 *N limitation severity hypothesis: More severe N limitation is more common at lower*  
344 *latitudes*

345 If there is a range of N limitation over which SNF is not cost-effective ( $S_{crit} < S_{co}$ , as in  
346 Fig. 2b, Appendix S2: Fig. S1b), then only the proportion of habitat where N limitation is  
347 sufficiently severe (i.e., SNF is cost-effective)—not the proportion that is N limited at all—  
348 predicts N-fixing tree success. The N limitation severity hypothesis states that even if N  
349 limitation is less common in lower- than higher-latitude forests, *more severe* N limitation is *more*  
350 common in lower- than higher-latitude forests (Fig. 2d, Appendix S2: Fig. S1d). Put another  
351 way, even if the mean trend is that higher latitudes are more N limited than lower latitudes,  
352 variance in the magnitude of N limitation across habitats could be greater at lower latitudes.

353 *N fixation benefit-cost hypothesis: SNF is cost-effective over a wider range of N*  
354 *limitation at lower latitudes*

355 If SNF is only cost-effective when N limitation is severe enough ( $S_{crit} < S_{co}$ ; Fig. 2b), the  
356 “severe enough” threshold itself ( $S_{co} - S_{crit}$ ) might vary across latitude. The N fixation benefit-  
357 cost hypothesis states that SNF is only cost-effective under more severe N limitation at higher  
358 latitudes, whereas it is cost-effective under more moderate N limitation at lower latitudes (Fig.

359 2e, Appendix S2: Fig. S1e). This general hypothesis class encompasses many specific  
360 mechanisms. Because plant traits modify the co-limitation threshold, the N fixation benefit-cost  
361 threshold, or both (Table S1), we can determine which specific mechanisms would support the N  
362 fixation benefit-cost hypothesis (Fig. 3, Appendix S1, Appendix S2: Fig. S2). Not all of these  
363 mechanisms are likely, but we list them for completeness. First, lower N use efficiency at higher  
364 latitudes would increase the co-limitation threshold but decrease the N fixation benefit-cost  
365 threshold compared to lower latitudes. Second, stronger tradeoffs between SNF and N use  
366 efficiency, soil N uptake, and turnover at higher latitudes decrease the N fixation benefit-cost  
367 threshold at higher latitudes. Third, a higher maximum growth rate, a lower susceptibility to non-  
368 N-based competition, or a lower soil N uptake rate at higher latitudes compared to lower  
369 latitudes would increase ecosystem N demand at higher latitudes.

370 *Differential regulation hypothesis: The SNF strategy differs across latitude*

371 The three hypotheses discussed above examine situations in which facultative N-fixers  
372 are more common at lower than at higher latitudes. If higher-latitude N-fixers are more obligate  
373 but lower-latitude N-fixers are more facultative (Menge et al. 2014, Sheffer et al. 2015), then a  
374 fourth hypothesis—the differential regulation hypothesis—emerges.

375 Obligate N-fixers are less competitive under mild N limitation than facultative N-fixers,  
376 for two reasons. First, the N fixation benefit-cost threshold is closer to the co-limitation threshold  
377 for facultative (or over-regulating, under-regulating, or incompletely down-regulating; Menge et  
378 al. 2015) than for obligate N-fixers (Fig. 2f, Appendix S1, Appendix S2: Fig. S1f). The second  
379 reason concerns relative growth rates when SNF is not cost-effective. When SNF is not cost-  
380 effective, obligate N-fixers have much lower relative population growth rates than non-fixers  
381 because they are wasting energy fixing N. On the contrary, facultative N-fixers that are not  
382 fixing have only slightly lower relative population growth rates than do non-fixers, depending on  
383 the costs of being facultative (Appendix S1).

384 **Discussion**

385 Four general hypothesis classes emerge from our graphical framework, all of which could  
386 explain why N-fixing trees are much more abundant at lower latitudes than at higher latitudes,  
387 and all of which could act in concert. These general hypothesis classes relate to our finding that,  
388 contrary to the intuition that N fixation is cost-effective under all degrees of N limitation, it is  
389 only cost-effective under sufficiently severe N limitation. This finding, which has also been

390 shown or suggested in previous studies (e.g., Vitousek & Howarth 1991, Ritchie & Tilman 1995,  
391 Vitousek & Field 1999, Rastetter et al. 2001, Menge et al. 2008), provides a graphical  
392 explanation for sustained N limitation, which has long been viewed as a paradox in ecosystem  
393 ecology (Vitousek & Howarth 1991). Although this understanding of sustained N limitation  
394 opens doors to many questions, our focus here is on understanding the latitudinal abundance  
395 pattern of N-fixing trees in the Americas. In that vein, we now draw on the literature to evaluate  
396 how likely each general hypothesis class is and which of the specific mechanisms we have  
397 highlighted might underlie them.

398 *N limitation is probably not more common at lower latitudes*

399 The latitudinal abundance pattern of N-fixing trees seems paradoxical (Houlton et al.  
400 2008, Hedin et al. 2009, Menge et al. 2014) precisely because N limitation is thought to be less  
401 common at lower latitudes, not more common (Vitousek & Sanford 1986, Hedin et al. 2009,  
402 Brookshire et al. 2012). The evidence for this, such as the 10-fold greater leaching of plant-  
403 available N in tropical compared to temperate forests (Hedin et al. 2009, Brookshire et al. 2012),  
404 suggests that our first hypothesis—the N limitation frequency hypothesis—is unlikely.  
405 Somewhat surprisingly, a meta-analysis of N fertilization studies found that N limitation was at  
406 least as strong in tropical forests as in temperate forests (LeBauer & Treseder 2008), although  
407 three factors mitigate this finding. First, few fertilization studies have been conducted in tropical  
408 forests, particularly before 2008. Second, site selection bias towards young and successional  
409 tropical forests may have amplified the N limitation signal compared to the true distribution of  
410 tropical forest types (LeBauer et al. 2008). In particular, only one mature tropical forest was  
411 included in that meta-analysis, and it did not show an NPP response to N fertilization. Mature  
412 tropical forests, which comprise 58% of Latin American tropical forest area (Chazdon et al.  
413 2016), are the tropical forests typically thought to be N-rich (Hedin et al. 2009). Studies in  
414 mature tropical forest studies since 2008 have found no ecosystem-level response to N additions  
415 (Wright et al. 2011, Alvarez-Clare et al. 2013). Many other tropical forests, such as those  
416 growing on young substrates (Vitousek & Farrington 1997) or those in early successional stages  
417 (Davidson et al. 2004, Batterman et al. 2013), are often N limited. Third, the response metric  
418 used—the response ratio—does not distinguish between the frequency and severity of N  
419 limitation, and as we discuss below, tropical forests might be more severely but less frequently N  
420 limited.

421 *More severe N limitation might be more common at lower latitudes*

422 The N limitation severity hypothesis—more severe N limitation is more common in  
423 lower- than higher-latitude forests—is new, to our knowledge, and is an intriguing possibility. It  
424 could explain why tropical forests appeared at least as N limited as temperate forests in a meta-  
425 analysis (LeBauer & Treseder 2008). Even if most tropical forests are not N limited, a few  
426 severely N-limited forests would inflate the average response to fertilization. On a mechanistic  
427 level, lower-latitude forests likely have a greater capacity for more severe N limitation than  
428 higher-latitude forests. Longer growing seasons, warmer temperatures, and ample rainfall  
429 stimulate N demand, so if N supply is greatly diminished—for example, due to large  
430 disturbance-mediated N losses—then lower-latitude forests can be more severely N limited than  
431 higher-latitude forests (Fig. 2d). For example, a recent modeling study (Ri & Prentice 2017)  
432 suggests that N demand that is unmet by recycling is much higher at lower than higher latitudes.

433 Variation in N limitation could occur at a variety of scales. On the successional timescale  
434 and the landscape spatial scale, a variety of studies suggest that N limitation—possibly severe N  
435 limitation—is common in young regenerating tropical forests (Davidson et al. 2004, 2007,  
436 Batterman et al. 2013), which comprise 22% of Neotropical forests (where “young” is <20 years  
437 old; Chazdon et al. 2016). However, N-fixing trees are also common in mature tropical forests  
438 (ter Steege et al. 2006, Batterman et al. 2013, Menge & Chazdon 2016). On smaller spatial  
439 scales, tree-fall gaps lead to greater understory light penetration at lower latitudes because of the  
440 sun angle (Canham et al. 1990). Greater light penetration—which increases N demand for the  
441 remaining trees—combined with reduced N supply in gaps (Vitousek and Denslow 1986) could  
442 drive a severe N demand-supply imbalance in gaps, even in mature forests. A study in Panama  
443 documented much higher nodulation rates in mature forest gaps than in the surrounding matrix  
444 (Barron et al. 2011), which could explain the continued success of N-fixing trees in mature  
445 tropical forests (Batterman et al. 2013).

446 *SNF might be cost-effective under a wider range of N limitation at lower latitudes*

447 Most of the previously proposed mechanisms that could constrain N-fixers in N-limited  
448 environments are, in essence, explanations for why SNF is only cost-effective when N limitation  
449 is sufficiently severe. An allocation tradeoff between soil N uptake and SNF (Rastetter et al.  
450 2001, Menge et al. 2008), lower N use efficiency or higher turnover as a consequence of SNF  
451 (Menge et al. 2008), and energetic or other resource (e.g., P or Mo) costs of SNF (Vitousek &

452 Howarth 1991, Vitousek & Field 1999, Rastetter et al. 2001, Uliassi & Ruess 2002) all make  
453 SNF cost-effective under more severe but not more moderate N limitation (Fig. 2b).

454 If any of these specific mechanisms change across latitude, and the change is in the right  
455 direction, they could support the N fixation benefit-cost hypothesis, and help explain why N-  
456 fixers are rare at higher latitudes. Two of these latitudinal changes relate to previously proposed  
457 mechanisms (Fig. 3). If temperature constrains the process of SNF (Houlton et al. 2008), then  
458 higher-latitude N-fixers would need to invest more carbon per unit N fixed than lower-latitude  
459 N-fixers. Such a carbon investment could strengthen the tradeoff between SNF and N uptake at  
460 higher latitudes because carbon used for SNF cannot be used for soil N uptake. Alternatively,  
461 such a carbon investment could make SNF more costly via an increased turnover rate.

462 The second previously proposed mechanism relates to herbivory. As explained above, N-  
463 fixers' higher N content could lead to higher herbivore pressure (if it is used for tasty, non-  
464 defensive compounds; Vitousek & Howarth 1991), or it could enable a greater capacity for  
465 chemical defense, by using N-based defensive compounds, by using their higher photosynthetic  
466 rates to synthesize more C-based defensive compounds, or both. If herbivory is a stronger  
467 selective force at lower latitudes (Coley & Barone 1996), then lower latitude N-fixers might have  
468 been selected for greater investment in anti-herbivore defense than higher latitude N-fixers  
469 (Vitousek & Field 1999, Menge et al. 2008). In this case, N-fixers might have a higher turnover  
470 cost of SNF, corresponding to higher mortality rates than non-fixers, at higher latitudes, but vice  
471 versa at lower latitudes (Figs. 1c, 3). In support of this prediction, N-fixing trees in the  
472 coterminous U.S.A. had higher mortality rates than non-fixing trees (Liao & Menge 2016),  
473 whereas N-fixing trees in Costa Rica had lower mortality than non-fixing trees (Menge &  
474 Chazdon 2016). This empirical mortality pattern is consistent with an herbivory mechanism, but  
475 does not pinpoint herbivory as the mechanism because it was not measured.

476 Curiously, one previously proposed specific mechanism—that N-fixers have a greater  
477 ability to produce P-liberating phosphatases (Houlton et al. 2008)—cannot explain N-fixer  
478 abundance in our model. The model that produced that hypothesis (Wang et al. 2007, Houlton et  
479 al. 2008) is much more complex than ours, so it is not surprising that it allows for possibilities  
480 that ours does not, but we note that many other specific mechanisms emerge from our model  
481 despite its simplicity.

482 In addition to the previously-proposed mechanisms that could underlie the N fixation  
483 benefit-cost hypothesis, three other possibilities emerge from our model (Fig. 3). First, the  
484 stronger the tradeoff between SNF and N use efficiency, the more severe N limitation must be to  
485 favor SNF (Fig. 3). Second, trees might experience weaker competition for non-N resources at  
486 higher latitudes. Third, trees might have lower soil N uptake rates at higher latitudes. However,  
487 even if these traits and tradeoffs change in the right direction, they can be offset by other trends.  
488 For example, trees are more N use efficient at higher latitudes (Vitousek 1984, McGroddy et al.  
489 2004), which lowers ecosystem-level N demand and enhances the benefit of fixed N (Fig. 3).

490 Although it is useful to think through these specific mechanisms, pinning down all the  
491 conditions needed to scale up to the overall balance of N fixation benefits and costs is  
492 challenging. The specific mechanisms interact, and trends for seemingly unrelated traits can  
493 cancel each other out (Appendix S1: Eqn. S11). Pursuing each of these specific mechanisms is a  
494 good goal, but a complementary way to evaluate the N fixation benefit-cost hypothesis is to test  
495 the theoretical predictions rather than the parameters. For example, Menge et al. (2015) found  
496 that a number of herbaceous plant species were “over-regulators.” These plants turned SNF off at  
497 N supply levels that were lower than their N demand, as predicted by our theory (Appendix S1,  
498 Appendix S1: Fig. S1). If these plants’ SNF rates accurately assess the cost-effectiveness of  
499 SNF, then “over-regulation” is evidence that the N fixation benefit-cost threshold is lower than  
500 the co-limitation threshold.

#### 501 *N-fixing trees might be more facultative at lower latitudes*

502 A variety of field observations suggest that higher latitude N-fixing trees are obligate  
503 (Mead & Preston 1992, Binkley et al. 1994, Menge & Hedin 2009), whereas lower latitude N-  
504 fixing trees are facultative (Pearson & Vitousek 2001, Barron et al. 2011, Batterman et al. 2013,  
505 Sullivan et al. 2014, Bauters et al. 2016, see also Andrews et al. 2011). Modeling suggests that a  
506 strategy transition across latitude can explain the latitudinal trend (Menge et al. 2014).  
507 Underlying climate effects on soil N deficits (Sheffer et al. 2015) or on the constraints and costs  
508 of regulating SNF (Menge et al. 2009a) can explain a transition in strategy. Although  
509 experimental evidence is still scant, the differential regulation hypothesis is promising.

#### 510 *Conclusions*

511 Given current evidence, the most likely reasons that N-fixing trees are more abundant at  
512 lower latitudes in the Americas are: a greater prevalence of more severe N limitation at lower

513 latitudes (the N limitation severity hypothesis), lower costs of SNF compared with other forms of  
514 N uptake at lower latitudes (the N fixation benefit-cost hypothesis), and a transition in SNF  
515 strategy across latitude (the differential regulation hypothesis) (Fig. 4). Evolutionary constraints  
516 on the biogeography of N-fixing trees and a higher frequency of N limitation at lower latitudes  
517 are unlikely to explain the latitudinal trend of N-fixer abundance in the Americas. Disentangling  
518 the relative importance of N limitation severity, N fixation benefits vs. costs, and differential  
519 regulation, and determining the specific mechanisms that underlie them, will help resolve the  
520 seemingly paradoxical latitudinal distribution of N-fixers that has puzzled scientists for over 65  
521 years. Furthermore, given the importance of N fixation for ecosystems' responses to rising  
522 atmospheric CO<sub>2</sub> and temperature, testing these hypotheses will greatly improve our  
523 understanding of how the fixation and carbon sequestration responses differ across latitude,  
524 which will improve our predictions of global climate change.

#### 525 **Acknowledgements**

526 This material is based upon work supported by the National Science Foundation under  
527 grant no. DEB-1457650. SAB was supported by a UK Natural Environment Research Council  
528 Independent Research Fellowship (NE/M019497/1).

#### 529 **Literature Cited**

- 530 Adams, M. A., T. L. Turnbull, J. I. Sprent, and N. Buchmann. 2016. Legumes are different: Leaf  
531 nitrogen, photosynthesis, and water use efficiency. *Proceedings of the National Academy of*  
532 *Sciences USA* 113: 4098-4103.
- 533 Alvarez-Clare, S., M. C. Mack, and M. Brooks. 2013. A direct test of nitrogen and phosphorus  
534 limitation to net primary productivity in a lowland tropical wet forest. *Ecology* 94: 1540-1551.
- 535 Andrews, M., E. K. James, J. I. Sprent, R. M. Boddey, E. Gross, and F. B. dos Reis Jr. 2011.  
536 Nitrogen fixation in legumes and actinorhizal plants in natural ecosystems: values obtained  
537 using <sup>15</sup>N natural abundance. *Plant Ecology & Diversity* 4: 131-140.
- 538 Barron, A. R., D. W. Purves, and L. O. Hedin. 2011. Facultative nitrogen fixation by canopy  
539 legumes in a lowland tropical forest. *Oecologia* 165: 511-520.
- 540 Batterman, S. A., L. O. Hedin, M. van Breugel, J. Ransijn, D. J. Craven, and J. S. Hall. 2013.  
541 Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* 502:  
542 224-227.

543 Bauters, M., N. Mapenzi, E. Kearsley, B. Vanlauwe, and P. Boeckx. 2016. Facultative nitrogen  
544 fixation in legumes in the central Congo basin is downregulated during late successional stages.  
545 *Biotropica* 48: 281-284.

546 Binkley, D., K. Cromack Jr., and D. D. Baker. 1994. Nitrogen fixation by red alder: Biology,  
547 rates and controls. Pages 57-72 *in* D. Hibbs, D. DeBell, and R. Tarrant, editors. *The Biology and*  
548 *Management of Red Alder*. Oregon State University Press, Corvallis.

549 Bordeleau, L. M. and D. Prévost. 1994. Nodulation and nitrogen fixation in extreme  
550 environments. *Plant and Soil* 161: 115-125.

551 Brookshire, E. N. J., S. Gerber, D. N. L. Menge, and L. O. Hedin. 2012. Large losses of  
552 inorganic nitrogen from tropical rainforests suggests a lack of nitrogen limitation. *Ecology*  
553 *Letters* 15: 9-16.

554 Canham, C. D., J. S. Denslow, W. J. Platt, J. R. Runkle, T. A. Spies, and P. S. White. 1990. Light  
555 regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. *Canadian*  
556 *Journal of Forest Research* 20: 620-631.

557 Ceuterick, F., J. Peeters, K. Heremans, H. de Smedt, and H. Olbrechts. 1978. Effect of high  
558 pressure, detergents and phospholipase on the break in the Arrhenius plot of *Azotobacter*  
559 nitrogenase. *European Journal of Biochemistry* 87: 401-407.

560 Chazdon, R. L., et al. 2016. Carbon sequestration potential of second-growth forest regeneration  
561 in the Latin American tropics. *Science Advances* 2: 1501639.

562 Ciais, P., et al. 2013. Carbon and other biogeochemical cycles. Pages 465-570 *in* T. F. Stocker, et  
563 al., editors. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I*  
564 *to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge  
565 University Press, New York.

566 Coley, P. D. and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual*  
567 *Review of Ecology and Systematics* 27: 305-335.

568 Crews, T. E. 1999. The presence of nitrogen fixing legumes in terrestrial communities:  
569 evolutionary vs. ecological considerations. *Biogeochemistry* 46: 233-246.

570 Davidson, E. A., et al. 2004. Nitrogen and phosphorus limitation of biomass growth in a tropical  
571 secondary forest. *Ecological Applications* 14: S150-S163.

572 Davidson, E. A., et al. 2007. Recuperation of nitrogen cycling in Amazonia forests following  
573 agricultural abandonment. *Nature* 447: 995-998.

574 Field, C. and H. A. Mooney. 1986. Photosynthesis-nitrogen relationship in wild plants. *in*  
575 Proceedings of the 6<sup>th</sup> Maria Moors Cabot Symposium, Evolutionary Constraints on Primary  
576 Productivity, Adaptive Patterns of Energy Capture in Plants. Cambridge University Press,  
577 Cambridge.

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

580 Gerber, S., L. O. Hedin, M. Oppenheimer, S. W. Pacala, and E. Shevliakova. 2010. Nitrogen  
581 cycling and feedbacks in a global dynamic land model. *Global Biogeochemical Cycles* 24:  
582 GB1001.

583 Hedin, L. O., E. N. J. Brookshire, D. N. L. Menge, and A. R. Barron. 2009. The nitrogen paradox  
584 in tropical forest ecosystems. *Annual Review of Ecology Evolution and Systematics* 40:613-635.

585 Houlton, B. Z., Y. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for  
586 dinitrogen fixation in the terrestrial biosphere. *Nature* 454: 327-330.

587 Hulme, P. E. 1996. Herbivores and the performance of grassland plants: A comparison of  
588 arthropod, mollusk and rodent herbivory. *Journal of Ecology* 84: 43-51.

589 Hungate, B. A., J. S. Dukes, M. R. Shaw, Y. Luo, and C. B. Field. 2003. Nitrogen and climate  
590 change. *Science* 302: 1512-1513.

591 Jenny, H. 1950. Causes of the high nitrogen and organic matter content of certain tropical forest  
592 soils. *Soil Science* 69: 63-69.

593 Knops, J. M. H., M. E. Ritchie, and D. Tilman. 2000. Selective herbivory on a nitrogen fixing  
594 legume (*Lathyrus venosus*) influences productivity and ecosystem nitrogen pools in an oak  
595 savanna. *Écoscience* 7: 166-174.

596 Kurokawa, H., D. A. Peltzer, and D. A. Wardle. 2010. Plant traits, leaf palatability and litter  
597 decomposability for co-occurring woody species differing in invasion status and nitrogen  
598 fixation ability. *Functional Ecology* 24: 513-523.

599 LeBauer, D. S. and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in  
600 terrestrial ecosystems is globally distributed. *Ecology* 89: 371-379.

601 Liao, W. and D. N. L. Menge. 2016. Demography of symbiotic nitrogen-fixing trees explains  
602 their rarity and successional decline in temperate forests. *PLoS ONE* 11: e0164522.

603 Liao, W., D. N. L. Menge, J. W. Lichstein, and G. Ángeles-Pérez. 2017. Global climate change  
604 will increase the abundance of symbiotic nitrogen-fixing trees in much of North America. *Global*  
605 *Change Biology* in press. doi: 10.1111/gcb.13716.

606 McGroddy, M. E., T. Daufresne, and L. O. Hedin. 2004. Scaling of C:N:P stoichiometry in  
607 forests worldwide: implications of terrestrial Redfield-type ratios. *Ecology* 85: 2390-2401.

608 Mead, D. J. and C. M. Preston. 1992. Nitrogen fixation in Sitka alder by <sup>15</sup>N isotope dilution  
609 after eight growing seasons in a lodgepole pine site. *Canadian Journal of Forest Research* 22:  
610 1192-1194.

611 Menge, D. N. L., S. A. Batterman, W. Liao, B. N. Taylor, J. W. Lichstein, and G. Ángeles-Pérez.  
612 2017. Nitrogen-fixing tree abundance in higher-latitude North America is not constrained by  
613 diversity. *Ecology Letters* 20: 842-851.

614 Menge, D. N. L. and R. L. Chazdon. 2016. Higher survival drives the success of nitrogen-fixing  
615 trees through succession in Costa Rican rainforests. *New Phytologist* 209: 965-977.

616 Menge, D. N. L. and T. E. Crews. 2016. Can evolutionary constraints explain the rarity of  
617 nitrogen-fixing trees in high-latitude forests? *New Phytologist* 211: 1195-1201.

618 Menge, D. N. L., J. L. DeNoyer, and J. W. Lichstein. 2010. Phylogenetic constraints do not  
619 explain the rarity of nitrogen-fixing trees in late-successional temperate forests. *PLoS ONE* 5:  
620 e12056.

621 Menge, D. N. L. and L. O. Hedin. 2009. Nitrogen fixation in different biogeochemical niches  
622 along a 120,000-year chronosequence in New Zealand. *Ecology* 90: 2190-2201.

623 Menge, D. N. L., S. A. Levin, and L. O. Hedin. 2008. Evolutionary tradeoffs can select against  
624 nitrogen fixation and thereby maintain nitrogen limitation. *Proceedings of the National Academy*  
625 *of Sciences USA* 105: 1573-1578.

626 Menge, D. N. L., S. A. Levin, and L. O. Hedin. 2009a. Facultative versus obligate nitrogen  
627 fixation strategies and their ecosystem consequences. *American Naturalist* 174: 465-477.

628 Menge, D. N. L., J. W. Lichstein, and G. Ángeles-Pérez. 2014. Nitrogen fixation strategies can  
629 explain the latitudinal shift in nitrogen-fixing tree abundance. *Ecology* 95: 2236-2245.

630 Menge, D. N. L., S. W. Pacala, and L. O. Hedin. 2009b. Emergence and maintenance of nutrient  
631 limitation over multiple timescales in terrestrial ecosystems. *American Naturalist* 173: 164-175.

632 Menge, D. N. L., A. A. Wolf, and J. L. Funk. 2015. Diversity of nitrogen fixation strategies in  
633 Mediterranean legumes. *Nature Plants* 1: 15064.

634 Nasto, M. K., S. Alvarez-Clare, Y. Lekberg, B. W. Sullivan, A. R. Townsend, and C. C.  
635 Cleveland. 2014. Interactions among nitrogen fixation and soil phosphorus acquisition strategies  
636 in lowland tropical rain forests. *Ecology Letters* 17: 1282-1289.

637 Pearson, H. L., and P. M. Vitousek. 2001. Stand dynamics, nitrogen accumulation, and symbiotic  
638 nitrogen fixation in regenerating stands of *Acacia koa*. *Ecological Applications* 11: 1381-1394.

639 Pellegrini, A. F. A., A. C. Staver, L. O. Hedin, T. Charles-Dominique, and A. Tourgee. 2016.  
640 Aridity, not fire, favors nitrogen-fixing plant across tropical savanna and forest biomes. *Ecology*  
641 97: 2177-2183.

642 Prévost, D., H. Antoun, and L. M. Bordeleau. 1987. Effects of low temperature on nitrogenase  
643 activity in sanfoin (*Onobrychis viciifolia*) nodulated by arctic rhizobia. *FEMS Microbiology*  
644 *Ecology* 45: 205-210.

645 Rastetter, E. B., P. M. Vitousek, C. B. Field, G. R. Shaver, D. Herbert, and G. I. Ågren. 2001.  
646 Resource optimization and symbiotic nitrogen fixation. *Ecosystems* 4: 369-388.

647 Ri, X. and I. C. Prentice. 2017. Modelling the demand for new nitrogen fixation by terrestrial  
648 ecosystems. *Biogeosciences* 14: 2003-2017.

649 Ritchie, M. E. and D. Tilman. 1995. Responses of legumes to herbivores and nutrients during  
650 succession on a nitrogen-poor soil. *Ecology* 76: 2648-2655.

651 Ruess, R. W., J. M. McFarland, L. M. Trummer, and J. K. Rohrs-Richey. 2009. Disease-  
652 mediated declines in N-fixation inputs by *Alnus tenuifolia* to early-successional floodplains in  
653 interior and south-central Alaska. *Ecosystems* 12: 489-502.

654 Sheffer, E., S. A. Batterman, S. A. Levin, and L. O. Hedin. 2015. Biome-scale nitrogen fixation  
655 strategies selected by climatic constraints on nitrogen cycle. *Nature Plants*: 15182.

656 Sokolov, A. P., D. W. Kicklighter, J. M. Melillo, B. S. Felzer, C. A. Schlosser, and T. W.  
657 Cronin. 2008. Consequences of considering carbon-nitrogen interactions on the feedbacks  
658 between climate and the terrestrial carbon cycle. *Journal of Climate* 21: 3776-3796.

659 Sprent, J. I. 2009. *Legume Nodulation: A Global Perspective*. Wiley-Blackwell. Ames, IA.

660 ter Steege, H., et al. 2006. Continental-scale patterns of canopy tree composition and function  
661 across Amazonia. *Nature* 443: 444-447.

662 Sullivan, B. W., et al. 2014. Spatially robust estimates of biological nitrogen (N) fixation imply  
663 substantial human alteration of the tropical N cycle. *Proceedings of the National Academy of*  
664 *Sciences USA* 111: 8101-8106.

665 Thornton, P. E., J.-F. Lamarque, N. A. Rosenbloom, and N. M. Mahowald. 2007. Influence of  
666 carbon-nitrogen cycle coupling on land model response to CO<sub>2</sub> fertilization and climate  
667 variability. *Global Biogeochemical Cycles* 21: GB4018.

668 Uliassi, D. D. and R. W. Ruess. 2002. Limitations to symbiotic nitrogen fixation in primary  
669 succession on the Tanana River floodplain. *Ecology* 83: 88-103.

670 Vitousek, P. M. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests.  
671 *Ecology* 65: 285-298.

672 Vitousek, P. M. and J. S. Denslow. 1986. Nitrogen and phosphorus availability in treefall gaps of  
673 a lowland tropical rainforest. *Journal of Ecology* 74, 1167-1178.

674 Vitousek, P. M., et al. 2002. Towards an ecological understanding of biological nitrogen fixation.  
675 *Biogeochemistry* 57: 1-45.

676 Vitousek, P. M. and H. Farrington. 1997. Nutrient limitation and soil development: Experimental  
677 test of a biogeochemical theory. *Biogeochemistry* 37: 63-75.

678 Vitousek, P. M. and C. B. Field. 1999. Ecosystem constraints to symbiotic nitrogen fixers: a  
679 simple model and its implications. *Biogeochemistry* 46: 179-202.

680 Vitousek, P. M. and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it  
681 occur? *Biogeochemistry* 13: 87-115.

682 Vitousek, P. M., D. N. L. Menge, S. C. Reed, and C. C. Cleveland. 2013. Biological nitrogen  
683 fixation: rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical*  
684 *Transactions of the Royal Society B* 368: 20130119.

685 Vitousek, P. M. and R. L. Sanford. 1986. Nutrient cycling in moist tropical forest. *Annual*  
686 *Review of Ecology and Systematics* 17: 137-167.

687 Wang, Y.-P., B. Z. Houlton, and C. B. Field. 2007. A model of biogeochemical cycles of carbon,  
688 nitrogen, and phosphorus including symbiotic nitrogen fixation and phosphatase production.  
689 *Global Biogeochemical Cycles* 21: GB1018.

690 Wårlind, D., B. Smith, T. Hickler, and A. Ameth. 2014. Nitrogen feedbacks increase future  
691 terrestrial ecosystem carbon uptake in an individual-based dynamic vegetation model.  
692 *Biogeosciences* 11: 6131-6146.

693 Wieder, W. R., C. C. Cleveland, D. M. Lawrence, and G. B. Bonan. 2015. Effects of model  
694 structural uncertainty on carbon cycle projections: biological nitrogen fixation as a case study.  
695 *Environmental Research Letters* 10: 044016.

696 Wright, J. J. J. B. Yavitt, N. Wurzbarger, B. L. Turner, E. V. Tanner, E. J. Sayer, L. S. Santiago,  
697 M. Kaspari, L. O. Hedin, K. E. Harms, and M. N. Garcia. 2011. Potassium, phosphorus, or  
698 nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest.  
699 Ecology 92: 1616-1625.  
700 Wolf, A. A., J. L. Funk, and D. N. L. Menge. 2017. The symbionts made me do it: Legumes are  
701 not hardwired for high nitrogen concentrations but incorporate more nitrogen when inoculated.  
702 New Phytologist, 213: 690-699.

### 703 **Figure legends**

704 Figure 1. Structure of the theoretical model and previously proposed trends. Red and blue curves  
705 indicate lower and higher latitudes, respectively. (a) The model is an ecosystem model that tracks  
706 nitrogen (N) in plants and soils, and carbon in plants. Plant growth can be limited by N, by  
707 another implicit resource such as light or phosphorus, or co-limited. (b) Previously proposed  
708 relationships between N fixation and other plant traits. The vertical axis is a relative trait value  
709 axis for each trait, so the absolute value has no meaning. Question marks by traits indicate that  
710 there are mechanisms suggesting both directions (the trait value might increase or decrease with  
711 N fixation). (c)-(e): Previously proposed changes in the relationship between N fixation and plant  
712 traits across latitude. The changes indicated are changes in slope. Although some are shown as  
713 switches in the sign of the slope (e.g., up vs. down), they could also be changes in slope without  
714 a change in sign (e.g., less down vs. more down). See text for specific mechanisms underlying  
715 these trends.

716 Figure 2. Framework for visualizing the mechanisms that can explain persistent N limitation and  
717 the hypotheses that can explain the latitudinal paradox. The horizontal axis, the N limitation  
718 index, is the difference between instantaneous soil N supply and N demand (in units such as kg  
719  $\text{N ha}^{-1} \text{y}^{-1}$ ). It represents N limitation to growth for both non-fixers and N-fixers that are not  
720 fixing, which in our model are equal, and therefore to net primary productivity. The vertical axis  
721 is the frequency of habitats (in proportion of area). Shading indicates that the relative population  
722 growth rate of facultative N-fixers exceeds that of non-fixers. The dashed vertical line at 0, the  
723 co-limitation threshold, corresponds to where N supply matches N demand. Habitats to the right  
724 of the dashed line are N rich and those to the left are N limited. Habitats immediately to the left  
725 of the dashed line are only moderately N limited, whereas those further to the left are more  
726 severely N limited. "SNF" indicates symbiotic N fixation. If N fixation is cost-effective

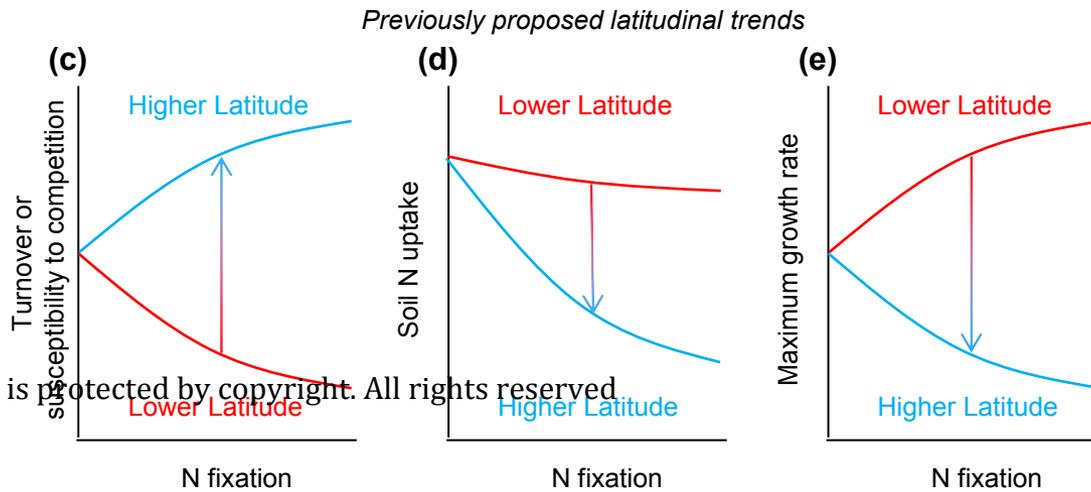
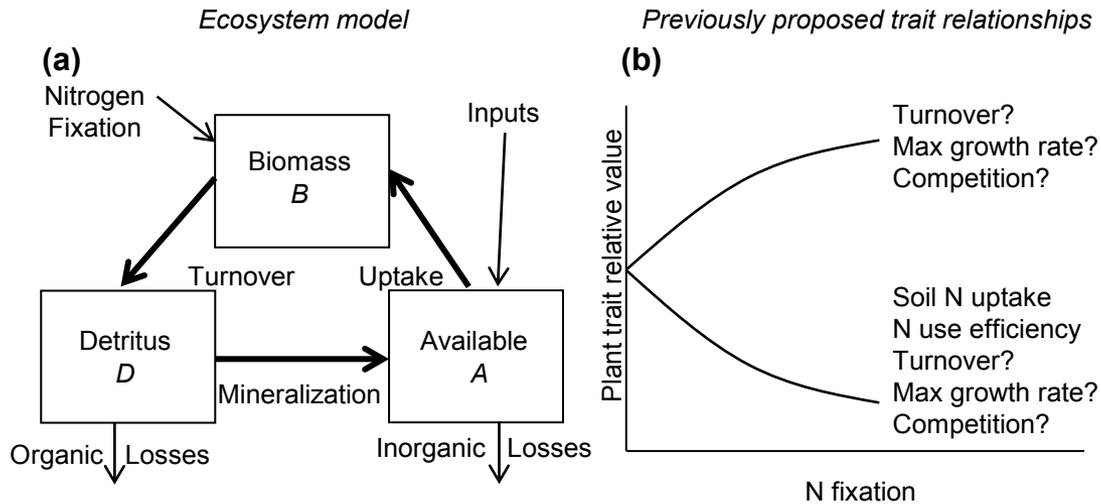
727 whenever N-fixers are N limited, the N supply rate at which the benefits of N fixation equal the  
728 costs of N fixation is the same as the co-limitation threshold (a). Alternatively, if N fixation is  
729 only cost-effective when N limitation is sufficiently severe, there is a benefit-cost threshold to  
730 the left of the co-limitation threshold (b). In panel (a), most of the habitat is N limited and N  
731 fixation is cost-effective whenever N is limiting. Panel (b) shows a scenario where most habitats  
732 are N limited but N fixation is only cost-effective when N limitation is sufficiently severe. This  
733 scenario can arise from a number of mechanisms (see text). Panels represent snapshots in time.  
734 Habitats are not defined at a particular spatial scale; they could be different forests across a  
735 continent or different patches across a forest. Lower latitude distributions and benefit-cost  
736 thresholds are shown in red, higher latitude distributions and benefit-cost thresholds are shown in  
737 blue. Panels (c)-(f) show four hypotheses that can account for greater abundance of N-fixers at  
738 lower latitudes. (c) *N limitation frequency hypothesis*: N limitation is more common at lower  
739 latitudes. (d) *N limitation severity hypothesis*: More severe N limitation is more common at  
740 lower latitudes, even though some degree of N limitation is more common at higher latitudes. (e)  
741 *N fixation benefit-cost hypothesis*: The N fixation benefit-cost threshold is at more moderate N  
742 limitation at lower latitudes, so N fixation is cost-effective across a wider range of N limitation at  
743 lower latitudes. In panels (c)-(e) the benefit-cost thresholds are shown for facultative N fixation  
744 only, whereas in panel (f) different thresholds are shown for facultative and obligate N fixation.  
745 (f) *Differential regulation hypothesis*: Facultative N fixation is more cost-effective than obligate  
746 N fixation (provided there is minimal cost to being facultative), and N-fixers can be somewhat  
747 abundant even in habitats where N fixation is not cost-effective because they turn fixation off  
748 (indicated by pink shading). The facultative and obligate thresholds do not need to correspond to  
749 latitude, so they are written in black.

750 Figure 3. Trends that would support the N fixation benefit-cost hypothesis. The horizontal axis is  
751 the same as Fig. 2. As in Fig. 2c-f, red and blue indicate lower and higher latitudes, respectively.  
752 “SNF” indicates symbiotic N fixation. Supporting the N fixation benefit-cost hypothesis means  
753 increasing the separation between the co-limitation threshold and the N fixation benefit-cost  
754 threshold at higher latitudes compared to lower latitudes (Fig. 2e). Arrows going from red to blue  
755 indicate this increasing separation with latitude. Either raising the co-limitation threshold  
756 (increasing N demand) or lowering the N fixation benefit-cost threshold (making N fixation less  
757 cost-effective) at higher latitudes support the N fixation benefit-cost hypothesis. The top row (N

758 use efficiency) both raises the co-limitation threshold and lowers the N fixation benefit-cost  
759 threshold. The middle row (tradeoffs between N fixation and plant traits) only lowers the N  
760 fixation benefit-cost threshold. The turnover and soil N uptake trends correspond to Fig. 1c, d.  
761 The bottom row only raises the co-limitation threshold. Trends that would support the benefit-  
762 cost hypothesis but are unlikely are crossed out.

763 Figure 4. Conceptual diagram of general hypothesis classes and specific mechanisms to explain  
764 the rarity of N-fixing trees at higher latitudes compared to lower latitudes. Questions are shown  
765 in blue, hypothesis classes in orange, and specific mechanisms for each hypothesis in yellow.  
766 Underlying drivers of specific mechanisms are in parentheses. Crossed out hypotheses are  
767 unlikely. Detailed explanations for each part of this figure can be found in the text.

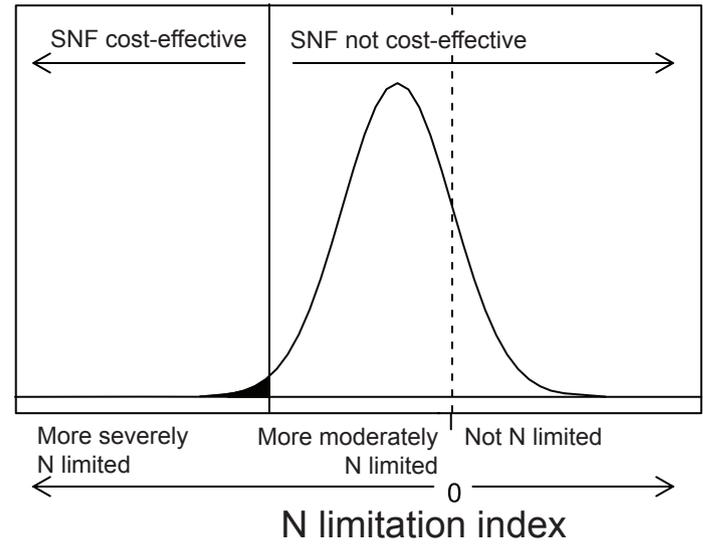
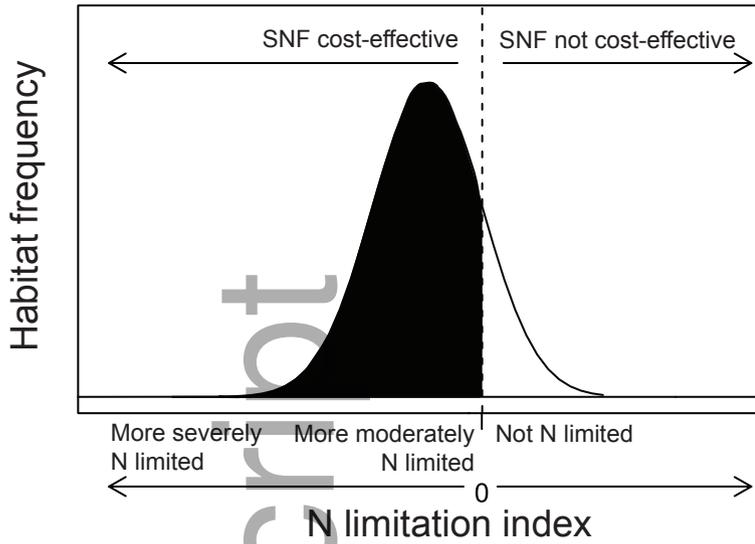
Author Manuscript



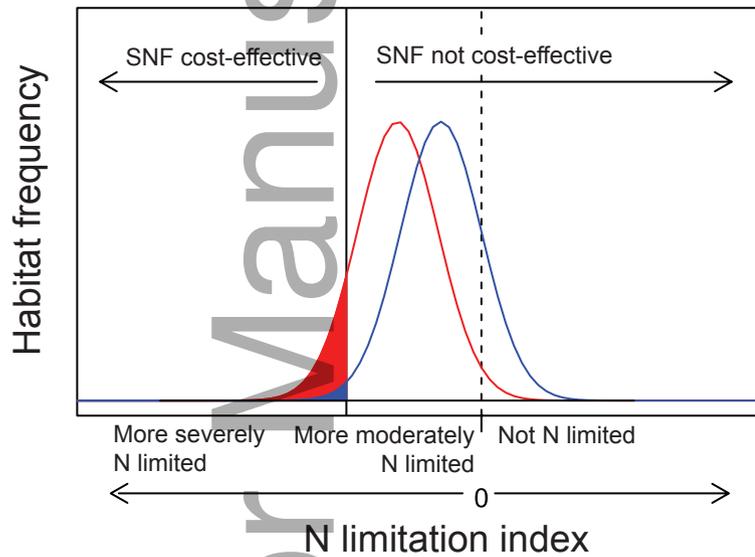
(a) Intuitive scenario

ecy\_2034\_f2

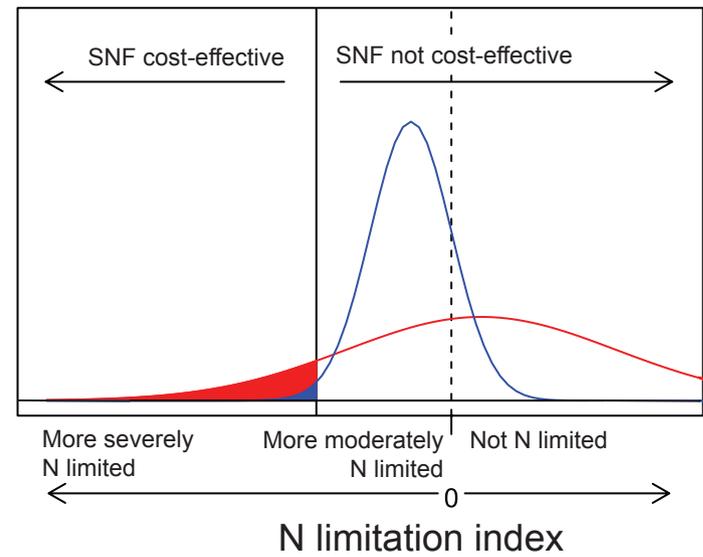
(b) N fixation is only cost-effective under severe N limitation



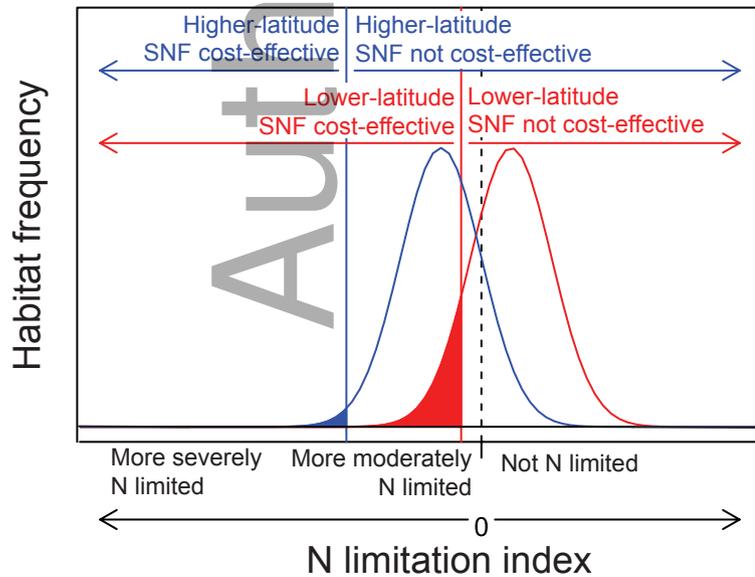
(c) N limitation frequency hypothesis



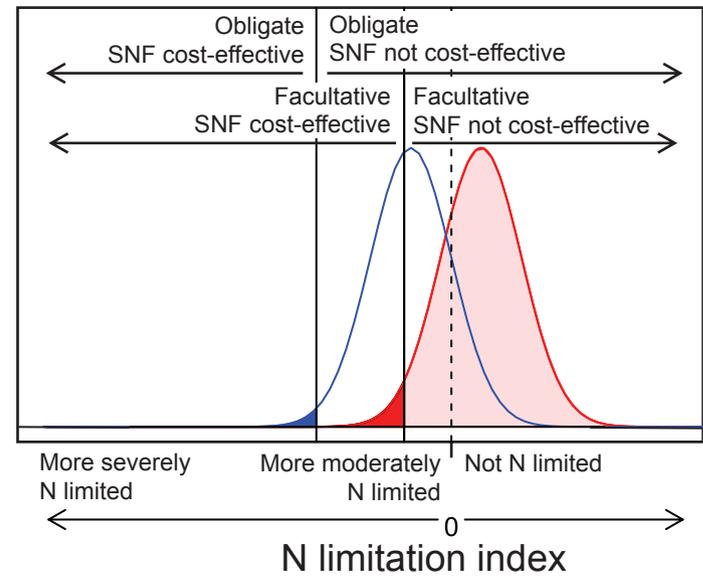
(d) N limitation severity hypothesis

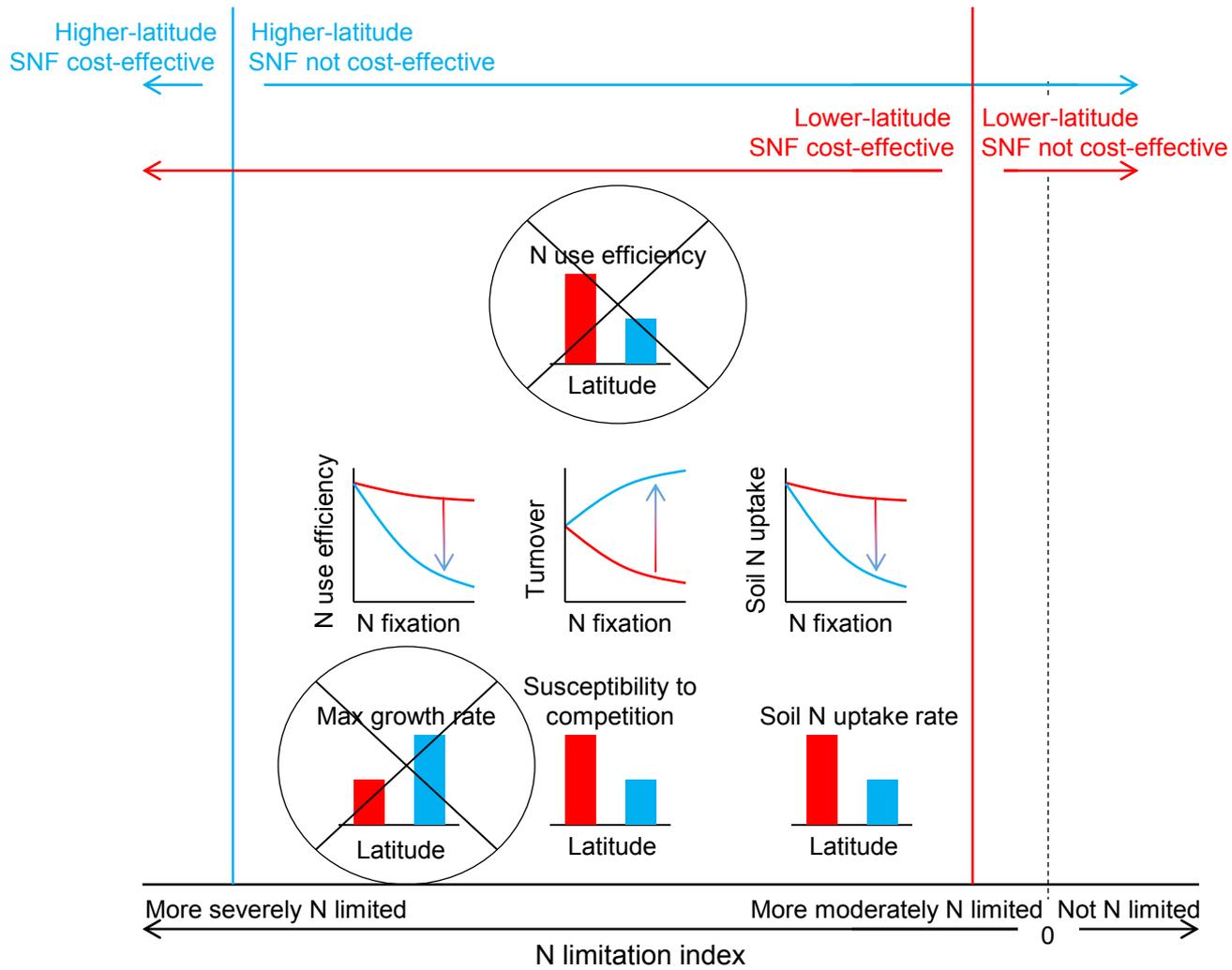


(e) N fixation benefit-cost hypothesis



(f) Differential regulation hypothesis





Why are N-fixing trees rare at higher compared with lower latitudes?

Hypothesis classes

~~Evolutionary constraints hypothesis~~

~~N limitation frequency hypothesis~~

N limitation severity hypothesis

N fixation benefit-cost hypothesis

Differential regulation hypothesis

Specific mechanisms

Lower potential N limitation at higher latitudes because of lower N demand (energy, water)

Lower disturbance-mediated N loss rates at higher latitudes

Stronger N fixation-N uptake tradeoff at higher latitudes (energy)

Stronger N fixation-turnover tradeoff at higher latitudes (herbivory)

Stronger N fixation-N use efficiency tradeoff at higher latitudes

Lower susceptibility to competition at higher latitudes (energy)

Lower N uptake rates at higher latitudes (energy, water)

Time lags constrain facultative N fixation at higher latitudes (energy)

Higher soil C:N ratios at higher latitudes favor obligate N fixation (energy)