

Nitrogen fixation strategies can explain the latitudinal shift in nitrogen-fixing tree abundance

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Abstract. The rarity of symbiotic nitrogen-fixing trees in higher-latitude compared to lower-latitude forests is paradoxical because higher-latitude soils are relatively N poor. Using national-scale forest inventories from the United States and Mexico, we show that the latitudinal abundance distribution of N-fixing trees (more than 10 times less abundant poleward of 35° N) coincides with a latitudinal transition in symbiotic N-fixation type: rhizobial N-fixing trees (which are typically facultative, regulating fixation to meet nutritional demand) dominate equatorward of 35° N, whereas actinorhizal N-fixing trees (typically obligate, maintaining fixation regardless of soil nutrition) dominate to the north. We then use theoretical and statistical models to show that a latitudinal shift in N-fixation strategy (facultative vs. obligate) near 35° N can explain the observed change in N-fixing tree abundance, even if N availability is lower at higher latitudes, because facultative fixation leads to much higher landscape-scale N-fixing tree abundance than obligate fixation.

Key words: actinorhizal N fixation; facultative N fixation; global pattern; legume; obligate N fixation; regulation; rhizobial N fixation; strategy; succession; symbiotic nitrogen fixation.

INTRODUCTION

Symbiotic nitrogen (N) fixation, the conversion of N₂ gas to bioavailable N by symbioses between bacteria and angiosperms, plays a key role in ecosystems (Vitousek et al. 2013). It has the capacity to bring over 100 kg N·ha⁻¹·yr⁻¹ (Binkley et al. 1994) into ecosystems. It also has the capacity to act as a “nitrostat” (Menge and Hedin 2009), ramping up when N is limiting and ramping down when N is not limiting. N availability often limits net primary production (LeBauer and Treseder 2008), so symbiotic N fixation can facilitate plant growth and carbon storage (Batterman et al. 2013). However, symbiotic N fixation can only occur if N-fixing symbioses are present, which is not the case in many ecosystems (all N fixation and N-fixers we discuss in this paper are symbiotic, so we drop the term “symbiotic” hereafter for brevity.) Although progress has been made in understanding the abundance distribution of N-fixers, there is much we do not understand.

In particular, the latitudinal abundance distribution of N-fixing trees presents a paradox. From the plant’s perspective, N fixation should be advantageous in N-limited soils because atmospheric N₂ is ubiquitous, but disadvantageous in non-N-limited soils because N fixation is energetically expensive (Vitousek and

Howarth 1991). Higher-latitude forests are, on average, more N limited than lower-latitude forests (Vitousek and Sanford 1986, Vitousek and Howarth 1991, Hedin et al. 2009, Brookshire et al. 2012a, b, though see LeBauer and Treseder 2008), suggesting that N-fixing trees should be more abundant at higher latitudes. However, the opposite pattern occurs in nature. For example, forest inventories show that N-fixing trees occupy ~1% of total tree basal area in the coterminous United States (Menge et al. 2010), compared with 6–14% in Amazonia (ter Steege et al. 2006). N fixation undoubtedly contributes to the N richness of many tropical forest soils (Hedin et al. 2009), but this does not explain how N-fixing trees maintain high abundance in the N-rich environment they helped to create. Furthermore, the latitudinal pattern presents a major conundrum: why are N-fixing trees less abundant at higher latitudes, where N limitation is presumably more common?

A previous hypothesis to explain the biome-level difference in N-fixing tree abundance focused on the temperature dependence of N fixation and the ability of N-fixers to invest N in phosphatase enzymes (Houlton et al. 2008). This hypothesis is consistent with higher abundance of N-fixing trees in warmer climates, but does not account for N-fixer adaptation to local temperature conditions (Prévost et al. 1987, Poinot et al. 2001), and fails to explain the frequent occurrence of N-fixing herbs, shrubs, and early-successional trees in cold ecosystems (Viereck et al. 1993, Sprent 2009).

Manuscript received 18 November 2013; revised 2 December 2013; accepted 9 January 2014; final version received 3 February 2014. Corresponding Editor: G. S. Gilbert.

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Furthermore, investment in phosphatases is a strategy employed by many plants and microbes (Marklein and Houlton 2012), not just N-fixers. Therefore, a phosphorus advantage for N-fixers is hard to reconcile with excess availability of soil N, which is cheaper for plants to use than N acquired via fixation (Gutschick 1981). This hypothesis also does not distinguish between the two fundamentally different types of N-fixing symbioses.

Rhizobial plants (primarily legumes that form symbioses with α - or β -*Rhizobia* bacteria) and actinorhizal plants (non-legumes that form symbioses with *Frankia*-type bacteria) are both globally distributed (Benson and Dawson 2007, Sprent 2009), yet their abundances vary greatly. Rhizobial trees are common in tropical forests (ter Steege et al. 2006), where they are common throughout succession (Gehring et al. 2008, Batterman et al. 2013), whereas they are rare at higher latitudes (Menge et al. 2010). In contrast, actinorhizal trees are found primarily at higher latitudes and appear to be early-successional specialists (Viereck et al. 1993, Richardson et al. 2004, Menge et al. 2010, but see Vitousek et al. 1987 for presence at lower latitudes).

Rhizobial and actinorhizal trees might employ different strategies of N fixation. Specifically, there is evidence that rhizobial trees are facultative, regulating fixation based on soil N supply relative to individual demand, whereas actinorhizal trees are obligate, maintaining similar fixation rates across the natural range of soil N supply. Our use of the term “obligate” does not suggest that these plants cannot survive without bacterial symbionts, nor that they cannot take up soil N. Rather, obligate here means only that plants continue to fix N at similar rates across the range of soil N supply and N demand they experience in nature (analogous to constitutive enzyme production within a cell). The phrase “across the range of soil N supply they experience in nature” is important, because there is evidence from greenhouse studies that actinorhizal plants downregulate N fixation at higher soil N than is observed in nature (Binkley et al. 1994). Direct evidence of differential regulation comes from observations that rhizobial fixation declines at high soil N availability (Barron et al. 2011) but actinorhizal fixation remains high at high soil N availability (Mead and Preston 1992, Binkley et al. 1994, Menge and Hedin 2009, Chaia and Myrold 2010; Fig. 1). Additional evidence comes from the observation that the percentage of N that N-fixers derive from fixation is less variable and higher in actinorhizal compared to rhizobial N-fixers (Andrews et al. 2011; Appendix B: Fig. B1).

Unfortunately, the cited field data suggesting obligate vs. facultative N fixation are confounded by location. The evidence for rhizobial trees being facultative comes from lower latitudes, whereas the evidence for actinorhizal trees being obligate comes from higher latitudes. Therefore, it is not clear whether the strategy difference stems from environmental factors, phylogenetic factors, or both. One piece of evidence that might suggest a

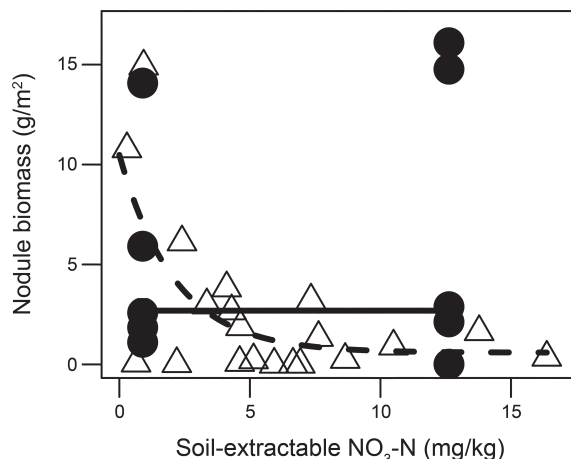


FIG. 1. Field evidence for differential regulation of symbiotic nitrogen fixation from Menge and Hedin (2009) and Barron et al. (2011). Solid circles and line represent the actinorhizal tree *Coriaria arborea* in the most N-poor and N-rich sites along a New Zealand forest chronosequence (43° S; Menge and Hedin 2009). Each point represents 10 400 cm² × 15 cm soil pits ($N = 5$ per site). Open triangles and dashed line represent the rhizobial tree genus *Inga* in the Barro Colorado Nature Monument, Panama (9° N; Barron et al. 2011). Each point represents a single tree ($N = 20$). Fits are from the published works. In each case, there was no trend in nitrogenase activity per nodule biomass, so N-fixation rates were proportional to nodule biomass.

phylogenetic association is that actinorhizal plants have different nodule morphologies corresponding to a lower ability to regulate nodule oxygen content (Tjepkema 1988); oxygen regulation is one of the key mechanisms used by rhizobial plants (Kiers et al. 2003). However, environmental factors might also play a role. Colder temperatures should lead to longer time lags in regulating N fixation, which would favor an obligate strategy (Menge et al. 2009). Shorter growing seasons might favor obligate N fixation if the annual costs of being facultative (e.g., building and maintaining the infrastructure required to regulate N fixation [Menge et al. 2009]) depend less strongly on growing season length than the energy supply (net photosynthesis) needed to pay these costs. One possibility, therefore, is that actinorhizal plants are predisposed to obligate N fixation for phylogenetic reasons, so they have higher fitness than rhizobial plants in environments that favor an obligate strategy. Regardless of the underlying driver, it is reasonable to ask what role a strategy difference might play in controlling the latitudinal abundance distribution of N-fixing trees.

Here, we present the first large-scale, systematic analysis of N-fixing tree abundance spanning the extratropical-to-tropical transition by combining national forest inventories from the United States and Mexico. We use these data to quantify (1) N-fixing tree abundance across latitude, (2) the relative dominance of the two symbiotic tree types (actinorhizal vs. rhizobial)

across latitude, and (3) the relationship between symbiotic type dominance and overall N-fixing tree abundance. We then use theoretical and statistical models to develop a new hypothesis. This differential regulation hypothesis states that the observed latitudinal abundance distribution of N-fixing trees can be explained by a latitudinal shift in the N-fixation strategy (facultative vs. obligate), even if there is a poleward increase in N limitation. The differential regulation hypothesis also explains the prevalence of early-successional N-fixing trees at higher latitudes.

METHODS

Forest inventory data

Data come from the U.S. Forest Service's Forest Inventory and Analysis (FIA), version 4.0 (data available online),⁵ and the Mexican Comisión Nacional Forestal's Inventario Nacional Forestal y de Suelos (INFiS; see Plate 1) 2004–2007. Sample plots were located systematically across the coterminous United States (one randomly located plot per ~2400 ha forested land) and Mexico (one per ~2500 ha). Trees were measured at diameter at breast height (dbh); 4.5 ft above ground for FIA, 1.3 m above ground for INFiS. Trees ≥ 12.7 cm (FIA) or 7.5 cm (INFiS) dbh were identified to species and measured on four subplots per plot (7.3 m [FIA] or 11.3 m [INFiS] radius); saplings (dbh 2.54–12.7 cm) were measured on four 2.1 m radius subplots (FIA). Results shown here exclude trees with dbh < 7.5 cm to ensure that different protocols do not influence results, although the results are nearly identical if these small saplings are included. We also excluded plantations, leaving 79 508 plots and 2 046 221 trees in the FIA and 15 358 plots and 1 148 474 trees in the INFiS. FIA and INFiS data span 25.4–49.3° N and 14.6–32.5° N latitude, respectively.

We classified species as N-fixers, non-fixers, or unknown using published reports (Huss-Danell [1997] for actinorhizal, Sprent [2009] for rhizobial) and, when additional information for rhizobial species was needed, the U.S. Department of Agriculture GRIN database (information available online).⁶ Species with no recorded N-fixing status (unknown) were classified as N-fixers if $\geq 60\%$ of reported congeners were N-fixers (according to Huss-Danell [1997], Sprent [2009], or GRIN) and non-fixers otherwise (any cutoff between 10–90% yielded nearly identical classifications). Although the GRIN database is likely to contain some errors (Sprent 2009), it is the best resource for filling database gaps in a large-scale study. Of the 180 750 individual trees in our database that were classified as N-fixing trees, 68% belong to species whose nodulation status is known from the most reliable sources (Huss-Danell 1997, Sprent 2009), 7% were classified as N-fixing trees based

directly on GRIN, and 25% were classified as N-fixing trees because $\geq 60\%$ of reported congeners were N-fixers.

Successional dynamics model

We used a deterministic theoretical model to predict successional trajectories of obligate or facultative N-fixers competing with non-fixers. This successional dynamics model, originally developed to examine biogeochemical consequences of N-fixation strategies (Hedin et al. 2009, Menge et al. 2009), uses differential equations to track plant biomass (counted in units of foliar C) and two soil nutrients (N and phosphorus) in two forms, plant-available (e.g., nitrate) and plant-unavailable (e.g., complex organic molecules). Obligate fixers fix N at a constant rate, whereas facultative fixers adjust fixation to balance nutrition when possible. Fixing N costs more than acquiring soil N if soil N is abundant, as in real plants (Gutschick 1981). Additionally, facultative fixers pay costs to be facultative (van Kleunen and Fischer 2005, Menge et al. 2009). All plants in our model take up plant-available soil N. The successional dynamics model includes abiotic nutrient inputs, losses from soil pools, and nutrient recycling.

Equations and parameter values come from Menge et al. (2009), with the following specifics and modifications. Eqs. 1–6 from Menge et al. (2009) were used for all simulations, modified to include a constant cost of being facultative (expressed as a turnover rate; γ in Eq. 10, $\gamma = 0.036$ per yr). There is very little direct information on γ , so we chose this value based on the other turnover rates (it increases plant turnover rate by 4%). Additionally, we evaluated how uncertainty in this cost affects our successional model results. The obligate N fixation rate was $0.03 \text{ kg N} \cdot (\text{kg foliar C})^{-1} \cdot \text{yr}^{-1}$, whereas the instantaneous facultative N fixation rate was set to balance nutrition (Eq. 7) unless the optimal rate was negative or $> 0.03 \text{ kg N} \cdot (\text{kg foliar C})^{-1} \cdot \text{yr}^{-1}$ (in these cases, respectively, it was set to 0 and $0.03 \text{ kg N} \cdot (\text{kg foliar C})^{-1} \cdot \text{yr}^{-1}$). The obligate N-fixation rate was chosen to represent an average N-fixation flux for actinorhizal trees: $0.03 \text{ kg N} \cdot (\text{kg foliar C})^{-1} \cdot \text{yr}^{-1}$ translates into $30 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ for 1 (Mg foliar C) $\cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ (the maximum fixer foliar biomass in our simulations; comparable to *Alnus rubra* foliar biomass [Binkley et al. 1992]). This is between estimates for *Alnus rubra* (Binkley et al. 1994) and *Coriaria arborea* (Menge and Hedin 2009).

We examined successional trajectories in three habitats; severely, moderately, and non-N-limited. In this model, N-limited vs. non-N-limited is defined by the equilibrium soil N status in the absence of N fixation, but we note that the limiting nutrient remains the same throughout succession in each simulation if N fixation is excluded. Severely vs. moderately N-limited habitats are distinguished from each other by starting conditions, and thus are transient phenomena. Starting conditions (per ha) for the severely N-limited habitat were 10 kg foliar C biomass for each plant type, 1 and 5 kg plant-

⁵ <http://www.fia.fs.fed.us/>

⁶ <http://www.ars-grin.gov/~sbmljw/cgi-bin/taxnodul.pl>

unavailable N and P, and 0.001 and 0.1 kg plant-available N and P. These conditions, meant to represent a 1-yr-old primary successional site, were estimated from the youngest (5-yr-old) primary successional site in the Franz Josef chronosequence (Richardson et al. 2004). Starting conditions for the moderately N-limited and non-N-limited habitats, approximating early secondary succession following a moderate disturbance, were 1000 kg foliar C biomass for each plant type, 50 and 5 kg plant-unavailable N and P, and 1 and 0.1 kg plant-available N and P. These values represent significant increases in the C and N stocks compared to the severely N-limited scenario. All parameters were the same for severely and moderately N-limited habitats, yielding N limitation in the absence of N fixation, whereas the non-N-limited habitat had a higher abiotic N input flux. Simulations were numerically integrated using the ode45 function in Matlab R2010b (MathWorks, Natick, Massachusetts, USA). Succession was run to 400 yr, which includes the overwhelming majority of FIA stand ages. Other reasonable parameter values and starting conditions gave similar results.

Landscape-scale abundances of the different N-fixing types in different habitats were derived from the successional dynamics model and used as inputs to the latitudinal abundance model (see *Latitudinal abundance model*). To translate successional trajectories from the successional dynamics model into landscape-level abundances, we weighted successional trajectories by the age distribution of FIA plots (Appendix B: Fig. B2) as well as several alternative age distributions.

Latitudinal abundance model

A key result of the successional dynamics model is that facultative N-fixing trees are common at the landscape scale whereas obligate N-fixing trees are rare. It follows that a latitudinal transition in N-fixation strategy (i.e., the proportion of fixation that is facultative vs. obligate) might help explain a latitudinal transition in N-fixing abundance, but the precise latitudinal strategy distribution, as well as the latitudinal distribution of habitats (severely, moderately, or non-N-limited), that yield good fits to the observed latitudinal N-fixing abundance pattern are unknown. Thus, the purpose of our latitudinal abundance model is to investigate which combination of latitudinal patterns of N-fixation strategy and habitat best explain the latitudinal abundance distribution of N-fixing trees.

To calculate predicted N-fixing abundance at each latitude, we first calculated the product of (1) successional biomass trajectories from the successional dynamics model for each strategy in each habitat and (2) the FIA stand-age distribution. This product (Appendix A: Table A1) was then weighted by (3) the latitudinal habitat distribution (percentage of the landscape comprised of severely, moderately, or non-N-limited habitats at different latitudes) and (4) the latitudinal strategy distribution (percentage of N-fixing trees that are

facultative vs. obligate at different latitudes). These calculations yielded a predicted latitudinal distribution of N-fixing tree abundance that was compared to the observed N-fixing tree abundance distribution from forest inventory data. Components 1 and 2 were inputs to the latitudinal abundance model, whereas components 3 and 4 were fitted functions that allowed us to assess which latitudinal distributions of habitat and N-fixation strategies best fit N-fixing tree abundance data. Due to the similar abundances in severely and moderately N-limited habitats, the latitudinal abundance model was under-constrained when it included all three habitats, so we used versions of the model with only two habitats: non-N limited and either severely or moderately N limited.

The latitudinal distribution of N-fixing tree basal area is given by

$$BA = O \times (N \times (BA | O, N) + M \times (BA | O, M)) \\ + F \times (N \times (BA | F, N) + M \times (BA | F, M)) \quad (1)$$

where O , F , N , and M denote the latitudinal distributions of percentages of N-fixing trees that are obligate (O) or facultative ($F = 1 - O$), and habitat that is N limited (N) or non-N limited ($M = 1 - N$). The $BA | S, H$ terms indicate N-fixing tree basal area given fixer strategy S (O or F) and habitat H (N or M) in Appendix A: Table A1. Latitudinal distributions were assumed to be the sigmoid functions

$$O(\text{latitude}) = s_1 + (s_2 - s_1) \\ \div \left(1 + \exp \left(-s_3 (\text{latitude} - s_4) \right) \right) \quad (2)$$

$$N(\text{latitude}) = h_1 + (h_2 - h_1) \\ \div \left(1 + \exp \left(-h_3 (\text{latitude} - h_4) \right) \right) \quad (3)$$

where the parameters are the minimum and maximum percentages (s_1 , s_2 , h_1 , and h_2 ; constrained between 0–100%), the maximum steepness (s_3 , h_3 , the signs of which determine whether s_1 vs. s_2 and h_1 vs. h_2 are the maxima or minima), and the latitudinal inflection point (s_4 , h_4). The assumption of a sigmoid function does not imply an increase or a decrease with latitude. The curves in Eqs. 2 and 3 can rise or fall with latitude depending on values of s_1 – s_3 and h_1 – h_3 . Additionally, these functions allow linear, concave, convex, or sigmoid shapes across the observed latitudinal range (15°–49° N), so the assumed sigmoid functions place minimal restrictions on the analysis.

The model was under-constrained by the abundance data, so we introduced additional constraints to allow convergence. We considered different versions of these constraints to assure that our qualitative results were robust (described in *Results* and Appendices A and B). Our base case assumed that the obligate N-fixing tree

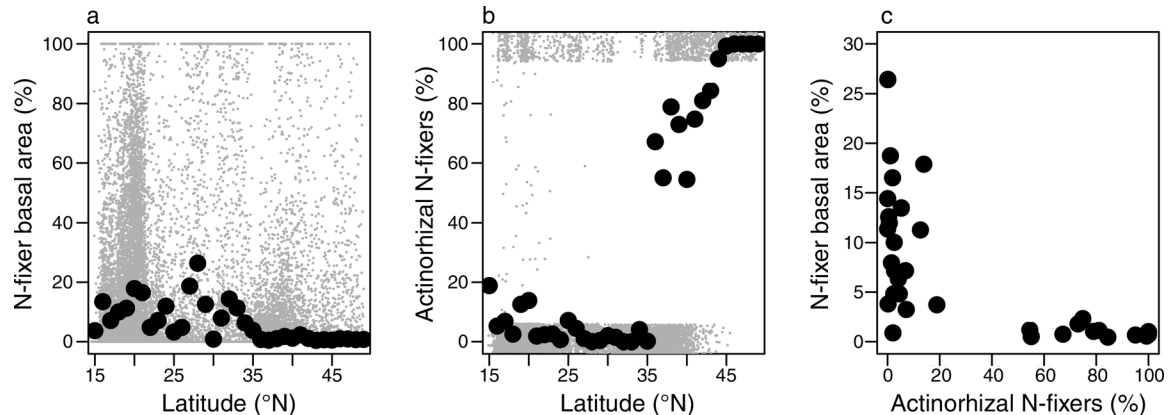


FIG. 2. Latitudinal distributions of N-fixing tree abundance and symbiotic type. Data are from systematic national forest inventories from the coterminous United States and Mexico. Gray dots denote inventory plots (of which there are 94 866) and black circles denote mean values of 1° grid cells in 1° latitudinal bands (of which there are 35). (a) Latitudinal pattern of N-fixing tree abundance. (b) Latitudinal pattern of dominance of actinorhizal vs. rhizobial N-fixing trees, defined as the percent of N-fixing basal area that is actinorhizal. Gray dots are vertically jittered for visual clarity; 99.5% of values are 0% or 100% actinorhizal. (c) N-fixing tree abundance as a function of actinorhizal dominance at the 1° latitude scale.

percentage increased with latitude ($s_1 = 0\%$, $s_2 = 100\%$, $s_3 = 1$ per degree latitude), although we did not specify where the transition occurred (s_4 was free). This is akin to asking “if there is a latitudinal increase in obligate N fixation, what combination of strategy, transition latitude (parameter s_4), and latitudinal abundance distribution of habitat N limitation (parameters h_1 – h_4) best explains the N-fixing tree abundance data?” Various versions of the latitudinal abundance model with different numbers of free s and h parameters (including cases where s_3 was free, i.e., percentage of N-fixing trees that are obligate was not assumed to rise with latitude) were fit with the nls function in R (R Development Core Team 2009). We then used AIC_c (Akaike’s information criterion, corrected for finite sample sizes; Anderson 2008) to compare the different latitudinal abundance model versions against each other and against standard sigmoid and linear regressions of N-fixing tree abundance vs. latitude. The standard sigmoid and linear regressions do not represent any specific hypotheses regarding the latitudinal abundance distribution, but simply provide benchmarks against which to compare the fit of our latitudinal abundance model, which is based on our differential regulation hypothesis. 95% confidence intervals on each model-fit curve were estimated by generating 1000 random draws from the approximate multivariate normal parameter distribution (Bolker 2008).

Because we do not have data for the stand-age distribution in Mexico, we ran the latitudinal abundance model with alternate stand-age distributions (United States distribution with ages halved and doubled) below 35° N latitude, which gave slightly different successional-weighted N-fixing tree abundances (Appendix A: Table A1). Additionally, we ran the latitudinal abundance model with $s_1 = 10\%$ rather than 0% to account for the possibility that some obligate N-fixers exist at all

latitudes. Source code files (in R and Matlab) for models are in the Supplement.

RESULTS

N-fixing tree abundance and dominance of symbiotic types

Forest service data from the United States and Mexico reveal an abundance threshold near 35° N latitude (Fig. 2a), substantially poleward of the tropical–temperate divide, with N-fixing trees occupying ~0.7% of basal area poleward and ~10% equatorward. Additionally, these data show a corresponding distribution of the two types of N-fixing symbioses: actinorhizal trees dominate the N-fixing tree community poleward of 35° N, whereas rhizobial trees dominate the N-fixing tree community equatorward of 35° N (Fig. 2b, c). Co-occurrence of the symbiotic types is also rare at finer scales; Mexican actinorhizal trees occur predominantly at high elevation (Appendix B: Fig. B3), and the two types co-occur in 63 of the 11 816 inventory plots that contain N-fixing trees (Fig. 2b).

Successional dynamics model

In the severely N-limited habitat, obligate N-fixing trees dominate early in successional simulations when soil N is negligible (Fig. 3a). However, N fixation and subsequent litterfall increase soil N, and obligate N-fixing trees are then displaced by non-fixing trees that do not pay fixation costs and are thus more competitive under high-N conditions. In the moderately N-limited habitat, displacement of obligate N-fixing trees occurs more rapidly due to the higher initial soil N supply (Fig. 3b). Facultative N-fixing trees dominate both N-limited habitats (Fig. 3 c, d), despite paying a cost for fixing N and an additional cost for being facultative, because they only fix as much N as they need, and therefore do not fertilize non-fixers excessively or waste energy.

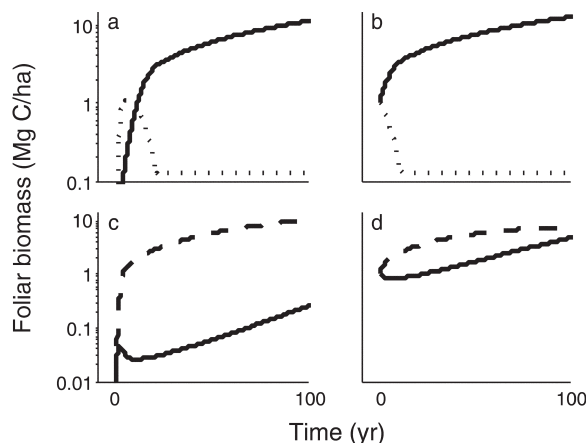


FIG. 3. Successional trajectories predicted by our successional dynamics model. Each panel shows a simulation of competition between N-fixing (dashed or dotted lines) and non-fixing tree populations (solid lines) in (a and c) severely and (b and d) moderately N-limited environments. (a and b) Obligate N-fixing trees (dotted lines) that cannot downregulate N fixation dominate early succession in (a) severely N-limited environments, but are constrained to relatively low biomass throughout succession in (b) moderately N-limited environments. (c and d) Facultative N-fixing trees dominate (c) severely and (d) moderately N-limited environments throughout succession. Habitat names (e.g., severely N-limited environments, moderately N-limited environments) denote limitation in the absence of N fixation. Our plant parameter values (e.g., stoichiometry, turnover) reflect foliage rather than total biomass, so biomass output numbers reflect foliar rather than total biomass. Note the logarithmic vertical scale.

Neither obligate nor facultative N-fixing trees became established in the non-N-limited habitat.

In terms of understanding the latitudinal abundance pattern of N-fixing trees, the key prediction from this theory is that, averaging across succession in N-limited habitats, obligate N-fixing trees are rare, but facultative N-fixing trees are abundant. If we weight the successional trajectories in Fig. 3 by the age distribution of United States forests (Appendix B: Fig. B2), then predicted landscape-scale N-fixing tree abundance in N-limited habitats is $\sim 2\%$ of biomass for obligate vs. $\sim 75\%$ for facultative strategies (Appendix A: Table A1).

A strategy transition can explain the latitudinal abundance distribution

The N-fixation strategy exerts enormous leverage over predicted landscape-scale N-fixing tree abundance (Appendix A: Table A1), so a strategy shift can explain the poleward decrease in N-fixing tree abundance (Fig. 2a) despite a poleward increase in N limitation (or a wide range of limitation trends, Fig. 4b, Appendix B: Figs. B4b–B7b). According to our latitudinal abundance model, two conditions are sufficient to explain the latitudinal N-fixing tree abundance distribution: (1) a transition from facultative to obligate fixation near 35° N (Fig. 4a) and (2) at least some N-limited habitat at lower latitudes (Fig. 4b).

The success of our latitudinal abundance model in predicting the latitudinal abundance pattern is insensitive to our specific assumptions regarding the steepness of the strategy curve (Appendix B: Fig. B4), the age distribution of lower-latitude forests (Appendix B: Figs. B5, B6), and the absence of obligate N-fixing trees at lower latitudes (Appendix B: Fig. B7). For example, a version of the model that assumes that 10% of tropical N-fixing trees are obligate (rather than 0%, as in Fig. 4) yields nearly identical results (Appendix B: Fig. B7).

The two best fit models (both 0.03 AIC_c units better than the nonmechanistic sigmoid fit and 2.7 units better than any other model we tried), shown in Fig. 4, had the steepness of each curve fixed at 1. Fits with s_3 fit as a free parameter also fit the data well (Appendix B: Fig. B4), although their AIC_c values were 2.7 units worse than those in Fig. 4. Despite the AIC_c increase, the fits in Appendix B: Fig. B4 are important because we made no

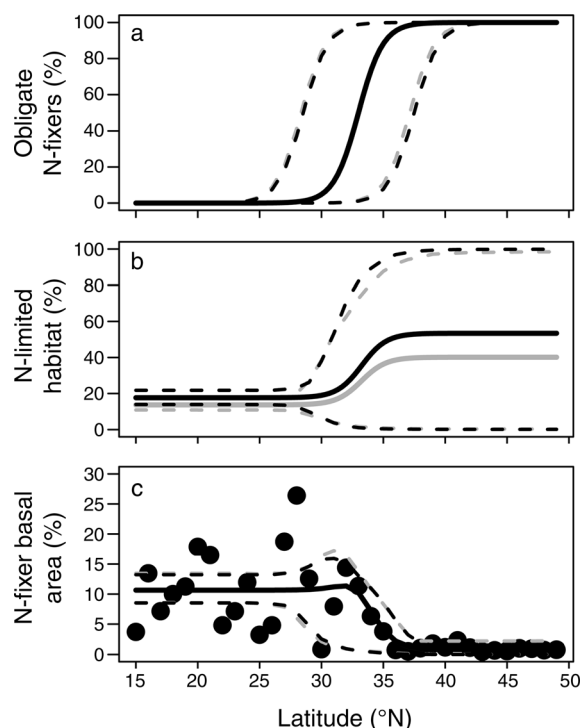


FIG. 4. Modeled latitudinal distribution of N-fixing tree abundance. The two best-fit versions of our latitudinal abundance model, which assume all N-limited habitat is severely (gray) or moderately (black) N limited, are shown on each panel. Solid lines are maximum-likelihood fits and dashed lines are 95% CIs. (a) Model-fit N fixation strategy distribution, shown as the percentage of N-fixing trees that are obligate. (b) Model-fit habitat distribution. Habitat names (i.e. N-limited vs. non-N-limited) denote limitation in the absence of N fixation. (c) Model-fit distribution of N-fixing tree abundance (shown as N-fixers as a percentage of total basal area), which combines the (a) strategy and (b) habitat distributions with the theoretically predicted landscape-scale abundance for each habitat–strategy combination (Appendix A: Table A1). Black circles are 1° latitudinal means (from Fig. 2a) to which statistical models were fit ($N = 35$).



PLATE 1. A forest in the Yucatan Peninsula, where legume trees capable of fixing nitrogen are often dominant. Seed pods are visible on the tree in the foreground. Photo credit: G. Angeles-Pérez.

assumptions about the strategy curve slope; the percentage of N-fixing trees that are obligate and the percentage of habitat that is N limited are both predicted by the statistical fit to increase with latitude. Maximum likelihood parameter estimates for the best fit model (with 95% CI) for the model with all N-limited habitat being severely N limited were $h_1 = 14\%$ (11–17%), $h_2 = 40\%$ (0–99%), $h_4 = 33^\circ \text{ N}$ (29–38° N), and $s_4 = 33^\circ \text{ N}$ (29–37° N). For the model with all N-limited habitat being moderately N limited they were $h_1 = 18\%$ (14–22%), $h_2 = 53\%$ (0–100%), $h_4 = 33^\circ \text{ N}$ (29–38° N), and $s_4 = 33^\circ \text{ N}$ (29–37° N). Analyses investigating sensitivity to stand age and minimum percentage of obligate N-fixing trees gave similar parameter estimates, although the habitat confidence intervals at lower latitudes were larger in the doubled age case (Appendix B: Figs. B5–B7).

DISCUSSION

Mexican and United States forest inventory data show that N-fixing trees are 10 times more abundant south of 35° N than they are to the north, and that the abundance transition is accompanied by a transition in the dominant symbiotic type. Our analysis suggests that a single phenomenon, a transition in the dominant N fixation strategy near 35° N latitude, can explain the 10-fold change in N-fixing tree abundance, because of the

large effect of strategy type on landscape-level abundance of N-fixing trees. Furthermore, a strategy transition can explain the abundance pattern even if there is a poleward increase in N limitation. Finally, our theoretical predictions for the successional patterns of obligate (Fig. 3a, b) and facultative (Fig. 3c, d) N-fixing trees match observed patterns in higher- and lower-latitude forests, respectively. N-fixing trees are largely confined to early succession at higher latitudes (Fig. 3a, b; Viereck et al. 1993, Richardson et al. 2004, Menge et al. 2010) but persist throughout succession at lower latitudes (Fig. 3c, d; Gehring et al. 2008, Batterman et al. 2013).

The best statistical fits to the N-fixing tree abundance data included poleward increases in N limitation (Fig. 4; Appendix B: Figs. B4–B7). This result provides a resolution to the paradox presented in the introduction: even if underlying biogeochemical factors produce an increase in N limitation at higher latitudes, N-fixation strategy has sufficient influence on plant fitness to cause a poleward decrease in N-fixing tree abundance. Our analysis did not assume that N limitation increases toward the poles; rather, this emerged as the most likely of a number of possible scenarios. The fact that the best fit included an increase in N limitation at higher latitudes, as seems to occur in forests in nature (Vitousek

and Howarth 1991, Hedin et al. 2009), lends credence to the differential regulation hypothesis.

The details of the statistical analysis provide a richer picture of N limitation and N fixation across latitude. At lower latitudes, our analysis suggests that ~16% of the landscape needs to be N limited to explain the observed N-fixing tree abundance (~10% of total tree basal area). Fertilization studies reveal that N limitation occurs in some tropical forests (LeBauer and Treseder 2008), despite the overall perception that most tropical forests are not N limited (Vitousek and Sanford 1986, Hedin et al. 2009, Brookshire et al. 2012a, b). Furthermore, small-scale gap dynamics might create pockets of N limitation where N-fixers thrive (Barron et al. 2011) in an otherwise N-rich landscape. These lines of evidence provide support for our statistical inference that at least some N-limited habitat occurs in lower-latitude forests. Because N limitation in our models is defined in the absence of N fixation, and because fixation can overcome this underlying N limitation, a habitat classified as N limited in our models might not be N limited with N-fixers present. Therefore, even if fewer than 16% of lower-latitude habitats appeared N limited empirically, this would not be inconsistent with our results. Moreover, given that facultative N-fixing trees dominate N-limited landscapes in our simulations, our analysis suggests that the relatively high abundance of N-fixing trees at lower latitudes (10%) could be much higher if underlying conditions favored more N limitation.

At higher latitudes, our best statistical fit implies that about half of habitats are N limited, but the confidence intervals are at least as interesting as the most likely fit. According to our analysis, obligate N-fixing trees are sufficiently rare at the landscape scale, even in severely N-limited habitats, that nearly the entire range of habitat possibilities (<1 to >99% N limited) is consistent with low landscape-level N-fixing tree abundance (Fig. 4b) when obligate N-fixing trees dominate the N-fixing community. Therefore, the perception that most higher-latitude habitats are N limited is consistent with our analysis, but a large range of possibilities would be as well.

As explained in the introduction, there is evidence for a relationship between symbiotic type (actinorhizal vs. rhizobial) and N-fixation strategy (facultative vs. obligate). Two new results reported here provide additional, indirect support for this relationship: (1) the latitudinal strategy transition that best fits the abundance data is similar to the latitudinal transition from rhizobial to actinorhizal dominance (Figs. 2b, 4a), and (2) mean N-fixing tree abundance is always low at latitudes where actinorhizals dominate (Fig. 2c). However, a relationship between symbiotic type and N-fixation strategy is not an essential component of the differential regulation hypothesis. If some lower-latitude actinorhizal species were facultative, or if some rhizobial species from any latitude were obligate, the hypothesis would still hold.

The key requirements for the hypothesis are that higher-latitude N-fixing trees be obligate across the range of conditions they experience in the field, and that some lower-latitude N-fixing trees be facultative.

One of the model parameters that merits exploration is the cost of being facultative. There is a large body of literature (Pigliucci 2005, van Kleunen and Fischer 2005, Auld et al. 2010) suggesting that physiological plasticity (equivalent to our facultative strategy) carries costs. As a loose proof by contradiction, if plasticity were not costly, all traits should be infinitely plastic, which is clearly not true. There is empirical evidence for trait plasticity being costly in realms outside of N fixation (van Kleunen and Fischer 2005), but these costs are difficult to quantify. In our successional dynamics model, we assumed that being facultative carries costs, but we know of no estimates of this cost. Therefore, we conducted a sensitivity analysis to this cost by running our successional dynamics model with all three types (facultative N-fixing, obligate N-fixing, and non-fixing trees) competing, with everything else the same as in the original simulations except that the costs of being facultative varied. As costs increase, non-fixers occupy a larger share of the N-limited habitat (Appendix B: Fig. B8a, b) and obligate N-fixing trees occupy a larger share of the N-fixing tree community (Appendix B: Fig. B8c, d).

These results are not surprising, but two features of the analysis are intriguing. First, the sigmoid response in Appendix B: Fig. B8c, d resembles the observed latitudinal transition from rhizobial to actinorhizal N-fixing trees (Fig. 2b), showing that a gradual change in the cost of being facultative with latitude (responding to temperature or growing season, for instance) could produce the observed sigmoid dominance trend. Second, the sensitivity of N-fixing tree abundance to the cost of being facultative (Appendix B: Fig. B8a, b) combined with uncertainty about the cost suggests two things: (1) the confidence intervals on the habitat at lower latitudes should be wider than in the results we report, and (2) if costs of being facultative are substantial, overall N-fixing tree abundance at higher latitudes can be low even if a significant fraction of N-fixing trees are facultative. Therefore, although the presence of facultative N-fixing trees at higher latitudes would reject the differential regulation hypothesis as laid out in *Introduction*, it would still be consistent with low overall N-fixing tree abundance if the cost of being facultative were relatively high at higher latitudes. This suggests, as an alternative to the differential regulation hypothesis, a cost-of-regulation hypothesis: the observed latitudinal abundance distribution of N-fixing trees can be explained by a latitudinal shift in the cost of regulating N fixation, even if there is a poleward increase in N limitation.

The differential regulation hypothesis (and the cost-of-regulation hypothesis) can explain the latitudinal N-fixing tree abundance trend under a wide variety of conditions, but they are not the only explanations. The

combination of temperature constraints on N fixation and a greater ability of N-fixers to produce phosphatase enzymes (Houlton et al. 2008) can also explain the trend. The historical biogeographic distribution of woody legumes has been mentioned as a potential constraint (Crews 1999), though the prevalence of non-woody legumes (Sprent 2009), actinorhizal plants (Fig. 2b), and trees closely related to N-fixers (Menge et al. 2010) suggest that historical constraints are unlikely to explain low N-fixing tree abundance at higher latitudes. Regardless, these explanations are not mutually exclusive, so evidence for one does not disprove the others. To our knowledge, though, the differential (and cost of) regulation hypotheses are the only hypotheses that capture not only the latitudinal abundance pattern, but also the latitudinal differences in succession described in the introduction (Viereck et al. 1993, Richardson et al. 2004, Gehring et al. 2008, Menge et al. 2010, Batterman et al. 2013).

Although the present work does not purport to explain the N-rich conditions encountered in many tropical forests (Vitousek and Sanford 1986, Hedin et al. 2009, Brookshire et al. 2012a, b), it is natural to wonder what drives N richness and how the differential regulation hypothesis relates to these drivers. One explanation for N richness, the “leaky nitrostat” hypothesis (Hedin et al. 2009), states that facultative N fixation combines with other N inputs to bring tropical forests out of N limitation. Facultative fixation does not on its own produce N richness, but continued N inputs, particularly via N-fixers living in N-poor niches such as epiphytic surfaces and the ground litter layer (Reed et al. 2008, Menge and Hedin 2009), can push forests over the edge into N richness (Hedin et al. 2009). Another possibility is that time lags in facultative fixation lead to enough “overshoot” (continued N fixation beyond when it is necessary) to account for N richness (Menge et al. 2009). Under either of these scenarios, the differential regulation hypothesis is consistent with N-rich conditions in tropical forests.

Global models designed to study interactions between the carbon and N cycles and climate change (e.g., Thornton et al. 2007) should account for the distribution of N-fixation strategies. N limitation to CO₂ fertilization (Reich et al. 2006) can be overcome more rapidly by facultative fixation due to higher N-fixing tree abundance at the landscape scale and the faster timescale of physiology (days–months for facultative fixation to increase via nodule construction) compared to forest community dynamics (decades–centuries for obligate fixation to increase via changes in species composition; Gerber et al. 2010). The differential regulation hypothesis provides a simple explanation for the decrease in N-fixing tree abundance from lower to higher latitudes in terms of the latitudinal distribution of N-fixation strategies. A better understanding of the mechanisms determining the distribution of N-fixation strategies is, therefore, critical to predicting how N-

fixing tree abundances and the process of N fixation itself will respond to global change, with important implications for climate change.

ACKNOWLEDGMENTS

D. N. L. Menge designed the study, developed models, analyzed data, and was primary writer. J. W. Lichstein provided and analyzed data and contributed to writing. G. A. Pérez provided and analyzed data. D. N. L. Menge was supported by the Carbon Mitigation Initiative, with funding from BP and Ford, during the genesis of this work. Mexico's Comisión Nacional Forestal provided the Mexican forest inventory data. We thank Peter Vitousek and the Vitousek lab for comments on drafts.

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SUPPLEMENTAL MATERIAL

Appendix A

Theory-predicted N-fixing tree abundance in different habitats (*Ecological Archives* E095-198-A1).

Appendix B

Additional evidence for differential regulation, stand-age distributions, inventory patterns with elevation, and sensitivity analyses (*Ecological Archives* E095-198-A2).

Supplement

Code for simulations and statistical models (*Ecological Archives* E095-198-S1).