

## LETTER

# Nitrogen-fixing tree abundance in higher-latitude North America is not constrained by diversity

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### Abstract

The rarity of nitrogen (N)-fixing trees in frequently N-limited higher-latitude (here, > 35°) forests is a central biogeochemical paradox. One hypothesis for their rarity is that evolutionary constraints limit N-fixing tree diversity, preventing N-fixing species from filling available niches in higher-latitude forests. Here, we test this hypothesis using data from the USA and Mexico. N-fixing trees comprise only a slightly smaller fraction of taxa at higher vs. lower latitudes (8% vs. 11% of genera), despite 11-fold lower abundance (1.2% vs. 12.7% of basal area). Furthermore, N-fixing trees are abundant but belong to few species on tropical islands, suggesting that low absolute diversity does not limit their abundance. Rhizobial taxa dominate N-fixing tree richness at lower latitudes, whereas actinorhizal species do at higher latitudes. Our results suggest that low diversity does not explain N-fixing trees' rarity in higher-latitude forests. Therefore, N limitation in higher-latitude forests likely results from ecological constraints on N fixation.

### Keywords

actinorhizal, America, diversity, latitude, legume, Mexico, nitrogen, nitrogen fixation, rhizobial, symbiosis.

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## INTRODUCTION

Biological nitrogen (N) fixation brings more N into terrestrial ecosystems than any other natural input (Vitousek *et al.* 2013). Trees that form symbioses with N-fixing bacteria have the capacity to fuel symbiotic N fixation fluxes in excess of 100 kg N ha<sup>-1</sup> year<sup>-1</sup> in tropical (Binkley & Giardina 1997), temperate (Binkley *et al.* 1994), and boreal (Ruess *et al.* 2009) forests. Such high fluxes far exceed the contribution from asymbiotic N-fixers (Reed *et al.* 2011) and abiotic N inputs in all but the most polluted ecosystems (Galloway *et al.* 2004), although realized fluxes of symbiotic N fixation are often much lower (e.g. Sullivan *et al.* 2014). Despite this huge capacity, many forest ecosystems remain N deficient (LeBauer & Treseder 2008), largely because of the absence or rarity of N-fixing trees (Vitousek & Howarth 1991). Hereafter, we refer to tree species capable of forming N-fixing symbioses as 'N-fixing trees' for convenience, regardless of whether they are actively fixing N.

One striking example of the rarity of N-fixing trees appears across a latitudinal gradient in the Americas. For decades, scientists have noticed that N-fixing trees are comparatively rare in higher-latitude relative to lower-latitude American forests (Jenny 1950; Rundel 1989; Vitousek & Howarth 1991; Crews 1999). Recent studies with government-sponsored forest inventories, which are systematic, broad in geographic scale, and large in total sampling effort, have quantified this pattern in

detail. N-fixing trees comprise around 10% of total trees in Amazonia (ter Steege *et al.* 2006) and around 10% of tree basal area in the USA and Mexico south of 35° N latitude, but around 1% of tree basal area in the coterminous USA north of 35° N (Menge *et al.* 2014).

While several hypotheses have been proposed to explain this transition in N-fixing tree abundance (Jenny 1950; Crews 1999; Houlton *et al.* 2008; Menge *et al.* 2014; Sheffer *et al.* 2015), our focus here is the possibility that low N-fixing tree diversity at higher latitudes might constrain N-fixing tree abundance (Crews 1999). N-fixing trees are undoubtedly less taxonomically rich at higher than lower latitudes, but the same is true for nearly all taxa (Hillebrand 2004). To test the hypothesis that taxonomic diversity limits N-fixing tree abundance at higher latitudes, it is critical to understand the *relative* diversity – the proportion of tree taxonomic richness – of N-fixing trees, and how their relative diversity compares to their relative abundance. The hypothesis that N-fixing tree diversity constrains N-fixing tree abundance at higher latitudes would be supported if N-fixing taxa comprised a much smaller fraction of total tree taxa at higher than lower latitudes. By contrast, a similar fraction of N-fixing taxa at higher and lower latitudes would suggest that they have diversified in and/or colonized higher latitudes as successfully as non-fixing trees, which would reject the hypothesis that their diversity constrains their abundance. However, there has yet to be a systematic assessment of how relative N-fixing tree

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diversity changes across latitude or how it relates to N-fixing tree relative abundance across latitude. Although our focus is on relative diversity, we also consider the role of absolute N-fixing tree diversity, which could factor in via sampling effects (Hector *et al.* 2002), by studying how lower-latitude islands compare to the continent. Diversity might be driven by different factors on islands vs. continents (MacArthur & Wilson 1967), so the comparison is not perfect, but the lower absolute richness on lower-latitude islands provides a natural test for the role of absolute richness.

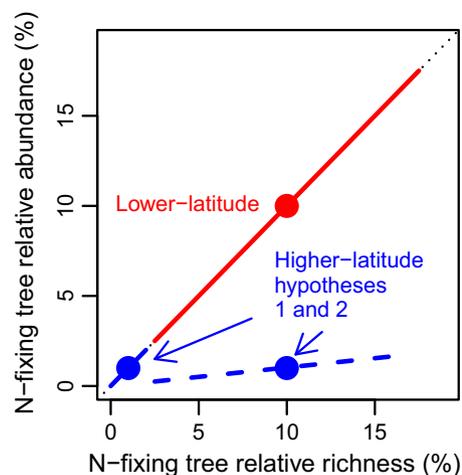
When considering the taxonomic diversity of N-fixing trees, it is important to note that there are two major types of symbiotic N-fixing tree, rhizobial and actinorhizal. Rhizobial N-fixers are legumes (and *Parasponia*) that form symbioses with *Rhizobia*-type bacteria (Sprent 2009), whereas actinorhizal N-fixers are plants from eight other plant families that form symbioses with *Frankia*-type bacteria (Huss-Danell 1997). Although rhizobial trees are incredibly diverse globally (Sprent 2009; Werner *et al.* 2014), they are species-poor outside the tropics (Rundel 1989; Crews 1999), leading Crews (1999) to suggest that there are too few N-fixing legume tree species to fill the available niche space for symbiotic N-fixers at higher latitudes. Given that all species are less diverse at higher latitudes, however, their contribution to relative diversity is not yet as clear. Actinorhizal species contribute more than legumes to tree diversity (both absolute and relative) at higher latitudes (Benson & Dawson 2007; Menge *et al.* 2010, 2014), but are largely confined to early stages of succession (Benson & Silvester 1993).

Here, we use national forest inventories from Mexico and the USA, including Alaska and tropical islands, to fill these gaps. Because we are using a newer and larger dataset than Menge *et al.* (2014), we first update the latitudinal pattern of N-fixing tree abundance and establish the lower-latitude continental vs. island pattern. We then ask two questions about patterns within the continent: (Q1) How does N-fixing tree diversity, as a proportion of total tree taxa, change across latitude? (Q2) How does the relationship between relative abundance and relative diversity of N-fixing trees change across latitude? We expect that the answers to these questions lie along a spectrum (Fig. 1). At one end of the spectrum, relative diversity of N-fixing trees might be much lower at higher than lower latitudes, but the relationship between abundance and diversity might be similar across latitudes. This end of the spectrum, depicted as a solid blue line compared to the solid red line in Fig. 1, would be consistent with the hypothesis that diversity is a major constraint on N-fixing tree abundance at higher latitudes. At the opposite end of the spectrum, relative diversity of N-fixing trees might be similar across latitudes, but N-fixing tree abundance might increase more slowly with N-fixing diversity at higher latitudes compared to lower latitudes. This other end of the spectrum, depicted as a dashed blue line compared to the solid red line in Fig. 1, would reject the hypothesis that diversity is a major constraint on N-fixing tree abundance at higher latitudes. Because these are two ends of a spectrum, we also ask a third question: (Q3) What fraction of the latitudinal abundance pattern can be explained by differential relative diversity versus differential abundance per relative diversity? Finally, we assess how tropical islands

compare to the lower-latitude continent, which helps disentangle the role of absolute diversity. The key results we document below are that the relative diversity of N-fixing trees at higher latitudes is nearly as high as it is at lower latitudes, that relative diversity is unlikely to drive much of the latitudinal abundance pattern of N-fixing trees, and that low absolute diversity does not limit N-fixing tree abundance on the islands.

## METHODS

We investigated our questions in a variety of ways. Symbiotic N fixation is largely a genus-level trait (Sprent 2009; Werner *et al.* 2014), so we investigated taxonomic diversity at the genus level as well as the species level. Due to the major functional and phylogenetic differences between rhizobial and actinorhizal N-fixers (Menge *et al.* 2014; Werner *et al.* 2014), we investigated these questions for all N-fixers together, and also for rhizobial fixers and actinorhizal fixers separately. In some sites, N-fixing trees comprise a distinctly different proportion of tree basal area than proportion of individual trees (Menge & Chazdon 2016), so we investigated both abundance metrics: relative basal area and relative individual density. For measurements where the total amount of area sampled might



**Figure 1** Conceptual model of competing hypotheses. The dotted black line indicates the 1 : 1 line. Values on this line indicate that relative nitrogen (N)-fixing tree abundance is proportional to relative N-fixing tree taxonomic richness, whereas values off the line indicate that N-fixing trees are disproportionately abundant (above the 1 : 1 line) or disproportionately rare (below the 1 : 1 line) compared to their richness. The red line indicates a hypothesized trend in lower-latitude forests, and the blue lines indicate competing hypotheses for higher-latitude forests. Circles are means of the hypothesized trends. Both competing hypotheses capture the established trend that lower latitudes have ten-fold higher mean abundance of N-fixing trees than higher latitudes (vertical values of red compared to blue circles). The hypotheses differ in the relationship between relative richness and relative abundance. The solid blue line (hypothesis 1) indicates that the rarity of N-fixing trees in higher-latitude forests results from reduced relative diversity (lower mean richness) but not disproportionate rarity (same slope as red line). At the opposite end of the spectrum, the dashed blue line (hypothesis 2) indicates that N-fixer rarity results from reduced abundance per diversity (lower slope) but not reduced relative diversity (same mean richness for blue and red).

matter, we used the classic species–area relationship (Preston 1962) to scale our data to a similar area.

### Forest inventory data

Forest inventory data come from the US Forest Service's Forest Inventory and Analysis (FIA), version 5.1 (data available online at <http://www.fia.fs.fed.us/>) and the Mexican Comisión Nacional Forestal's Inventario Nacional Forestal y de Suelos (INFyS) 2004–2007. In both datasets, plots are systematically located across the land surface, at a density of one randomly located plot per ~ 2400 and ~ 2500 ha forested land in the US and Mexico, respectively. We excluded plots listed as plantations. Our dataset includes 331 447 plot records and 11 962 355 individual tree records (Table 1, Figs 2a and S1a). Menge *et al.* (2014) used the same INFyS dataset, but an earlier version of the FIA dataset that did not include plots in Alaska or tropical islands. Plot record densities in individual 1° latitude × 1° longitude grid cells are displayed in Fig. 2a. Details of plot structure and sampling can be found in Menge *et al.* (2014). As in Menge *et al.* (2014), results here use only individual tree stems ≥ 7.5 cm diameter at breast height (dbh) to standardize the comparison across the FIA and INFyS datasets. When quantifying total sampling effort (e.g. in Table 1) we list all plot records (including multiple measurements from some plots), whereas for calculations that concern area, we only count each unique plot once.

### N-fixing species determination

We classified taxa as N-fixers, non-fixers, or unknown according to Huss-Danell (1997) for actinorhizals and Sprent (2009) for rhizobials. Because N fixation is essentially a genus-level trait (Sprent 2009; Werner *et al.* 2014) and there are many species that have not been examined, we classified all species with congeners listed in Sprent (2009) or Huss-Danell (1997) as capable of N fixation. This differs from the classification scheme in Menge *et al.* (2014), which also used information from the GRIN database, but our current method excludes only five species, representing 155 individual trees, that would be included from the GRIN criterion used in Menge *et al.*

**Table 1** Plot records, individual tree records, and taxa by region

Region	No. plot records	No. tree records*	No. genera*	No. species*
Coterminous US	312 332	10 713 005 (79 635)	100 (11)	370 (21)
Mexico	15 305	1 097 517 (147 378)	771 (61)	2585 (337)
Southeastern Alaska	2865	111 858 (526)	11 (1)	17 (1)
Puerto Rico	405	13 667 (2093)	208 (19)	341 (29)
Hawaiian islands	205	14 211 (1769)	55 (9)	77 (9)
Palau	110	4761 (29)	101 (5)	136 (5)
Guam	86	2352 (291)	50 (3)	59 (4)
US Virgin Islands	65	2558 (722)	90 (9)	121 (14)
American Samoa	41	1234 (25)	60 (2)	79 (3)
Total	331 447	11 962 355 (232 694)	950 (68)	3348 (378)

\*Numbers out of parentheses include all trees; those in parentheses are N-fixing trees only.

(2014). The only species we classified as an N-fixer that did not have congeners listed in Huss-Danell (1997) or Sprent (2009) was *Morella faya*, which was synonymous with *Myrica faya* in 1997 and is well known to be an actinorhizal N-fixer. We have not classified species as exotic vs. native; our analysis includes all species in the FIA and INFyS datasets.

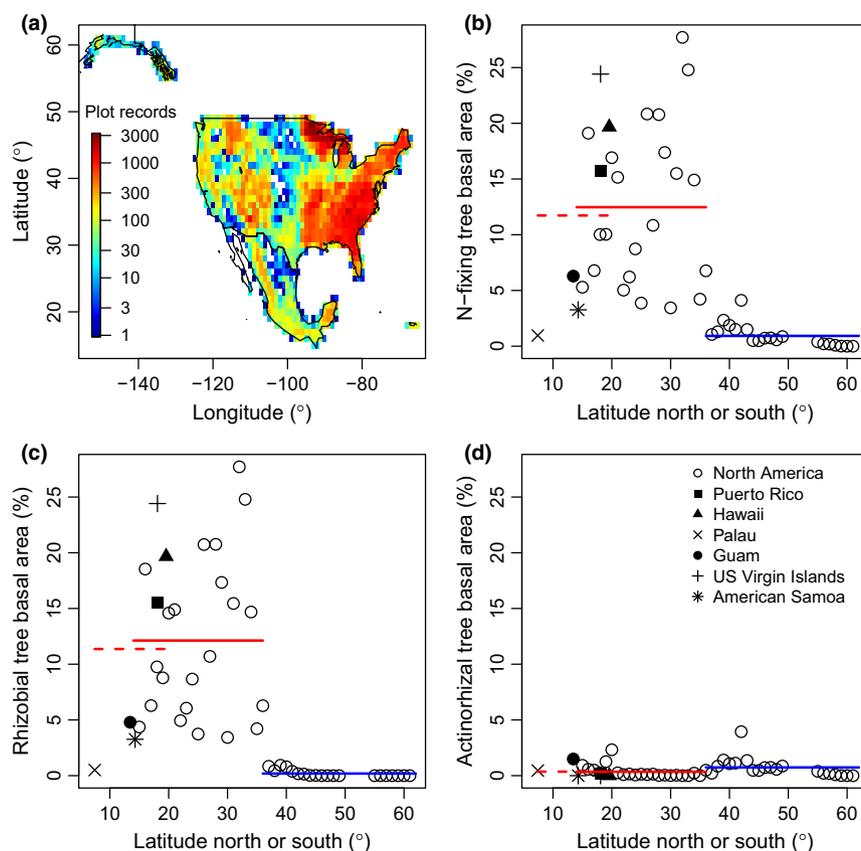
### Relative abundance, relative taxonomic richness, and absolute taxonomic richness

We calculated relative abundance as both the percentage of basal area and the percentage of individuals. To calculate basal area (BA) for each tree, we assumed circular stems and used each tree's recorded dbh:  $BA = \pi \left(\frac{dbh}{2}\right)^2$ . For both metrics, we calculated relative abundance for each grid cell as the fraction of basal area or individual trees comprised of N-fixing (or rhizobial, or actinorhizal) taxa. Latitudinal means are the means of all grid cells in the continent with the same latitude. Island means are the means within each island or island group. Relative taxonomic richness, for either species or genera, was calculated as the fraction of total taxa comprised of N-fixing (or rhizobial, or actinorhizal) taxa in an island or degree latitude.

To compare the total numbers of taxa (absolute richness), we extrapolated to a standard area for each degree of latitude for continental plots. We also extrapolated to the same standard area for each island group. We used the classic power law relationship from Preston (1962),  $S = cA^z$ , where  $S$  is the number of taxa (species or genera),  $A$  is area sampled, and  $c$  (taxa per area to the  $z$ th power) and  $z$  (unitless) are parameters defining the relationship between area and taxa. Area sampled,  $A$ , was calculated as the sum of standard subplot area in a given region, where the standard subplots are those used to sample adult trees of standard size in the INFyS and FIA datasets (see above). We used a common value of  $z$ , 0.25, fit  $c$  for each degree of latitude and taxonomic group, and extrapolated the number of taxa we would expect for the average area sampled per degree latitude in our data. We conducted these analyses for each taxonomic scale (species or genera) and N-fixing group (all taxa, all N-fixers, rhizobial N-fixers and actinorhizal N-fixers). We did not make these corrections for relative taxonomic richness because it is a proportion, and thus independent of sampling area.

### Statistical tests

To test whether N-fixing tree taxa are more or less abundant and diverse at lower latitudes vs. higher latitudes vs. islands, we conducted anovas and post hoc Tukey HSD tests on latitude- and island-scale data. To test whether N-fixing tree taxa are disproportionately rare compared to their taxonomic richness at higher vs. lower latitude vs. islands, we compared the slopes of linear regressions forced through zero of relative abundance as functions of relative taxonomic richness. For both analyses, we used 35° as our primary latitudinal cutoff because 35° is the transition point for N-fixing tree abundance in North America (Menge *et al.* 2014). To assess sensitivity to this cutoff, we conducted analyses using every two latitudinal degrees from 30° to 40° as the cutoff.



**Figure 2** Nitrogen (N)-fixing tree relative abundance across latitude. Data are from systematic national forest inventories in the United States and Mexico. (a) The total number of plot records in each 1° latitude by 1° longitude grid cell is shown with color on a log scale. (b) Relative abundance (% of total tree basal area) of all N-fixing trees. Open circles are means of all 1° latitude by 1° longitude grid cells on the continent. Other symbols are means within islands or island chains, none of which spans more than 1° latitude. Means are shown for islands (dashed red) and for lower- (solid red) and higher- (blue) latitude continental data, using 35° as the cutoff. Data are also displayed for (c) rhizobial N-fixers and (d) actinorhizal N-fixers.

## RESULTS

We conducted our statistics at the latitude scale ( $n = 55$ ), which vastly underestimates the true power of our data (331 447 plot records, 11 962 355 tree records; Table 1), yet still gives statistically significant results ( $P < 0.05$ ) for all the trends we report in the text. However, because statistical significance does not necessarily indicate biological importance, we focus on effect sizes.

### N-fixing taxa

Overall, our dataset included 3348 species and 950 genera (Table 1). Of these, we classified 378 species (11%) and 68 genera (7%) as N-fixing taxa. Table S1 lists the species we classified as N-fixers.

### N-fixing trees are an order of magnitude less abundant at higher latitudes

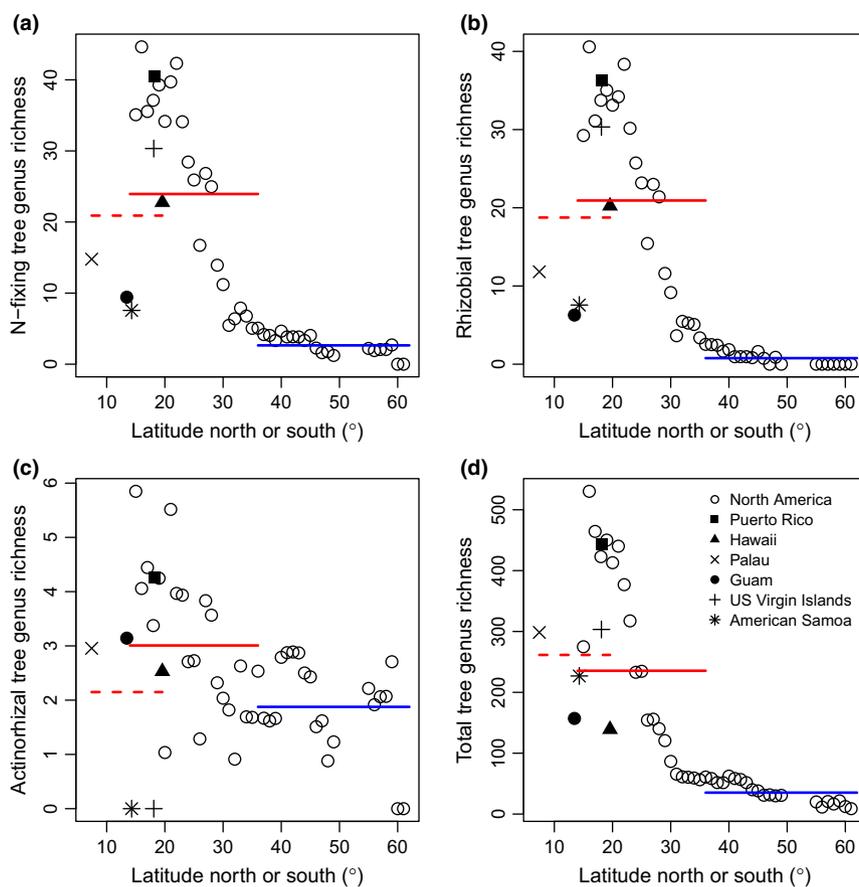
Our analyses confirm a threshold transition of N-fixing tree relative abundance at 35° latitude in North America. N-fixing trees are an order of magnitude more abundant (10.6-fold for basal area, 9.4-fold for individual trees) at lower (12.7% of

basal area) than at higher (1.2% of basal area) latitudes (Figs 2b and S1b, Table S2). The proportion of trees and the proportion of basal area show similar trends throughout our analyses, so we present basal area data in the main text and individual tree data in the Supporting Information. The tropical islands in the inventory have average relative abundances (11.7% of basal area) that are similar to the lower-latitude continent (Figs 2b and S1b, Table S2). The higher latitudes of Southeastern Alaska, from 54° N to 61° N, continue the trend of low relative abundance observed from 35° to 49° in the coterminous US (Figs 2b and S1b), although N-fixer abundance is even lower in Alaska (0.1% of basal area) than from 35° to 49° (1.7%).

Rhizobial N-fixers (Figs 2c and S1c) show similar trends to all N-fixers, although the latitudinal transition is even starker than it is for all N-fixers. Actinorhizal N-fixers are rare at all latitudes, and do not show a distinct latitudinal relative abundance trend (Figs 2d and S1d).

### N-fixing trees are less taxonomically rich at higher latitudes, as are all trees

There are nine times more N-fixing tree genera (30.6 vs. 3.4; Fig. 3a) and 20 times more N-fixing tree species (93.1 vs. 4.8;



**Figure 3** Tree genus richness in a standardized area across latitude. (a) Genus richness of all N-fixing trees is shown as the number of genera in each 1° latitude. Area sampled differs substantially across latitude, as shown in Fig. S1a, so these data are standardized to the mean area sampled for a given latitude. Uncorrected genus richness data, which are more relevant to comparing islands to the continent, are shown in Fig. S2. Symbols and fits follow Fig. 2. Genus richness of (b) rhizobial and (c) actinorhizal N-fixers as well as (d) all trees are also shown.

Fig. S2a) at lower latitudes than there are at higher latitudes (Table S3). This latitudinal disparity is sharper for rhizobial taxa (26- and 67-fold; Figs 3b and S2b). By contrast, actinorhizal species are only 1.6 times more genus-rich and 2 times more species-rich at higher latitudes than at lower latitudes (Figs 3c and S2c). These patterns hold whether or not we correct for different sampling areas (Fig. S2e–l), and for a range of cutoffs used to define higher vs. lower latitudes (Table S3). Like N-fixing trees, all trees are also more taxon-rich (seven- and six-fold for genera and species) at lower latitudes (Figs 3d and S2d), so we focused our subsequent analyses on the relative taxonomic richness of N-fixing trees (percent of total genera or species comprised by N-fixing trees). Tropical islands are 84% and 39% as genus- and species-rich as the lower-latitude continent when we standardize to the same area (Fig. 3a), but 38% and 17% as genus- and species-rich without standardizing (Fig. S2e,i).

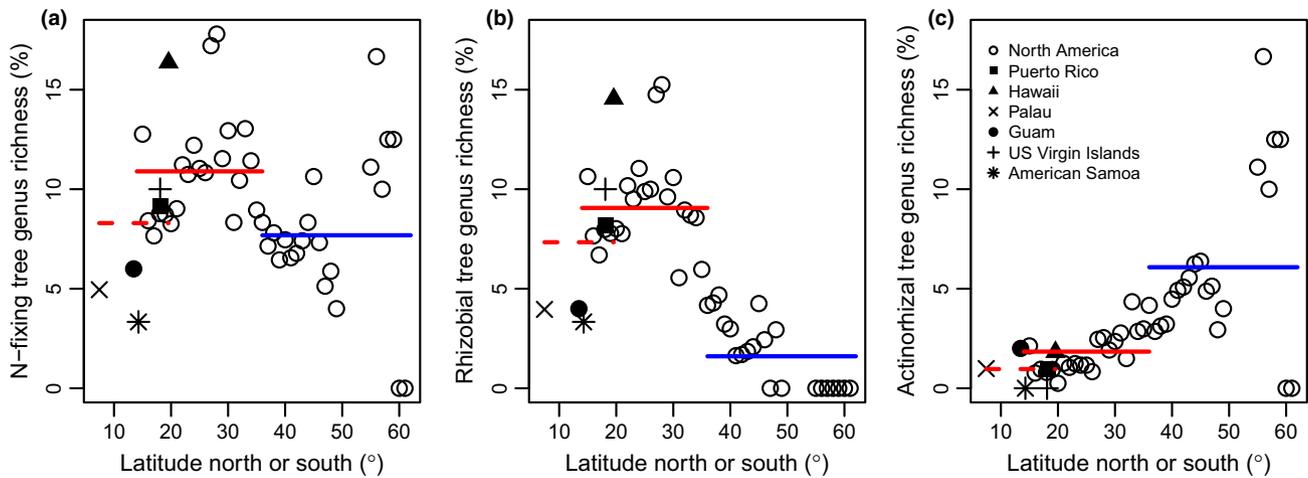
#### Relative taxonomic richness of N-fixing trees varies across latitude to different degrees, depending on N-fixer type and taxonomic scale

Although N-fixing trees are less taxonomically rich at higher latitudes, their lower diversity scales almost proportionally with total tree taxonomic richness. As a proportion of tree

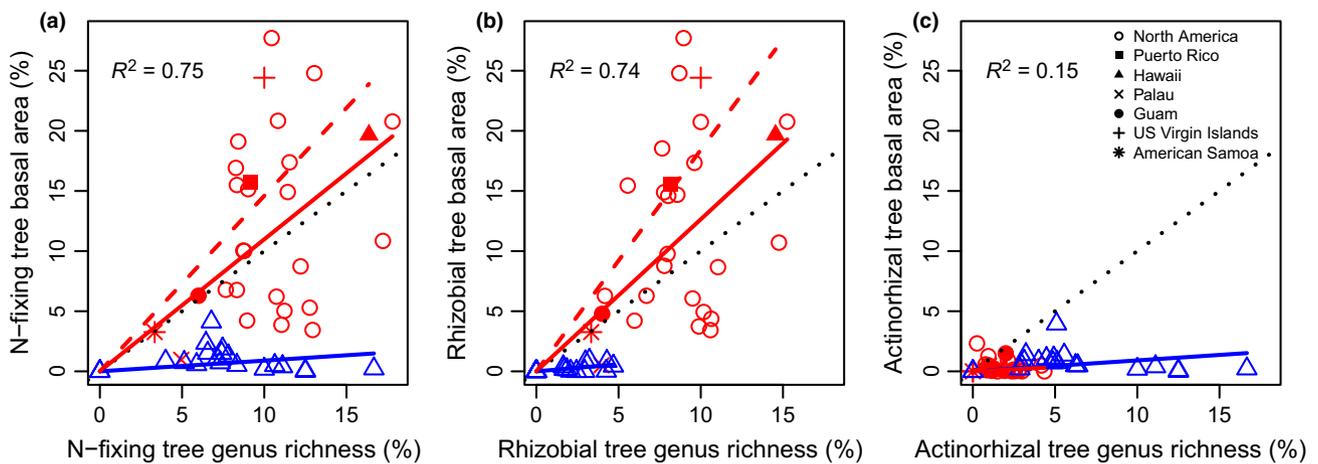
genera at a given latitude, N-fixing trees are only 1.4-fold more diverse at lower latitudes (11%) than at higher latitudes (7.7%; Fig. 4a, Table S4), compared to the 10.6-fold greater relative abundance. Relative species richness differs slightly more across latitude (2.4-fold; 11.1% vs. 4.6%; Fig. S3a, Table S4) than relative genus richness. Relative rhizobial richness of genera and species shows a starker latitudinal decline (5-fold for genera, 16-fold for species; Figs 4b and S3b, Table S4), whereas relative actinorhizal richness is actually higher at higher latitudes (3- and 4-fold for genera and species), driven largely by Alaska, where the lone N-fixing genus, *Alnus*, is one of only 11 total tree species in the dataset (Figs 4c and S3c, Tables 1 and S4). Relative diversity on islands is not significantly different than the lower-latitude continents for all N-fixer types (Figs 4 and S3, Table S4).

#### N-fixing tree rarity per taxon, not low N-fixing tree diversity, explains most of the abundance trend of N-fixing trees

All N-fixing trees have a similar range of relative genus richness at higher and lower latitudes and the tropical islands (Figs 5a and S4a–S8a). However, the relationship between relative richness and abundance differs substantially across latitudes. At lower latitudes, N-fixing tree abundance lies near



**Figure 4** Relative genus richness of N-fixing trees across latitude. The percent of all tree genera that are (a) N-fixing, (b) rhizobial, and (c) actinorhizal are shown as a function of latitude. Symbols and fits follow Fig. 2.



**Figure 5** N-fixing tree relative basal area as a function of relative genus richness of N-fixing trees. Lower-latitude (red open circles, red solid line;  $< 35^\circ$ ) and higher-latitude (blue triangles, blue line;  $> 35^\circ$ ) continental data and fits are shown along with tropical islands (filled symbols and red dashed line).  $R^2$  values shown in each panel are adjusted  $R^2$ s for the overall model (all lines in the panel). The 1 : 1 line is shown as a dotted black line. Data and fits are shown for (a) all N-fixers, (b) rhizobial N-fixers, and (c) actinorhizal N-fixers. Figures S4–S6 show alternate versions with the proportion of individual trees instead of basal area, and species richness instead of genus richness. Figures S7–S8 show alternate cutoffs for higher vs. lower latitude ( $35^\circ$  in this figure).

the 1 : 1 line with taxonomic richness (slope of 1.1), whereas at higher latitudes N-fixing trees are rare even when they comprise a large fraction of the taxon pool (slope of 0.12; Fig. 5a, Table S5). The ratio of slopes for higher vs. lower latitudes is 0.11 (Table S5), meaning that only about 11% of the abundance pattern across latitude can be explained by changes in taxonomic richness. This slope ratio is similar (0.08–0.15) for different metrics of abundance, taxonomic richness, and latitude cutoffs (Figs S4–S8a, Table S5). On the tropical islands, the slope (1.5) is even higher than the lower-latitude continent (Figs 5a and S4–S8a, Table S5).

The pattern is somewhat different for rhizobial (Fig. 5b) and actinorhizal (Fig. 5c) trees. Rhizobial trees do not have the same range of relative taxonomic richness at higher latitudes as do all N-fixing trees, so the slope ratio is more dependent on which metrics of relative abundance and richness we use. For basal area and genera, the slope ratio is 0.25

(Fig. 5b), whereas for other combinations, it ranges from 0.09 to 1.00 (Table S5, Figs S4b–S8b). As with all fixers, rhizobial trees had higher slopes on tropical islands than at lower latitudes on the continent.

Actinorhizal trees diverge from the pattern for all N-fixing trees in a different way. Actinorhizal trees are never abundant at the latitude scale, so all slopes are well below the 1 : 1 line. Furthermore, actinorhizals occupy a narrow range of relative taxonomic richness at lower latitudes, in contrast to rhizobials, so the lower-latitude slopes vary more. Overall, the actinorhizal slope ratios range from 0.17 to 0.81 (Table S5, Figs 5c and S4c–S8c).

## DISCUSSION

When we consider all N-fixing trees as a single group, our results do not support the hypothesis that evolutionary

constraints explain the low N-fixing tree abundance at higher latitudes. Relative taxonomic richness of N-fixing trees does not change much across latitude in North America, particularly at the genus level, whereas relative abundance of N-fixers declines dramatically above 35° N. The finding is somewhat different, however, if we focus on rhizobial and actinorhizal trees separately. Although N-fixing trees (rhizobial and actinorhizal combined) comprise a similar proportion of total tree taxa at higher vs. lower latitudes, rhizobial trees comprise a much smaller proportion of taxa at higher latitudes, where actinorhizal trees are the majority of N-fixing tree taxa. The slopes in Figs 5b and S4b indicate that rhizobial genera are disproportionately rare at higher latitudes even given this low diversity, but the species level results (Figs S5b and S6b) suggest that low diversity is the dominant driver. By contrast, actinorhizal genera are similarly rare at lower vs. higher latitude regardless of their diversity (Figs 5c and S4–S8c).

Based on the results presented here, therefore, the argument for diversity limitation only holds if rhizobial diversity, not actinorhizal diversity, is the limiting factor. This argument would require two components. First, actinorhizal N-fixing tree taxa would need to be inherently limited in their capacity to fill a wide range of niche space over evolutionary time. Second, rhizobial N-fixing tree taxa would need to be inherently limited in their capacity to reach higher latitudes over evolutionary time. Neither component is particularly plausible. Actinorhizal trees come from a large clade of angiosperms (Soltis *et al.* 1995; Werner *et al.* 2014) that is well represented in all successional stages of North American forests (Menge *et al.* 2010), so the idea that N fixation has not appeared with the other traits needed to succeed in a broader range of habitats is unlikely. It is possible that actinorhizal trees are evolutionarily confined to an obligate N fixation strategy, which might prevent them from filling a wide array of niche space (Crews 2016). However, it is at least as plausible that they have specialized in an obligate N fixation strategy for ecological reasons (Menge *et al.* 2009; Sheffer *et al.* 2015), which would indicate that the niche space for N-fixing trees is simply narrow. On the rhizobial side, a model of neutral trait evolution estimated that thousands of species of higher-latitude woody N-fixing legumes would have evolved if N fixation were as adaptive at higher latitudes as it is at lower latitudes (Menge & Crews 2016). That study also argued that post-glacial dispersal limitation (Svenning & Skov 2007) is unlikely to preferentially affect legumes, based on dispersal mechanisms and the observation that legumes are equally rare at high altitudes in Mexico as they are at higher latitudes (Menge *et al.* 2014). Therefore, the idea that diversity limits N-fixing tree abundance at higher latitudes lacks support. Rather, the most likely scenario is that the available niche space for N-fixing trees at higher latitude is narrow and filled by actinorhizal species.

What is the niche for N-fixing trees at higher latitudes? It has long been observed that N-fixing trees outside the tropics are pioneer species (e.g. Wardle 1980; Gutschick 1981; Boring *et al.* 1988; Vitousek & Howarth 1991; Chapin *et al.* 1994), as analyses with the US FIA data confirm (Menge *et al.* 2010). However, N-fixing trees are still fairly rare in young forests in the USA (~0.75% of basal area in the east and ~5% in the

west for forests 0–50 years old; Menge *et al.* 2010), so their niche is a small subset of early successional forests. The combined roles of light and N likely play major roles in defining this niche, and other factors such as herbivory, pathogens, or the availability of other nutrients might also play roles (Vitousek & Howarth 1991).

The observation that N fixation is energetically expensive (Gutschick 1981) has been used to argue that light availability might help constrain N-fixing trees to early-successional niches (Vitousek & Howarth 1991; Vitousek & Field 1999; Rastetter *et al.* 2001). N-fixing trees in the US FIA plots are shade intolerant (Menge *et al.* 2010), supporting this idea, but a number of lines of evidence suggest that light is not the only factor. First, as mentioned above, N-fixing trees are rare even in young forests (Menge *et al.* 2010), where most canopy trees have regenerated under high-light conditions. Second, N-fixing trees in the canopy of US FIA plots have lower growth rates and higher mortality rates than non-fixing trees in the canopy, just as they do in the understory (Liao & Menge 2016). Third, even when exposed to plenty of light, trees do not always fix N. In a lowland tropical forest in Panama, fixation rates were 15-fold lower in mature forests than in young successional forests, even though N-fixing trees in both forest types had similar access to the canopy (Batterman *et al.* 2013). Together, these observations suggest that there is another major constraint in addition to light.

Soil N availability is another obvious factor that could explain niche constraints on N-fixing trees at higher latitudes. At the beginning of secondary succession, N availability is often high because N mineralization continues despite a drop in soil N uptake (Vitousek & Reiners 1975; Houlton *et al.* 2003). This temporary flush of soil N could disfavor N-fixing trees during the initial stages of succession, so even if N availability declines later in succession, N-fixing trees might be sufficiently shaded that they cannot recover (Vitousek & Howarth 1991). Overall, the niche for N-fixing trees is likely to be the subset of young forests that have both high light penetration and extremely low soil N availability.

Most of the above light- and N-based mechanisms would influence the cost-effectiveness of N fixation itself, but would not necessarily influence the plant as a whole. A shady understory or high soil N availability might make N fixation cost-ineffective, but that would not matter if N-fixing plants can use soil N instead of fixed N without incurring a cost. Therefore, a key but often unspecified component of this niche argument is that N-fixing trees must either continue to rely on N fixation when it is cost-ineffective, or they must incur some cost of being able to fix N (Menge *et al.* 2009). There is some evidence that N-fixing trees at higher latitudes continue to fix N at high rates even under high soil N conditions (Mead & Preston 1992; Binkley *et al.* 1994; Menge & Hedin 2009), suggesting that they are either ecologically obligate (they fix at similar rates under natural conditions, even if they do not need to for survival) or that they incompletely down-regulate N fixation (Menge *et al.* 2015). By contrast, many N-fixing trees at lower latitudes seem to be facultative (Barron *et al.* 2011; Batterman *et al.* 2013; Sullivan *et al.* 2014), down-regulating N fixation under high N conditions, which would help explain their greater prevalence (Menge *et al.* 2014;

Sheffer *et al.* 2015). A possible cost of being able to fix N is that exposure to symbiotic bacteria leads to lower N use efficiency, even for plants that are not fixing (Menge *et al.* 2015; Wolf *et al.* 2017).

Our focus so far has been on relative diversity, but we now consider absolute diversity. In tropical forests where the species pools are larger, N-fixing trees differ widely in their successional habits (Batterman *et al.* 2013), N fixation rates (Wurzburger & Hedin 2016), foliar N contents (Bhaskar *et al.* 2016), and other traits (Rundel 1989; McKey 1994). With a smaller overall species pool at higher latitudes, could a sampling effect (e.g. Hector *et al.* 2002), whereby random chance has selected a series of poor-performing N-fixing tree species, explain the low abundance of N-fixing trees? We find an absolute diversity constraint unlikely for two reasons: (1) Our tropical islands results, and (2) The potential species pool. (1) Tropical islands have low absolute diversity of N-fixing trees like higher latitudes, but a tropical environment like lower latitudes, so they provide a natural way to disentangle the effect of absolute diversity from the effect of different environments. The fact that relative N-fixing tree diversity and abundance are similar in the islands and the lower-latitude continent suggests that low absolute diversity does not constrain N-fixing tree abundance. (2) A sampling effect concerns the potential species pool, not the existing species pool. The estimate that thousands of rhizobial N-fixing tree taxa have had the chance to colonize higher-latitude forests (Menge & Crews 2016) suggests that a wide species pool has been available, but has been unsuccessful. Given this large potential species pool, it is unlikely that the species that have colonized higher latitudes successfully are poor performers by random chance.

We have focused on higher vs. lower latitudes, but the trends within lower latitudes are also interesting. Relative abundance is proportional to relative diversity for all N-fixing trees and for rhizobial trees at low latitudes, but with wide variation around the trend. We have not directly addressed drivers of this variation, but aridity is an intriguing possibility. Our lower-latitude sites range from arid to exceptionally wet (Liao *et al.* 2017), and recent work has established that N-fixing and particularly rhizobial trees are more abundant in drier sites, both in these datasets (Liao *et al.* 2017) and elsewhere in the tropics (Pellegrini *et al.* 2016).

Overall, we find the argument that narrow niche space explains low N-fixing tree abundance in higher-latitude forests to be much more persuasive than the argument that diversity constrains N-fixing tree abundance. Additional ways to test these conclusions include assessing patterns on other continents or in the paleo-ecological record. For example, if N-fixing trees are rare at higher latitudes in other continents – and if N-fixing trees were rare at higher latitudes during previous interglacials and other periods with similar climate – despite comprising a similar fraction of tree diversity at higher and lower latitudes, a niche-based explanation would seem even more likely.

The approach we develop here – comparing slopes of relative abundance of a functional group against its relative diversity across categories – could be used in a variety of contexts to assess whether diversity of a group limits its abundance. For example, lianas (Schnitzer 2005), arbuscular (as opposed

to ecto or ericoid) mycorrhizal associations (Allen *et al.* 1995) and C<sub>4</sub> (as opposed to C<sub>3</sub>) photosynthetic pathways (Still *et al.* 2003) are common at lower latitudes but rare at higher latitudes.

The rarity of N-fixing trees in higher-latitude forests is a key component of a central biogeochemical paradox, the persistence of N limitation (Vitousek & Howarth 1991). Our results here support the idea that niche-based mechanisms are the culprit, and should therefore help focus future efforts to understand both the rarity of N-fixing trees and, ultimately, the persistence of N limitation. Understanding of N limitation in general (Hungate *et al.* 2003; Sokolov *et al.* 2008; Gerber *et al.* 2010; Zaehle *et al.* 2010; Thomas *et al.* 2013; Wårlind *et al.* 2014), and of the role of symbiotic N-fixers in particular (Stocker *et al.* 2016), have been highlighted as key uncertainties in global carbon-cycle and climate projections. Our results suggest that future efforts to study N-fixing trees and N fixation, and thus to better understand global carbon storage and climate, should focus on niche-based ecological mechanisms.

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#### AUTHORSHIP

DM developed the project, analyzed data and wrote the first draft. SB helped develop the project and edited the manuscript. WL and BT analyzed data and edited the manuscript. JL and GAP provided data and edited the manuscript.

#### DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.2d4t3>.

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