Tansley insight

Can evolutionary constraints explain the rarity of nitrogen-fixing trees in high-latitude forests?

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Contents

Summary

The rarity of symbiotic nitrogen (N)-fixing trees in temperate and boreal ('high-latitude') forests is curious. One explanation – the evolutionary constraints hypothesis – posits that high-latitude N-fixing trees are rare because few have evolved. Here, we consider traits necessary for high-latitude N-fixing trees. We then use recent developments in trait evolution to estimate that > 2000 and > 500 species could have evolved from *low-latitude* N-fixing trees and high-latitude N-fixing *herbs*, respectively. Evolution of N-fixing from *nonfixing* trees is an unlikely source of diversity. Dispersal limitation seems unlikely to limit high-latitude N-fixer diversity. The greater number of N-fixing species predicted to evolve than currently inhabit high-latitude forests suggests a greater role for ecological than evolutionary constraints.

I. Introduction

Why does nitrogen (N) limitation persist in some but not all ecosystems? This question is one of the enduring challenges in ecosystem ecology (Vitousek & Howarth, 1991). Symbiotic N fixation (SNF), in which bacteria living in symbiosis with other organisms convert atmospheric N_2 to ammonium, can bring huge quantities of N into ecosystems (> 100 kg N ha^{-1} yr^{-1}; Binkley *et al.*, 1994; Carlsson & Huss-Danell, 2003). Vitousek & Howarth (1991) posited that N-fixing symbioses should experience a competitive advantage under N limitation, such that their populations and activities would increase until N limitation disappeared. In some ecosystems this appears to be what happens, as with legume trees in many low-latitude forests (Hedin *et al.*, 2009). By contrast, legume trees are rare in high-latitude (here,

> 35°) forests globally (Rundel, 1989), even though these forests tend to be N-limited (Vitousek & Howarth, 1991; Thomas *et al.*, 2010). High-latitude North American forests, for example, have one-tenth the abundance of N-fixing trees of low-latitude (< 35°) American forests (ter Steege *et al.*, 2006; Menge *et al.*, 2014). In the southern hemisphere, where far less high-latitude land mass exists, N-fixing trees are also common < 35°S (Adams *et al.*, 2010). Woody legumes can be locally common somewhat higher than 35°S where climates are moderate, such as in Tasmania (*Acacia* spp.; Kitchener & Harris, 2013) and the Monte region of Argentina (*Prosopis* spp.; Roig *et al.*, 2009), but otherwise, high-latitude forests in South America (D. Ravetta, A. Vilela, pers. comm.) and New Zealand (D. Menge, pers. obs.) appear to maintain similarly low abundances of N-fixing trees as their latitudinal counterparts in the North.

Why are N-fixing trees ten times less abundant in high- compared to low-latitude forests? Recent work addressing this latitudinal pattern (Menge *et al.*, 2014; Sheffer *et al.*, 2015) has focused on what we will refer to as ecological constraints. Ecological constraints explain why the niche for symbiotic N-fixers might be restricted at high latitudes. By contrast, what we will call evolutionary constraints focus on organisms' underlying genetic capacity. In reality ecology and evolution are linked via selection, but we treat selection as an ecological constraint. As noted by Crews (1999, 2016), ecological explanations can only play out once the evolutionary stage has been set. Here, we consider this evolutionary stage. The two main contributions in this paper, as distinct from previous work discussing evolutionary constraints (Crews, 1999, 2016; Menge *et al.*, 2010), are that we delineate pathways by which high-latitude N-fixing trees might have evolved and quantify the possibility that evolutionary constraints explain the rarity of N-fixing trees in high-latitude forests.

II. The role of diversity

Crews' (1999, 2016) argument for evolutionary constraints focused on the low total diversity of woody N-fixing legumes at high latitudes, not a complete lack of diversity, implicitly invoking a sampling effect (e.g. Hector *et al.*, 2002). Other traits aside from SNF affect fitness, so even in an environment where SNF is adaptive, plants require additional traits to be successful. By random sampling, it is much more likely that SNF would appear with the right combination of other traits when there are many potentially N-fixing taxa than when there are few potentially N-fixing taxa (Fig. 1). So, even if species like *Robinia pseudoacacia* in the USA (Menge *et al.*, 2010) and *Acacia melanoxylon* and *A. dealbata* in Tasmania (Kitchener & Harris, 2013) are N-fixing trees that have colonized high-latitude forests, their other traits might confine them to particular habitats.

N-fixer traits vary widely across species within an ecosystem. Batterman *et al.* (2013) found that different species of legume trees in Panama varied in their successional patterns of SNF: some fixed more N in early succession, others in mature forests. Also in

Panama, Wurzburger & Hedin (2016) found enormous variation in SNF rates across different species. Perhaps the 'superfixers' in Panama (*Inga* spp. and *Tachigali versicolor*, taxa that contribute disproportionately to SNF; Wurzburger & Hedin, 2016) or the highly successful *Pentaclethra macroloba* in Costa Rica, which has remarkably high survival (Menge & Chazdon, 2016), are simply lucky rolls of the evolutionary dice that have the right combination of SNF and other traits. The more dice rolls there are, the greater the likelihood of N-fixers succeeding in a variety of habitats.

III. An evolutionary framework for the success of high-latitude N-fixing woody symbioses

There are a variety of evolutionary pathways by which woody symbiotic N-fixers might have colonized high-latitude forests (Fig. 2). High-latitude woody N-fixers must be capable of (1) surviving at high latitudes and (2) forming N-fixing symbioses. They must also (3) have an SNF strategy that is more facultative than obligate (Fig. 3) and (4) be woody. If these traits evolve outside of high-latitude forests, they must (5) disperse to high-latitude forests. Below, we describe each of these five pathways, use recent literature to estimate their roles, and give a hypothetical example of each. We do not consider symbioses such as cycads, *Gunnera*, pteridophytes and lichens, as they strike us as unlikely progenitors of a large diversity of trees compared to the far more speciose, woody and widely distributed Rosids.

1. Low-latitude facultative N-fixing trees evolve high-latitude traits

In this scenario, a low-latitude facultative N-fixing tree species such as *Inga* (Barron *et al.*, 2011) evolves traits that allow it to colonize high latitudes. Living at high latitudes involves dealing with a variety of environmental factors. Episodic freezing is particularly challenging, as it poses risks of embolism (Sperry & Sullivan, 1992) and snow damage (Nykänen *et al.*, 1997). Zanne *et al.* (2014) recently showed that woody angiosperms have colonized freezing-prone habitats by evolving small hydraulic
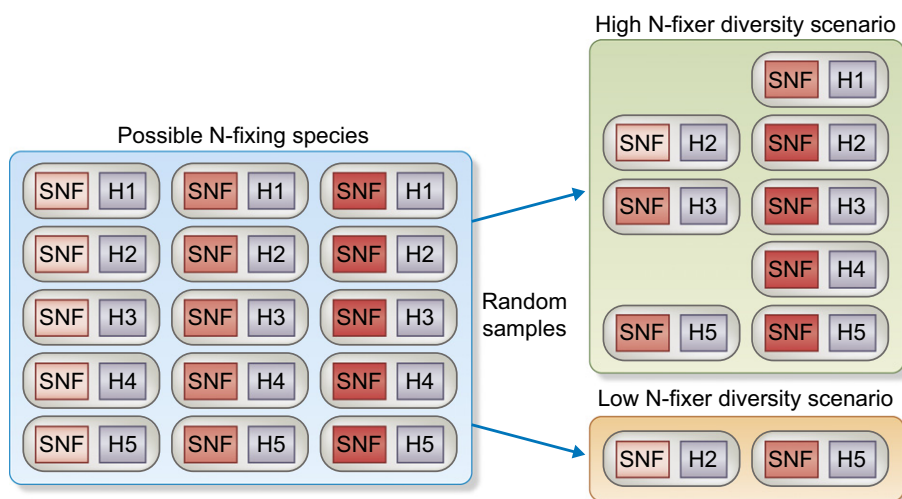

Fig. 1 The importance of diversity. The trait of symbiotic nitrogen fixation (SNF) varies from low (pink) to high (dark red) effectiveness. Other traits are adapted to different habitats, labeled here H1–H5. The set of possible species could range across SNF effectiveness and adaptation to different habitats. If species in a flora are sampled randomly from the set of species, high-diversity communities of symbiotic N-fixers are much more likely than their low-diversity counterparts to have traits that range across many habitats and to contain some species with highly effective SNF.

Fig. 2 Possible pathways to high-latitude woody facultative nitrogen (N)-fixers. The first four pathways involve genetic changes, represented by black solid arrows, that confer (1) high-latitude traits (symbolized by snowflakes), (2) symbiotic N fixation (SNF, symbolized by pink root nodules) with facultative regulation (symbolized by variable nodule size, which is meant to indicate regulation rather than actual nodule size), (3) facultative regulation of SNF (from obligate SNF), and (4) wood (symbolized by a tree instead of an herbaceous plant). Numbers by trait arrows indicate the approximate number of times that this set of traits is likely to have evolved and the number of extant species this would have produced without ecological constraints; see text for explanation of calculations. The fifth pathway is (5) spatial movement into a new habitat (dispersal), represented by a yellow dotted arrow. The purple dashed arrow indicates possible influence that symbiotic bacteria might have. The green arrow indicates selection, which can only act once plants have the genetic capacity for woodiness, survival at high latitudes, and facultative SNF. Selection is not the focus of this work.
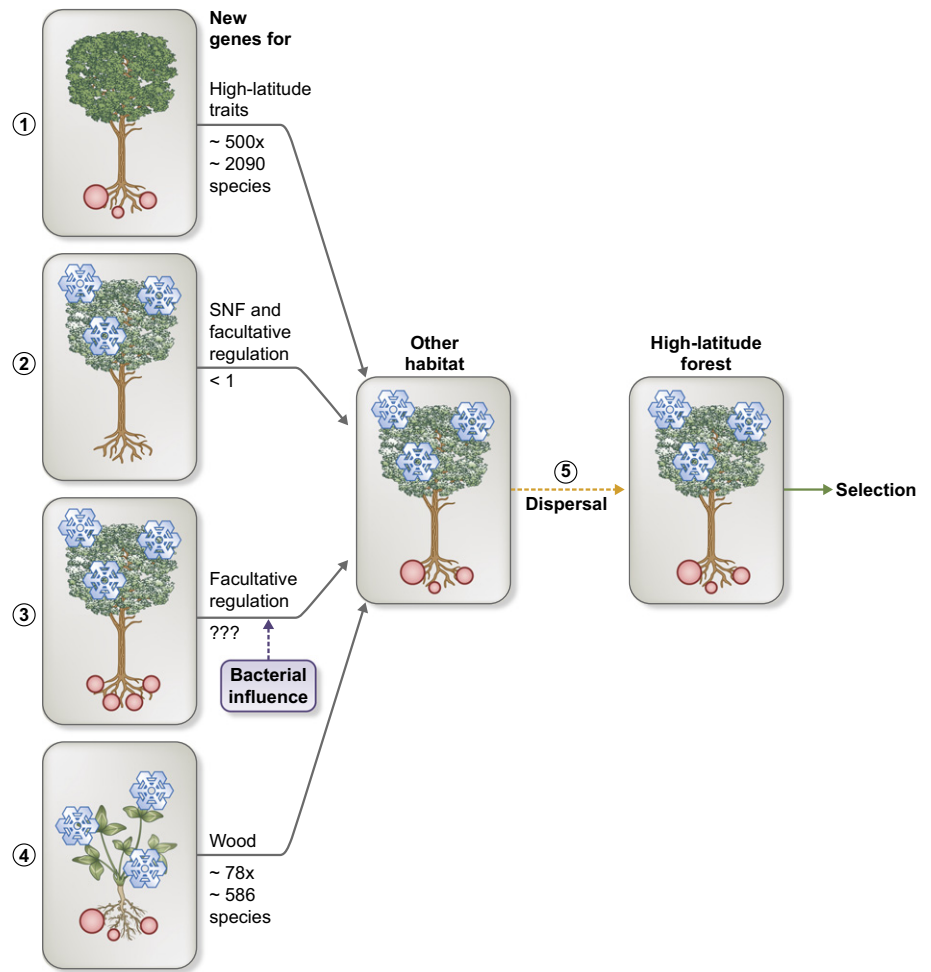
conduits, which minimize embolism risk, and deciduousness, which minimizes embolism risk and snow damage (Nykänen *et al.*, 1997).

What does this mean for N-fixing plants? According to the analysis of Zanne *et al.* (2014), woody angiosperms in the Superrosidae (in which all N-fixers reside) have colonized freezing-prone habitats at $0.01\,\text{Myr}^{-1}$ (Supporting Information Table S1). This rate, along with information about the legume phylogeny, can provide a rough estimate of the number of extant high-latitude woody facultative N-fixers there would be if there were no ecological constraints on SNF in freezing-prone habitats. Our approach, a neutral trait evolution model detailed in Methods S1, assumes that there were no ecological constraints on SNF in freezing-prone habitats, that the phylogenetic tree suggested by Zanne *et al.* (2014) is accurate, and that the basal legume (c. 51 Myr ago (Ma); Werner *et al.*, 2014) lived in a nonfreezing-prone habitat (this last assumption is meant to give a conservative estimate). The approach also assumes that freezing-prone and nonfreezing-prone environments correspond to high and low latitude, which is not strictly true, but allows us to use the results from Zanne *et al.* (2014) to address our questions. Under these assumptions, we estimate that low-latitude woody facultative N-fixing species would have

successfully colonized freezing-prone habitats c. 500 times. Each of these 500 could have subsequently radiated, and we estimate radiation to > 2000 species (Methods S1). These calculations are rough, with a lot of assumptions, but > 2000 species is a ballpark estimate of how many low-latitude woody facultative N-fixers like *Inga* should have colonized high latitudes in the absence of ecological constraints.

2. Nonfixing high-latitude trees evolve facultative symbiotic N fixation

A second possibility is that tree species which have already colonized high latitudes – but cannot fix N – evolve SNF. The capacity to evolve SNF requires a 'precursor' that has evolved only once, 100 Ma (Werner *et al.*, 2014). Werner *et al.* (2014) found that, subsequent to the evolution of this precursor, SNF itself has evolved about eight times. Candidates for our scenario 2 could be legumes – like *Gleditsia* – or nonlegumes – like *Betula* – that already exist in the flora of high-latitude forests, that are in the N-fixing clade, and that retain the precursor (Werner *et al.*, 2014). However, if SNF has evolved only eight times in 100 Myr across all angiosperm taxa, this pathway is unlikely to have generated much diversity of high-latitude N-fixing trees.
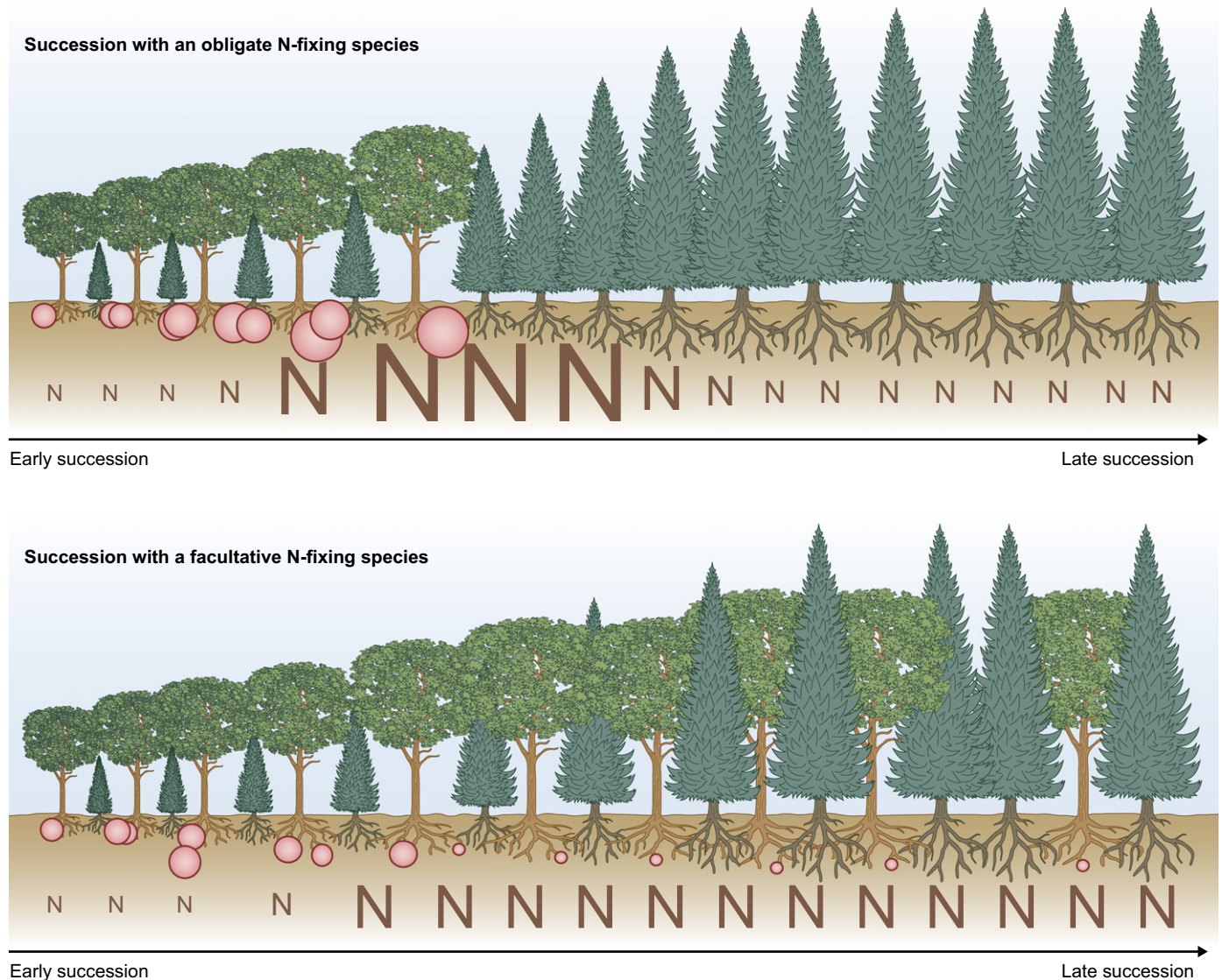
Fig. 3 Successional differences between obligate and facultative nitrogen (N)-fixing trees. These dynamics are based on the theoretical results of Menge *et al*. (2014, 2015). Nitrogen-fixing (flat-topped) and nonfixing (pointy-topped) trees are shown along with plant-available soil N across successional time. Pink circles on N-fixer roots indicate active nodules, i.e. N fixation. Sizes of each symbol (trees, nodules, soil N) are proportional to pool size. Obligate N-fixers (upper panel) maintain N fixation rates regardless of soil N supply, whereas facultative N-fixers (lower panel) adjust N fixation rates based on soil N supply relative to plant N demand. An obligate N fixation strategy does not mean that these plants need the symbionts to survive. When soil N availability is low, both facultative and obligate N fixation strategies derive most of their N from symbiotic N fixation (SNF). As soil N builds up during succession, facultative N-fixing trees downregulate SNF and rely primarily on soil N, whereas obligate N-fixing trees continue SNF and take up soil N. Because SNF is more expensive than soil N uptake when soil N is plentiful (Gutschick, 1981), obligate N-fixing trees spend more energy acquiring N than do facultative N-fixing trees when soil N availability is higher. This higher energy expenditure, combined with the possibility of fertilizing nonfixing competitors with excess N, leads to obligate N-fixing trees being outcompeted by nonfixing trees. Overall, an obligate N-fixing strategy is much less abundant than a facultative strategy across a range of habitats, although an obligate N-fixing strategy can be more successful in exceptionally N-poor conditions if there are costs of being facultative.

3. Obligate high-latitude N-fixing trees evolve a facultative strategy

Although rare, high-latitude N-fixing tree species exist. The ones that extend farthest poleward, such as the actinorhizal (nonlegume) N-fixers *Alnus* and *Coriaria*, are primarily early successional (Viereck *et al.*, 1993; Richardson *et al.*, 2004; Menge *et al.*, 2010), and seem to have obligate SNF strategies (Binkley *et al.*, 1994;

Menge & Hedin, 2009). We know little about SNF strategies (facultative, obligate or in between) for the overwhelming majority of woody N-fixers, but the existing evidence suggests that actinorhizal (nonlegume) N-fixers are obligate (Binkley *et al.*, 1994; Menge & Hedin, 2009), whereas rhizobial (legume) N-fixers are facultative (Barron *et al.*, 2011; Batterman *et al.*, 2013; Sullivan *et al.*, 2014). If actinorhizal N-fixers are confined to obligate SNF, and thus to early succession, then the presence of actinorhizal

N-fixers or potential actinorhizal N-fixers in high-latitude forests might not lead to high abundance of high-latitude woody N-fixers (Crews, 2016).

In this third pathway, a high-latitude, woody, obligate N-fixer such as *Alnus* evolves a facultative strategy, which allows it to colonize a greater range of successional habitats. Successional traits are labile across high-latitude tree species (Menge *et al.*, 2010), so diversifying into different successional niches seems reasonable. But how difficult is it to evolve *facultative* SNF from obligate SNF? Obligate N-fixers have some capacity to regulate SNF, given that they maintain nodulation at a particular level (Binkley *et al.*, 1994; Menge & Hedin, 2009). Therefore, it seems likely that relatively few genes would be needed to convert to facultative SNF.

On the contrary, symbiotic bacteria might pressure plants to retain obligate SNF. For rhizobial bacteria, colonizing root nodules lead to a 10^5–10^8-fold abundance increase (Denison & Kiers, 2011), so symbiotic bacteria have a strong incentive to maintain nodulation. Although some plants can impose sanctions on bacteria (Kiers & Denison, 2008), not all do (Marco *et al.*, 2009), so it is conceivable that bacteria might constrain the evolution of facultative N-fixing plants. Without additional information on SNF strategies across the angiosperm phylogeny it is difficult to quantify scenario (3), but it is intriguing.

4. Herbaceous high-latitude facultative N-fixing plants evolve wood

In this fourth scenario, a high-latitude herbaceous facultative N-fixing legume such as *Medicago polymorpha* evolves woodiness and colonizes forests. Although legume trees that can nodulate are uncommon in high-latitude forests, the majority of legume species globally occur in high latitudes (Sprent, 2009). Most of these are herbaceous, and these can reach high abundance. For example, in ungrazed grasslands burned annually at the Konza Prairie, which is located at 39° latitude in Kansas, USA, legumes comprise 5–7% cover in upland landscapes and 22–36% in lowlands (Towne & Kemp, 2003).

Are many herbaceous N-fixing plants facultative? A great deal of research has been carried out on annual herbaceous legume species used in agriculture for food or fertility-building rotations, and some level of facultative strategy is the rule rather than the exception (Peoples *et al.*, 2012). Although less research has been conducted on perennial legumes, Carlsson & Huss-Danell (2003) reported that several forage species partially downregulated N fixation in response to N fertilization. In glasshouse conditions, some wild herbs such as *Medicago polymorpha* shut SNF off entirely at moderate N supply (Menge *et al.*, 2015). Overall, facultative regulation seems possible in herbaceous legumes.

How likely is it that high-latitude facultative N-fixing herbs could evolve woodiness and colonize forests? According to Zanne *et al.* (2014), herbaceous plants in the Superrosidae have evolved into woody plants at 0.008 Myr^{-1} in freezing-prone habitats. If 10% of N-fixing legume species are high-latitude facultative N-fixing herb species (see Methods S1 for details), then something like *Medicago* would have evolved wood *c.* 78 times, and subsequently radiated into 586 extant species if there were no

ecological constraints on high-latitude woody facultative N-fixers. Combining scenarios (1) and (4) suggests > 2500 extant high-latitude woody facultative N-fixing species. A more complicated but more realistic model that we explored, which allows woodiness and habitat to evolve simultaneously (Methods S1), suggested that > 3300 extant high-latitude woody facultative N-fixing species would be expected to evolve in the absence of ecological constraints (Fig. 4). Not all woody species are trees, of course, but even half of this number is still thousands of species, and many of our assumptions were intentionally conservative.

5. Facultative N-fixing trees with high-latitude traits disperse to high latitudes

In this fifth scenario, a woody facultative N-fixing tree that already possesses high-latitude traits, such as a hypothetical frost-tolerant species of *Inga*, disperses to high latitudes. In this case, the rarity of facultative N-fixing trees at high latitudes is not the result of low genetic diversity, but of dispersal limitation. As such, this fifth pathway concerns shorter timescales – the thousands of years that characterize glacial/interglacial cycles – than the previous four pathways, which consider the millions of years of angiosperm evolution.

How likely is dispersal limitation for woody N-fixer colonization of high latitudes? Dispersal limitation plays an important role in postglacial tree distributions generally, with most trees dispersing at < 100 m yr^{-1} (Svenning & Skov, 2007). Might legume trees disperse more slowly than other trees? Some legume tree seeds engage in ballistic dispersal (Guimarães *et al.*, 2008), which averages about a meter per generation (Thomson *et al.*, 2011). However, other legume trees are dispersed by animals (Guimarães *et al.*, 2008), which can transport seeds farther (100s–1000s of meters per generation) than other dispersal mechanisms (Thomson *et al.*, 2011). Overall, consideration of dispersal distances does not provide strong evidence that legume trees disperse more slowly than other trees.
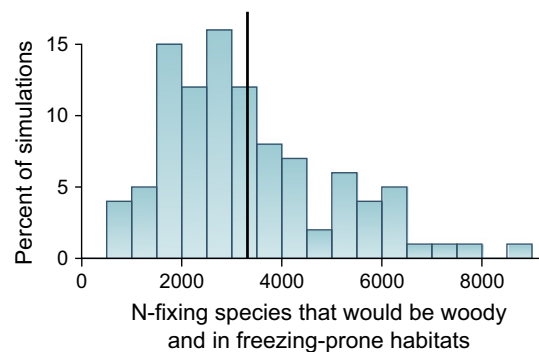

Fig. 4 Number of woody, high-latitude nitrogen (N)-fixing species expected in the absence of ecological constraints. These are simulation results from a stochastic trait evolution model that allows both woodiness and habitat (freezing-prone or nonfreezing-prone, which roughly corresponds to high vs low latitude) to evolve, based on the transition model from Zanne *et al.* (2014). The simulation used the conservative assumption that the ancestral legume was a low-latitude herb. The mean number of high-latitude woody N-fixing species that would be extant is shown with a thick black line.

A second way to gauge the likelihood of dispersal limitation is by examining elevation gradients. Dispersing up mountains in the tropics should be easier than dispersing to high latitudes. Forest inventory data from Mexico, however, show that N-fixer and particularly rhizobial N-fixer abundance declines at higher elevations in mountains in much the same way as it does across latitude in the Americas (Menge *et al.*, 2014).

A third way to gauge dispersal limitation is by comparing woody to herbaceous taxa. Myriad species of herbaceous N-fixing legumes have colonized high latitudes (Sprent, 2009), suggesting that they were not dispersal-limited. Herbaceous legumes have seeds that are 2–20 times smaller than those of woody legumes (Corby *et al.*, 2011), suggesting that they might disperse farther. However, woody plants are also taller than herbs, and seeds of taller plants disperse farther. Thomson *et al.* (2011) found that the difference in dispersal distance associated with a 100-fold difference in seed mass was matched by a five-fold difference in plant height, so it seems likely that woody N-fixing legumes disperse farther than herbaceous legumes per generation. Woody plant generation times are longer, but even so, it seems unlikely that herbs have a major dispersal advantage. Therefore, the diversity of herbaceous legumes at high latitudes argues against postglacial dispersal limitation as a primary factor limiting the abundance of woody N-fixing legumes at high latitudes.

IV. Conclusions

In this Insight we have discussed pathways by which high-latitude facultative N-fixing trees might have evolved, or might evolve in the future. Using some admittedly bold assumptions, we find that two evolutionary pathways are likely, one might be, and one is not. In the absence of ecological constraints, > 2000 low-latitude facultative N-fixing tree species would have evolved high-latitude traits and > 500 high-latitude facultative N-fixing herbs would have evolved woodiness. These results depend on the phylogenetic structure and trait evolution rates within angiosperms (Werner *et al.*, 2014; Zanne *et al.*, 2014), so updated understanding within these rapidly growing areas of study could modify our findings. The evolution of facultative N-fixing trees from obligate N-fixing trees that already exist in high-latitude forests also seems possible, although pressure from the bacterial symbionts to maintain nodulation might temper this possibility. Given the low rate at which symbiotic N fixation has evolved, it is unlikely that novel N-fixing taxa have contributed much. Postglacial dispersal seems unlikely to be a barrier.

Overall, our findings indicate that many more high-latitude woody facultative N-fixers should have evolved than currently persist today, suggesting a greater role for ecological than evolutionary constraints. These ecological constraints might include temperature or energetic constraints on SNF (Vitousek & Howarth, 1991), constraints of other resources such as phosphorus on symbiotic N fixation (Vitousek & Howarth, 1991), preferential herbivory on N-fixers (Vitousek & Howarth, 1991), or climatic constraints on facultative regulation of symbiotic N fixation (Menge *et al.*, 2014; Sheffer *et al.*, 2015). Our findings

suggest that these ecological constraints function on evolutionary timescales, inhibiting speciation or causing extinction at high latitudes.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Table S1 Superrosidae trait transition rates

Methods S1 Details of calculations and simulations.

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