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Research Article

Robinia pseudoacacia, a nitrogen-fixing tree, facilitates the future growth of neighboring trees in Black Rock Forest

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Abstract

Trees that form symbioses with nitrogen (N)-fixing bacteria (N fixers) have long been thought to facilitate neighbors' growth by providing new N, but some data do not show facilitation. Two hypotheses to explain the lack of facilitation are that (1) N fixers may decrease the amount of N they fix over time, reducing the N supply to their neighbors and (2) the effect of facilitation might take longer than the 1–5-year interval of most studies. In this study, we tested these hypotheses with the N-fixing tree *Robinia pseudoacacia* L. and its nonfixing neighbors, using tree ring N isotopes to estimate past N fixation and tree ring width to estimate past size and growth. Where the isotopes allowed a constrained estimate of N fixation, we did not find support for the first hypothesis: 51% of *Robinia*'s N came from fixation throughout its 60-year lifespan, with little variation across age. In support of the second hypothesis, we found that *Robinia* enhanced growth of neighboring trees in the future (up to 14 years) more than in the present. This delayed effect matches the expected timing of facilitation by N fixers and indicates that single-census studies might underestimate the degree to which N fixers facilitate forest growth.

Key words: isotope, natural abundance, delay, legume, nitrogen fixation

Introduction

Forests are a large carbon sink, absorbing much of the carbon emitted via human activities (Pan et al. 2011). The size of this carbon sink is thought to depend on the supply of nitrogen (N) (Hungate et al. 2003; Wieder et al. 2015), which commonly limits forest productivity (LeBauer and Treseder 2008). Symbiotic N fixation, where specialized prokaryotes in root nodules of certain plant species convert atmospheric N_2 gas to plant-available NH_3 (Vitousek et al. 2013), has an immense capacity to supply new N. Where N-fixing trees (hereafter, we refer to the trees and the symbioses as "N-fixing trees" or "N fixers") are abundant, they can bring in over 100 kg N ha^{-1} $year^{-1}$ (Binkley et al. 1994; Ruess et al. 2009) to ecosystems.

Given this large capacity to supply new N to forests, symbiotic N-fixing trees could stimulate forest growth, as suggested by a number of studies that quantify C and N budgets or model coupled C–N cycles (Binkley et al. 1992; Forrester et al. 2006; Batterman et al. 2013; Brookshire et al. 2019; Levy-Varon et al. 2019; Sulman et al. 2019). Despite this budgetary and modeling evidence, studies that directly compare forest growth across gradients of N fixer abundance have yielded mixed results. A meta-analysis of 14 studies showed that N-fixing trees enhanced the growth of nonfixing trees in plantations (Piotto 2008). Another meta-analysis of eight studies showed that N-fixing trees enhanced plantation productivity, where plantations consisted of nonfixers and N fixers together (Hulvey et al. 2013). However, studies in unplanted boreal (Chapin et al. 2016), temperate (Staccone et al. 2021), and tropical (Taylor et al. 2017; Lai et al. 2018; Xu et al. 2020) forests have shown mixed effects, no effect, or even a negative effect of N-fixing trees on forest and neighbor growth. Of particular relevance to the present work is a country-wide analysis (Staccone et al. 2021) of millions of trees in unplanted temperate forests across the USA, in which the most common N-fixing tree species was *Robinia pseudoacacia* L. (the focus of our study, and hereafter referenced by its genus name only). In that study the average effect was that *Robinia* enhanced the growth of neighboring nonfixing trees but had no net effect on overall forest growth (Staccone et al. 2021) because its own lower growth rate relative to its neighbors (Liao and Menge 2016) counteracted the enhanced growth of its neighbors. Staccone et al. (2021) also found wide variation across sites: *Robinia* facilitated forest growth in some sites, inhibited it in others, and had no effect in others.

Why might *Robinia* and other N-fixing trees, despite the large potential for supplying N, have such variable effects on forest growth? One possibility is variation in the amount of nitrogen they fix, as low rates of N fixation would not provide enough N to stimulate forest growth. N fixation rates often (Boring and Swank 1984*a*, 1984*b*; Pearson and Vitousek 2001;

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Wurzburger et al. 2022) but not always (Menge and Hedin 2009) decline as trees grow older, presumably due to some combination of changing resource availability across forest succession and ontogenetic effects. For example, *Robinia* trees in southern Appalachia fixed much more N when they were 15–20 years old than in later decades (Wurzburger et al. 2022).

A second possible explanation for why N-fixing trees might facilitate forest growth in some areas but not others is variation in site-specific environmental conditions, such as steep slopes that limit the pooling of nutrients in an area or high amounts of rainfall, which may re-distribute the soil nutrients, that determine the competitive balance of N-fixing versus nonfixing trees (Taylor et al. 2017; Staccone et al. 2021). N-fixing trees can outcompete nonfixing trees in some conditions, such as low soil N supply and high light availability, but tend to lose out in competition in other conditions such as high soil N supply (Staccone et al. 2021) and low light availability (Taylor and Menge 2018). Because N-fixing trees can transform ecosystems from low N conditions to high N conditions, one intriguing scenario is delayed facilitation, as observed in a number of models (Vitousek and Field 1999; Menge et al. 2009, 2015; Fisher et al. 2010; Levy-Varon et al. 2019; Kou-Giesbrecht et al. 2021). In these models, delayed facilitation happens for three reasons. First, whereas N-fixing trees have immediate access to their fixed N, competitors must wait until N-fixing tree tissues turn over and decompose. Second, N-fixing trees also take up soil N, and their initial rapid growth and N uptake can keep soil N availability low until soil organic N levels are sufficiently high and mineralization fluxes satisfy their demand. Third, in the models that include light competition, N-fixing trees' initial rapid growth shades the understory, but the less light-demanding nonfixers are better poised to grow when the initial cohort of N-fixing trees turns over. Other mechanisms not included in these models could lengthen the delay, such as Zinke-type (Zinke 1962; Waring et al. 2015) spatial structure of leaf litter, which might result in an individual tree recapturing a substantial fraction of the nutrients from its own leaf litter.

Given the challenges of measuring N fixation (Winbourne et al. 2018*a*; Soper et al. 2021) and the challenges of studying timescales relevant to forest succession (Chazdon et al. 2007; Feldpausch et al. 2007), it is not a surprise that we do not have a definitive answer to these questions. There is a growing number of studies looking at symbiotic N fixation through forest succession (e.g., Pearson and Vitousek 2001; Batterman et al. 2013; Sullivan et al. 2014; Bauters et al. 2016; Winbourne et al. 2018*b*; Taylor et al. 2019), including excellent work with *Robinia* (Boring and Swank 1984*a*, 1984*b*; Wurzburger et al. 2022). Uncertainties surrounding the nodule-counting method (Winbourne et al. 2018*a*; Soper et al. 2021) and the space-for-time approach inherent to chronosequences (Chazdon et al. 2007; Feldpausch et al. 2007), though, make it difficult to determine the degree to which N fixation declines during succession. There is undoubtedly substantial real variation on top of this uncertainty. A number of inventory studies have examined effects of N-fixing trees on their neighbors' growth (Taylor et al. 2017; Lai et al. 2018; Xu et al. 2020; Staccone et al. 2021), but they have not examined delayed effects, a clear gap in our understanding of these effects.

Tree rings provide decades-long time series, offering the possibility to examine long-term effects, but have not been used to explore N fixation or the competitive effects of N fixers. The growth aspect of tree rings is well established; widths of tree rings have been used to estimate stem growth rates for decades (Stokes 1996; Beghin et al. 2011). Tree rings have not, to our knowledge, been used to estimate past N fixation, but the two components needed to use this method—using natural abundance isotopes to measure N fixation and measuring N isotopes in tree rings—are well established. The natural abundance method of estimating symbiotic N fixation, which compares the isotopic signals of a focal N-fixing plant to a neighboring nonfixing ("reference") plant (Shearer and Kohl 1986), gives integrated estimates of symbiotic N fixation if the right circumstances are met (Shearer and Kohl 1986; Soper et al. 2021). Specifically, the natural abundance method requires that neighboring nonfixing trees have isotopic signatures that are sufficiently different from the signature of N fixation, which may or may not be true in a given site. The natural abundance method is typically used with foliar tissues, for which it captures fixation integrated over the lifespan of the leaf. Consequently, foliar tissues provide information on fixation of the past few months to years, but not deeper in the past.

Several studies have pioneered methods of measuring nitrogen isotope ratios in tree cores (Saurer et al. 2004; Beghin et al. 2011; Hietz et al. 2011). Because N can be recovered from dying cells and re-translocated to form new tissues during the process of converting from sapwood to heartwood (Merrill and Cowling 1966; Meerts 2002), this method is not ideal for pinpointing exact years, but over spans of multiple years it gives useful information (McLauchlan et al. 2010). Using tree cores to measure N fixation would complement the use of nodule sampling, which is currently the dominant method for measuring N fixation in mature trees (Soper et al. 2021; Wurzburger et al. 2022). Using tree cores to measure N fixation would also open up the wide array of possibilities associated with time series, such as studying delayed effects.

In this study, we used tree rings from *Robinia* trees and their neighbors to calculate the proportion of N from fixation, annual growth rates, the degree of crowding by neighboring trees, and the proportion of that crowding by *Robinia*. Following Canham et al. (2004) and others, we use the term "crowding", which combines the number, size, and proximity of neighbors, rather than competition, for two reasons. First, it is more precisely what we measured, and second, the term crowding allows for the possibility of facilitative effects as well as competitive effects, though competition for light and other resources is undoubtedly important. Using these data, we asked two questions: (1) How did the fraction of N from fixation change throughout the lifespan of *Robinia* trees? (2) To what degree were growth rates of neighboring trees facilitated or inhibited by past and current crowding from *Robinia*? We were particularly interested in the possibility that effects on tree neighbors might have delayed effects.

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Methods

Field sites

Our work took place in Black Rock Forest (41.42°N, −74.02°W), a 1550 ha forest preserve dominated by oak (*Quercus* spp.) (Schuster et al. 2008). The soils are loamy, acidic, and rocky, derived from glacial till, with low-nutrient availability (Schuster et al. 2008; Natural Resources Conservation Service 2023). Bedrock is typically found at 10–30 cm (Akana et al. 2023). The mean annual precipitation is 1200 mm and the mean monthly temperature ranges from −2.7 °C in January to 23.4 °C in July (Schuster et al. 2008). We collected data from two sites within Black Rock Forest, the Stone House (SH) site and the Upper Reservoir (UR) site. The SH site contained a total of 12 *Robinia* trees and 21 nonfixing trees (1 *Acer saccharum* Marshall, 1 *Fraxinus americana* L., 1 *Juglans nigra* L., 1 *Liriodendron tulipifera* L., 2 *Picea abies* L. H. Karst., 12 *Picea glauca* Moench Voss, and 3 *Prunus serotina* Ehrh). The SH site had been clear cut in the 1930s and was planted with *P. glauca* in the 1940s. The *Robinia* trees and other species at the SH site likely grew in naturally. The UR site contained a total of 37 *Robinia* and 46 nonfixing trees (16 *Acer rubrum* L., 1 *Betula lenta* L., 17 fm. *americana*, 2 *P. serotina*, 1 *Salix nigra* Marshall, and 3 *Quercus rubra* L, with a basal area of 48 $m^2 \cdot ha^{-1}$ (Akana et al. 2023). The UR site was burned in 1911 and by the 1930s was a pasture and farm. *Quercus*, *Fraxinus*, *Prunus*, and *Acer* were later planted on the site; *Robinia* likely grew naturally. The UR site is located near a stream and a reservoir, with various depressions. Soils in the UR plot are classified as Erie extremely stony soils (Natural Resources Conservation Service 2023). The UR site has higher rates of net N mineralization and nitrification than a nearby oak-dominated site, with a mass-based C:N ratio of 17.9 and a bulk density of 576 $kg \cdot m^{-3}$ (Akana et al. 2023). It is located at a lower elevation (280–286 m above sea level) than the SH site (between 384 and 390 m). We analyzed the sites separately because of these differences, and because the N isotopic signature of nonfixing trees only allowed a meaningful estimate of N fixation in one site (SH; see below).

Sample collection

At each site we mapped trees that were ≥10 cm diameter at breast height (1.3 m) (DBH). Specifically, we mapped all *Robinia* trees ≥ 10 cm DBH, all trees ≥ 10 cm DBH within 10 m of a *Robinia* tree ("*Robinia* neighbors"), and all trees ≥ 10 cm DBH within 10 m of a *Robinia* neighbor (to define the neighborhood crowding index (NCI) of each *Robinia* and each *Robinia* neighbor). We measured tree DBH with DBH tape. At least one core was taken at 1.3 m height from any tree that was not visibly rotten. Tree ring widths from cores were used to estimate stem growth rates and tree diameters throughout each individual tree's lifespan.

For $\delta^{15}N$ isotopic analysis, we selected *Robinia* trees that had suitable reference trees. Reference trees are critical for the success of this method since many of the assumptions of the $\delta^{15}N$ method depend on a suitable reference. Ideally, reference trees should sample from the same soil N pool as the N fixer and exhibit similar within-tissue fractionation (Shearer and Kohl 1986). Reference trees should also have lived throughout the same timespan as their corresponding N fixer because we hoped to match 5-year tree ring segments for *Robinia* trees with the same 5-year intervals for references trees. Thus, we chose focal trees that had reference trees that met the following conditions: close to the focal trees (within 10 m), alive during similar timespans (based on tree ring data; reference trees were 28–64 years and *Robinia* were 49–69 years), and species known to be the same mycorrhizal association as the focal *Robinia* trees (arbuscular mycorrhizae).

These criteria resulted in five *Robinia* and five neighboring reference trees for $\delta^{15}N$ isotopic analysis: three *Robinia* and three *Prunus* from SH, and two *Robinia* and two *Prunus* from UR. For each tree that we selected for $\delta^{15}N$ isotopic analysis, we took two cores perpendicular to each other. The trees we selected for as reference trees were all *Prunus*. More *Robinia* trees were chosen from the SH site than the UR site for $\delta^{15}N$ isotopic analysis, as there were fewer *Robinia* trees located near reference trees with arbuscular mycorrhizae at the UR site. Cores were collected from both sites between December 2018 and August 2019.

Pre-processing of tree cores and measurement of N isotopes

The *Robinia* and reference (*Prunus*) tree cores that were analyzed for $\delta^{15}N$ isotopic analysis were pre-processed by methods adapted from Sheppard and Thompson (2000). Two cores were collected for each *Robinia* tree and each reference tree. Each core was divided into 5-year segments because temporal resolution finer than 5 years could be confounded by radial translocation of N across rings after ring formation (Gerhart and McLauchlan 2014). The 5-year segments for each of the two paired cores per tree were combined for isotopic analysis. Each pair of 5-year segments was submerged in a 1:1 toluene and ethanol mixture for two nights, with the mixture replaced after the first 24 h. The mixture was decanted, and the paired segments were submerged in ethanol over one night and deionized (DI) water over the next night. This process removed any labile N compounds, secondary plant compounds, inorganic salts, and small polysaccharides. Lastly, the paired segments were dried at 60 °C and ground into a fine powder using a ball mill (GlenMills, Clifton, NJ). Each milled, paired 5-year segment sample was analyzed for $\delta^{15}N$ at the UC Davis Isotope laboratory using a PDZ-Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), giving a single $\delta^{15}N$ value for each 5-year segment for each tree. $\delta^{15}N$ was calculated based on $^{15}N/^{14}N$ isotopic ratios, using the standard formula:

$$(1) \qquad \delta^{15}N = \left(\frac{\frac{^{15}N}{^{14}N}\,\text{sample}}{\frac{^{15}N}{^{14}N}\,\text{atmospheric } N_2} - 1 \right) 1000$$

Percent of nitrogen derived from fixation

When soil N is sufficiently more ^{15}N-enriched than atmospheric N, the percent of nitrogen derived from fixation ($\%N_{dfa}$) can be determined by comparing the isotopic signature of N fixer tissue to that of nearby reference nonfixing

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plants (which are assumed to sample the same plant-available soil N pool as N fixers; Shearer and Kohl 1986). Specifically, $\%N_{dfa}$ was calculated for each *Robinia* individual i for each 5-year segment T as:

$$(2) \quad \%N_{dfa,iT} = \left(\frac{\delta_{r,jT} - \delta_{f,iT}}{\delta_{r,jT} - \delta_b}\right) 100$$

where $\delta_{r,jT}$ is the $\delta^{15}N$ value of 5-year segment T for the nonfixing reference tree(s) j matched to *Robinia* individual i, $\delta_{f,iT}$ is the $\delta^{15}N$ value from 5-year segment T from *Robinia* individual i, and δ_b is the isotopic signature of foliar N from pure N-fixation. For δ_b we used a mean of −1.2‰ (Mantovani et al. 2015) and a standard deviation of 0.5‰, given the literature range of −3‰ to 1‰, with the most common values near −1‰ (Kohl and Shearer 1980; Doughton et al. 1992; Boddey et al. 2000; Marron et al. 2018).

Despite our efforts to match reference trees to focal *Robinia*, no pairing is perfect. Furthermore, the isotopic signature of pure fixation is not known perfectly. To account for this uncertainty, we treated the end members (the reference value of $\delta_{r,jT}$ and the atmospheric value of δ_b) as distributions rather than point values. Specifically, we bootstrapped 10 000 times for each *Robinia* tree i at each 5-year time segment T to calculate distributions of $\%N_{dfa,iT}$, which we then used to calculate means and 95% confidence intervals of $\%N_{dfa,iT}$ (Taylor and Menge 2018). We assumed normal distributions of $\delta_{r,jT}$, $\delta_{f,iT}$, and δ_b. The means and standard deviations of $\delta_{r,jT}$ for the SH site were calculated from all reference trees present on the site. The means and standard deviations of $\delta_{r,jT}$ for the UR site were specific to the individual reference trees, as they were more than 10 m apart from each other and were paired with specific N fixers. Please see Lines 97–172 in supplementary file cjfr-2024-0183supple.R for details.

Measurement of ring widths and growth rates

The sample sizes for trees used to quantify ring widths (and therefore growth rates) were much higher than those used for $\delta^{15}N$ analysis. At the SH site, there were 12 *Robinia* trees and 21 nonfixing trees; at the UR site there were 37 *Robinia* trees and 46 nonfixing trees. Tree cores used for width measurements were fully dried before being glued on to wooden mounts with the vessels facing upwards. Once dry, the cores were sanded with increasingly fine sandpaper until rings were clearly visible under a sliding microscope. Years were assigned to rings starting from the outermost ring and moving inward to the pith. Annual ring widths were measured using a sliding microscope and the iMeasure computer program and confirmed using COFECHA (Holmes 1997). Annual ring widths were then used to calculate the relative diameter growth rates of each individual tree i (*Robinia* or nonfixer) in each year t, calculated as

$$(3) \quad gr_{it} = \frac{\ln(d_{i,t+\Delta t}) - \ln(d_{i,t})}{\Delta t}$$

where $d_{i,t+\Delta t}$ was twice the width of the annual ring for year t to $t + \Delta t$ (twice because diameter is twice the radius) for individual tree i. In our study, Δt was always 1 year, and diameters were measured in meters. Because relative diameter growth rate quantifies the proportional growth (given by the logarithms, which give unitless values), it has units of $year^{-1}$, unlike absolute growth rates, which would have the units of $m \cdot year^{-1}$. In cases where multiple cores per tree were taken, their mean annual ring widths were used. The number of rings present was used to determine the minimum age of the trees at coring height, as it is not always possible to core to the pith of a tree.

Neighborhood crowding index

The NCI is a metric used to quantify the degree of crowding experienced by a tree (Canham et al. 2004). In this study, as in others (e.g., Canham et al. 2004; Taylor et al. 2017; Staccone et al. 2021; and many others), crowding was used as a catch-all term to integrate potential competitive effects (such as competition for light, water, and soil nutrients) with potential facilitative effects (such as increased soil N) experienced by a tree. Crowding increases with the number of neighbors within a given radius (here, 10 m; following Canham et al. 2004), their size, and their proximity to the focal tree. Specifically, NCI was calculated for each tree i in each year t as

$$(4) \quad NCI_{total,it} = \sum \frac{DBH_{jt}^2}{d_{ji}^2}$$

where NCI of a focal tree i in year t is the sum, over all neighbors j within 10 m, of the squared DBH of neighbor j in year t divided by the squared distance (d) between neighbor j and focal tree i (Canham et al. 2004).

Our primary question for this section was about how crowding from N fixers, not crowding of any sort, affects tree growth. To determine the effect of crowding from N fixers, we calculated the proportion of NCI from N fixers for tree i in year t, following Taylor et al. (2017) and Staccone et al. (2021):

$$(5) \quad NCI_{prop,it} = \frac{NCI_{N-fixer,it}}{NCI_{total,it}}$$

Here $NCI_{total,it}$ is eq. 4, considering all neighbors, and $NCI_{N\text{-}fixer,it}$ is a version of eq. 4 including neighboring N fixers but not neighboring nonfixers.

We sought to study how crowding at various times in each tree's lifetime affected growth. Using tree rings to estimate past diameters allows us to estimate past crowding, with one important caveat. We do not have the diameters of trees that have died and fallen down, so our approach likely misses some of the past crowding, particularly for times in the more distant past.

Data analysis

To address our first question, how N fixation by *Robinia* changed through tree age, we followed the likelihood approach in Menge et al. (2015), which models how $\%N_{dfa}$ changes along a gradient (in this case, age) and incorporates uncertainty in the distribution of the end members of an isotopic mixing model (see also Phillips and Gregg 2001). Specifically, we compared two models of how $\%N_{dfa}$ might change

with tree age: a linear change with tree age and no change with tree age. Both models considered the SH and UR sites separately.

Specifically, we minimized the negative log likelihood of the *Robinia* δ^{15}N data ($\delta_{f,\,iT}$) for each model (no change in $\%N_{dfa}$ with age vs. a linear change in $\%N_{dfa}$ with age), including variation in both the end member δ^{15}N and the sample δ^{15}N as follows:

$$\text{(6)}\qquad \text{NLL} = -\sum_{iT} \ln\left(\text{Normal}\left(\delta_{f,iT} | \delta_{f,iT,\text{mean}}, \delta_{f,iT,sd}\right)\right)$$

where the expectation (mean) for a δ^{15}N sample from *Robinia* individual i at 5-year time segment T is given by a mixing model:

$$\text{(7)}\qquad \delta_{f,iT,\text{mean}} = \left(\frac{\%N_{\text{dfa},\,iT,\text{mean}}}{100}\right)\delta_{b,\text{mean}} + \left(1 - \frac{\%N_{\text{dfa},\,iT,\text{mean}}}{100}\right)\delta_{r,iT,\text{mean}}$$

and the standard deviation of the same sample, $\delta_{f,\,iT,\text{sd}}$ is given by a function of the standard deviations of pure fixation ($\delta_{b,sd}$) and the reference tree ($\delta_{r,iT,\text{sd}}$; calculated independently, as described above), along with an estimated standard deviation parameter (δ_{sd})

$$\text{(8)}\qquad \delta_{f,iT,\text{sd}} = \sqrt{\left(\left(\frac{\%N_{\text{dfa},\,iT,\text{mean}}}{100}\right)\delta_{b,\text{sd}}\right)^2 + \left(\left(1 - \frac{\%N_{\text{dfa},\,iT,\text{mean}}}{100}\right)\delta_{r,iT,\text{sd}}\right)^2 + {\delta_{\text{sd}}}^2}$$

The two alternative models are no change in $\%N_{\text{dfa},i,T,\text{mean}}$ with age, meaning that $\%N_{\text{dfa},i,T,\text{mean}}$ is a constant, and a linear change in $\%N_{\text{dfa},i,T,\text{mean}}$ with age (see Lines 173–536 in supplementary file cjfr-2024-0183supple.R, supplementary file cjfr-2024-0183supplf.xlxs). Therefore, the parameters estimated for the model where $\%N_{dfa}$ is constant with age were the mean $\%N_{dfa}$ parameter, $\%N_{\text{dfa},i,T,mean}$, and the standard deviation parameter δ_{sd}. For the model where $\%N_{dfa}$ changes with age, the parameters estimated were the slope and intercept of $\%N_{\text{dfa},i,T,\text{mean}}$ along with the standard deviation parameter δ_{sd}. For each of these models, we considered the SH and UR sites separately, given the different degrees of isotopic separation between reference plants and fixed N at the two sites.

To address our second question, how crowding by *Robinia* influenced the growth of its neighbors, we controlled for factors known to influence growth, then isolated the proportion of crowding by *Robinia* as our target driver variable. Specifically, we modeled relative growth rate (log-transformed to ensure normality) as a function of the size (DBH) of the individual (which influences radial growth via geometric relationships), the total crowding (NCI) around an individual (which is known to influence growth through competition and facilitation), and the proportion of NCI from *Robinia* (our target variable) (see Lines 24–170 in supplementary file cjfr-2024-0183supplb.R, supplementary file cjfr-2024-0183supplc.xlxs). We considered a variety of different versions of the model, including and excluding interaction terms and quadratic terms. For this model, all trees from both sites were included.

To address the possibility of delayed effects of *Robinia* on neighbor growth rates, we also considered the relationship between relative growth rate and past crowding, using separate models from 1 year ago, 2 years ago, and so on until 20 years ago (see Lines 400–521 in supplementary file cjfr-2024-0183supplb.R, supplementary file cjfr-2024-0183suppld.xlxs). We compared multiple versions of these delay models: one where both the proportion of NCI from *Robinia* and total NCI had delayed effects, and one where only the proportion of NCI from *Robinia* had a delayed effect. For example, for the version where both had a delayed effect, and the version where the delay was 5 years, we modeled relative growth rate of an individual (log-transformed, as in the nondelay model) as a function of the individual's current DBH, the total NCI from 5 years ago, and the proportion of NCI from *Robinia* from 5 years ago. As with the crowding models, the delayed effect models also used all trees from both sites. See Supplementary Text S1 for details.

We determined the best model without a delay based on the lowest Akaike information criterion (AIC) values, and also presented any other model within two ΔAIC units. AIC values were calculated using the bblme package in R. For models with effects of past crowding, we used the structure of the best fit model from the model without a delay, but with the proportion of NCI from fixers (and total NCI, in some versions) from past years. The reason we did not compare AIC values for delay models is that they contained different numbers of datapoints from the current models and from each other because not all trees were the same age, and it is inappropriate to compare models with different numbers of datapoints with information criteria. All analyses were implemented in R (R Core Team 2018).

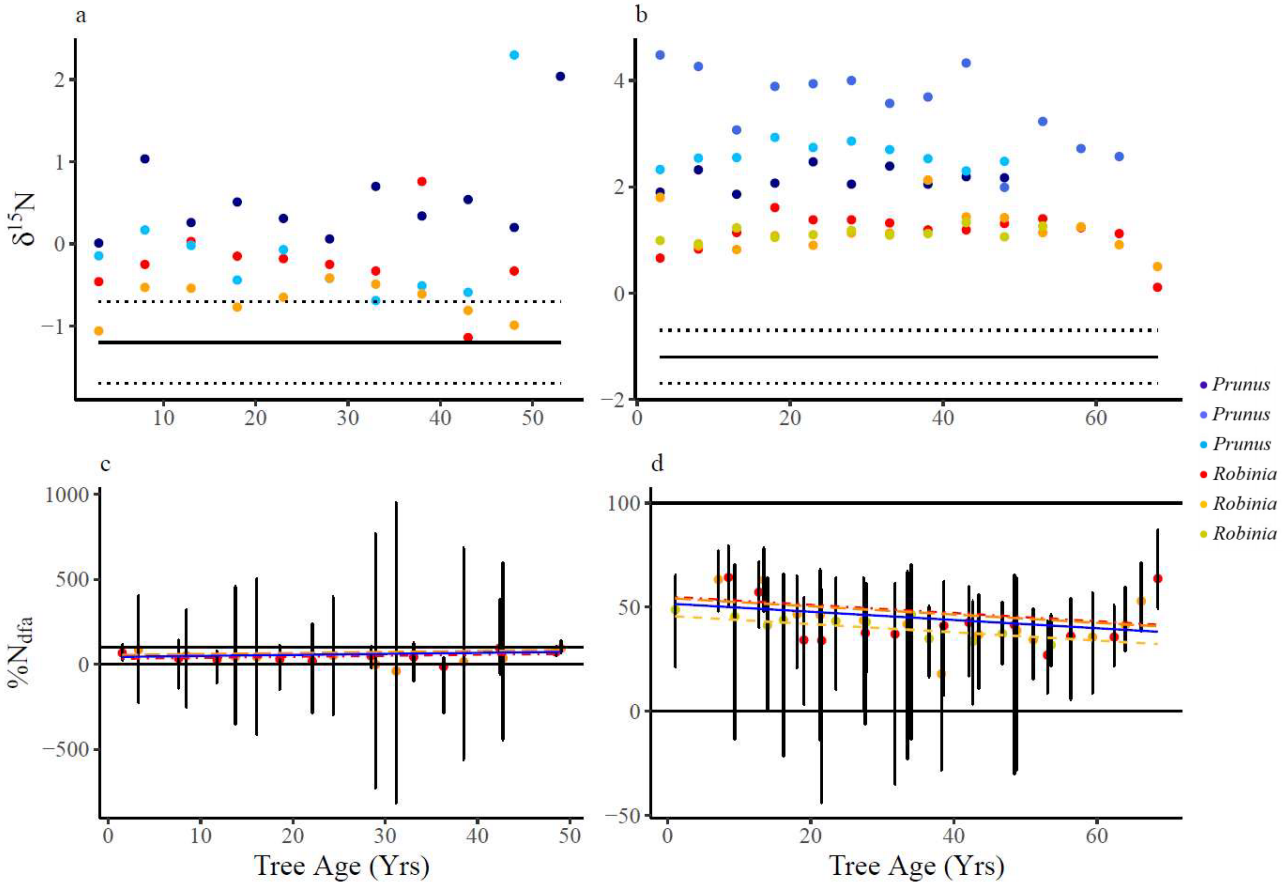
Results

How did N-fixation by Robinia trees change throughout their lifespan?

At the UR site, the estimated means of $\%N_{dfa}$ for two *Robinia* trees were 32% and 54%, with an average of 43%N_{dfa} (Supplementary Table S1; Figs. 1*a* and 1*c*). However, we have little confidence in these values, and present them mostly as a caution against mean-only calculations of $\%N_{dfa}$. We have little confidence because the isotopic signatures of the reference trees at the UR site (mean δ^{15}N of 0.3‰) were not sufficiently distinct from the signature of pure fixation (mean −1.2‰). The mean *Robinia* isotopic values for both trees combined were found to be −0.46‰ (Fig. 1*a*), leading to reasonably sound mean $\%N_{dfa}$ values, but the bootstrapped confidence

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Fig. 1. Nitrogen isotope ratios (δ^{15}N) in tree cores of *Robinia pseudoacacia* and reference trees (*Prunus serotina*) and the corresponding estimated percent of nitrogen derived from fixation (%N_{dfa}) in *Robinia* trees at different ages. Two sites within Black Rock Forest are shown: (*a* and *c*) the Upper Reservoir site and (*b* and *d*) the Stone House site. (*a* and *b*) δ^{15}N values were measured from 5-year segments of tree cores for all trees. Blue points are reference trees. Red, orange, and yellow points are *Robinia* trees. Each color corresponds to an individual tree. The black solid and dotted lines represent the mean ± standard deviation of the end member we used for the pure nitrogen fixation signal. The δ^{15}N values in (*a* and *b*) were used to calculate the %N_{dfa} values and their 95% confidence intervals in (*c* and *d*). (*c* and *d*) Each point represents the mean %N_{dfa} value for a given 5-year segment within an individual tree. Horizontal values were jittered for visibility. Each color is an individual *Robinia* tree, corresponding to their colors in (*a* and *b*). The black error bars represent the upper and lower 95% confidence intervals of the mean %N_{dfa} value. The red dash-dotted, orange long-dashed, and yellow-dashed lines are the mean %N_{dfa} values for the individual trees based on the best fit model. The blue solid line shows the mean %N_{dfa} values for all trees combined based on the best fit model. Horizontal black solid lines are placed at 0 and 100 to emphasize the difference in vertical scales in (*c*) versus (*d*).



limits on each plant's %N_{dfa} extended below 0% and above 100% (Fig. 1*c*), hence our lack of confidence in the values.

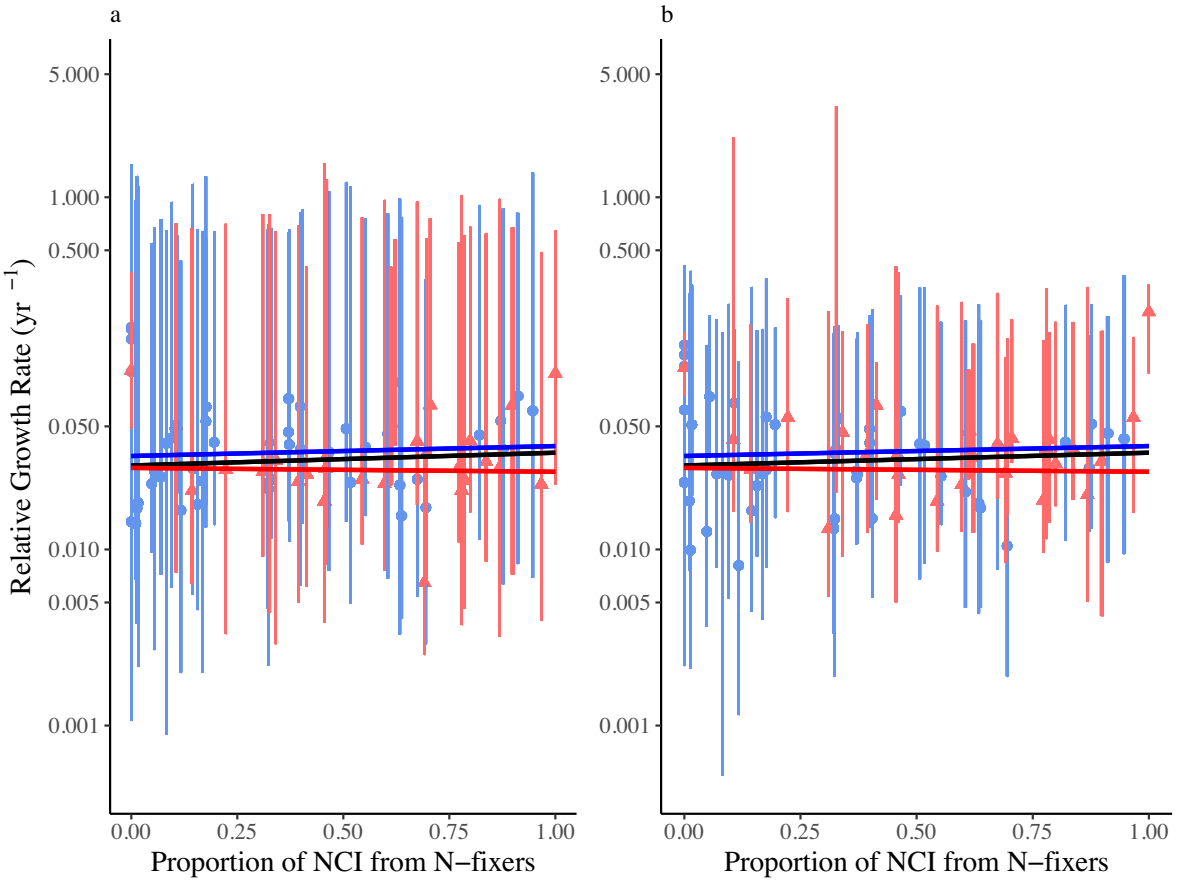
At the SH site, the mean reference δ^{15}N value was 2.8‰, which was sufficiently distinct from the pure fixation signal to give meaningful results, although the uncertainty of each estimate was still wide (Fig. 1*d*). The modeled mean value was 51%N_{dfa}, from a mean *Robinia* δ^{15}N value of 1.1‰ (Supplementary Table S1; Figs. 1*b* and 1*d*). At the SH site, the best fit model included a decline in %N_{dfa} with tree age, although the decline was very small (0.1%N_{dfa} $year^{-1}$). Numerically, this decline means that a tree that derives 60% of its N from fixation when it is 5 years old gets 50% from fixation when it is 105 years old. While this decline is statistically significant (Supplementary Table S1), it is too small to be biologically meaningful given the inherent uncertainty of this method. Therefore, our primary conclusion from this section is that a substantial fraction of *Robinia*'s N comes from N fixation throughout its lifespan.

To what degree were growth rates of neighboring trees facilitated or inhibited by past and current crowding from *Robinia*?

Our first task in analyzing the effect of *Robinia* on neighbors' growth was to determine the best-fit models for growth, so we could factor out the effects of tree size and total crowding. After that, we used the model results to address our questions about how *Robinia* affected neighbors' concurrent and future growth. Five of the models we compared (Supplementary eqs. S1–S5) did not allow N fixers and

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Fig. 2. Effect of crowding from N fixers on concurrent relative diameter growth rate (year^{-1}) for all *Robinia* trees and their nonfixing neighbors at the Stone House site or the Upper Reservoir site. Blue circles are the median growth rates of nonfixing trees of various species and red triangles are the median growth rates of *Robinia pseudoacacia* trees. Medians are across all the years of an individual tree's lifespan. Vertical lines represent the range of values for an individual tree's annual relative growth rate. (*a*) The best fit model, with the lowest AIC, had a single interaction (between the control drivers, DBH and NCI) and no quadratic term (eq. 9). The black line is the best fit for all trees together. The blue and red lines are the best fits for nonfixers and N fixers, respectively. The black, red, and blue lines are all plotted at the mean values of NCI and DBH. (*b*) The best fit models as shown in (*a*) plotted against the datapoints for average tree size with average crowding, which were determined by finding the residual for the mean growth rates and mean NCI per individual tree. This reduces noise due to tree size and total crowding. These plots show the overall effect N fixers have on neighboring trees, nonfixers, and N fixers alike, independent of tree size and total tree crowding. NCI, neighborhood crowding index.



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nonfixers to have different growth rates, whereas the other five (Supplementary eqs. S6–S10) did. We show results for both of these model types, as the former shows how *Robinia* affects the growth of its neighborhood as whole and the latter shows how *Robinia* affects the growth of nonfixers and other *Robinia* trees separately. For both model types, the one with the lowest AIC ($R^2 = 0.50$, Supplementary Table S2, black line on Fig. 2 for Supplementary eq. S3; $R^2 = 0.50$, Supplementary Table S4, red and blue lines on Fig. 2 for Supplementary eq. S8) included total crowding (NCI) and tree size (DBH) as covariates, along with an interaction between NCI and DBH (Supplementary eq. S3). For both model types, there was one additional model with ΔAIC < 2. This second-best model (ΔAIC = 1.85, $R^2 = 0.50$, Supplementary Table S3, Supplementary Table S8, black line on Supplementary Fig. S1 for Supplementary eq. S4; ΔAIC = 0.61, $R^2 = 0.50$, Supplementary Table S5, Supplementary Table S8, red and blue lines on Supplementary Fig. S1b for Supplementary eq. S9) included all the same covariates as the best model, but also included a quadratic term for crowding.

The covariate effects in these models were unsurprising. Crowding reduced tree growth (negative coefficient values for NCI_{total}, Supplementary Tables S2–S6), as expected under scenarios where trees are competing for light, water, nutrients, and other resources. Larger trees had lower relative growth (negative coefficient values for DBH, Supplementary Tables S2–S6), as expected: diameters of small trees increase by much higher percentages annually than diameters of large trees. Larger trees were less impacted by crowding than small trees (positive coefficient values for the NCI × DBH interaction, Supplementary Tables S2–S6). After accounting for these general properties of tree growth, we focused on how *Robinia*

Table 1. Expected changes in relative growth rates between a neighborhood comprised of all nonfixers and a neighborhood comprised of all *Robinia*, holding total crowding constant.

Model	N fixer effect on relative growth rate (−SE, +SE)
All tree growth (best-fit model; Supplementary eq. S3, Supplementary Table S2)	18.2% (12.5%, 24.2%)
All tree growth (second-best model; Supplementary eq. S4, Supplementary Table S3)	18.3% (12.5%, 24.2%)
N fixer growth (best-fit model; Supplementary eq. S8, Supplementary Table S4)	−5% (−20.3%, 13.5%)
N fixer growth (second-best model; Supplementary eq. S9, Supplementary Table S5)	−6.2% (−21.5%, 12%)
Nonfixer growth (best-fit model; Supplementary eq. S8, Supplementary Table S4)	13.9% (6.8%, 21.4%)
Nonfixer growth (second-best model; Supplementary eq. S9, Supplementary Table S5)	14.5% (7%, 22%)
1 year delay model, all tree growth (Supplementary eq. S11, Supplementary Table S6)	20.4% (14.8%, 26.3%)
14 year delay model, all tree growth (Supplementary eq. S11, Supplementary Table S7)	40.4% (35.6%, 45.2%)

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affected the growth of its neighbors, for concurrent growth in the following paragraph and future growth afterwards.

Being near *Robinia* rather than nonfixers enhanced concurrent tree growth. To discuss the magnitude of this *Robinia* effect, we use the term "N fixer effect" (Staccone et al. 2021), which shows how much higher (or lower) the growth of a tree would be in a neighborhood comprised entirely of *Robinia* compared to a neighborhood comprised entirely of nonfixing species, holding total crowding constant. Considering all trees together, the N fixer (*Robinia*) effect was 18% (Table 1). When we considered N fixers (*Robinia*) and nonfixers separately, we found that *Robinia* enhanced the growth of its nonfixing neighbors by 14%, but that it decreased growth of neighboring *Robinia* by 5% (Table 1).

To determine the effect of *Robinia* on future growth of its neighbors, we used the form of the best-fit concurrent model (crowding, tree size, and their interaction as covariates), but with the crowding and proportion of crowding from *Robinia* at various times in the focal tree's past rather than current values (Supplementary eq. S11, Supplementary Table S6). This analysis showed that *Robinia* increased the future growth of its neighbors more than the concurrent growth. Whereas the *Robinia* effect was 18% for concurrent growth, it was 20% for 1 year in the future (Table 1), and it continued to rise until 14 year in the future, when it was 40% (Fig. 3, Supplementary Fig. S2). That is, if a tree was exclusively crowded by *Robinia* 14 years ago it would grow 40% faster than if it was exclusively crowded by nonfixing trees 14 years ago.

Discussion

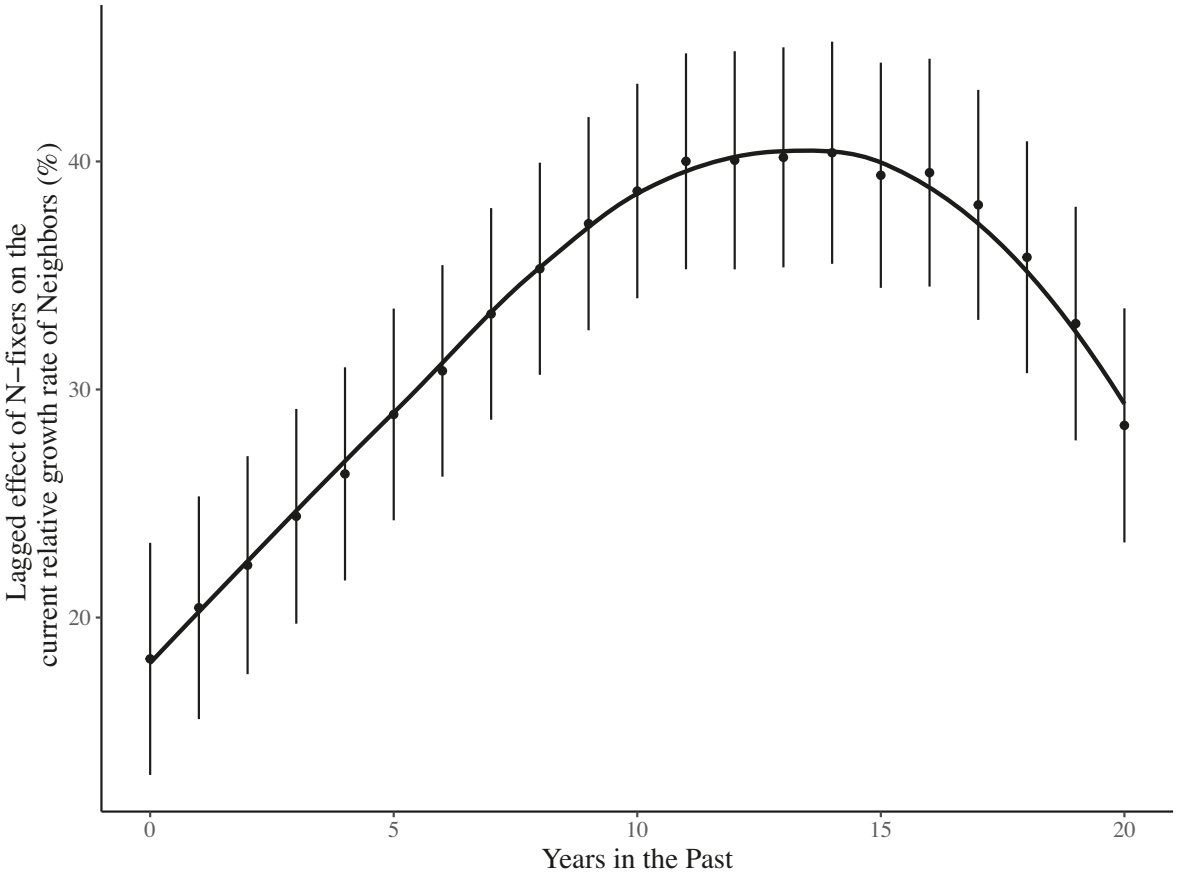
Our first question asked how N fixation by *Robinia* changed as the trees aged. In the site where we were able to answer this question, about half of the N in *Robinia* trees came from fixation throughout their lifetime as mature trees, with a negligible change as they aged from ∼5 to ∼70 years old. Below we discuss an important caveat, that our method is not as well suited to quantifying fixation in the early years of a tree's life as direct measurements during the early years. Regardless, our results suggest continued fixation at some level throughout the tree's lifespan, and this new infusion of N into the forest throughout the tree's life provided the capacity for facilitation of neighboring trees, which we addressed with our second question. We found that *Robinia* trees facilitated the growth of neighboring nonfixing trees, though they inhibited the growth of neighboring *Robinia* trees. A particularly novel aspect of our study was that we sought and detected a delay in the facilitation effect: *Robinia* presence had a larger effect on future neighbor growth than on current neighbor growth. The facilitation effect was highest with a 14-year delay.

N fixation through succession

The $\%N_{dfa}$ value we measured (51%, in the SH site) was at the lower end of the range reported in the literature for *Robinia*: averages of 59%–94% of its N from fixation (Danso et al. 1995; Mantovani et al. 2015; Marron et al. 2018; Menge et al. 2023). Why are the N fixation ($\%N_{dfa}$) values in our study at the low end of the range? We expect the main reasons are the age and environmental settings of the trees, but first, we consider other differences ranging from location to experimental treatment to isotopic technique to tissue type. Location is likely not a major driver of the low values we observed, as a study in the same forest in New York where we worked (Menge et al. 2023) found a similar range of $\%N_{dfa}$ values as studies in Austria (Danso et al. 1995), Germany (Mantovani et al. 2015), and France (Marron et al. 2018). Experimental treatment is also not likely the explanation: although fertilization (Menge et al. 2023) and drought (Mantovani et al. 2015) treatments caused some differences in $\%N_{dfa}$, control treatments in those studies were at the higher end of the $\%N_{dfa}$ range, and all trees in the present study were effectively controls. Isotopic technique is also not a likely driver of the relatively low values we observed, as previous studies using natural abundance (Mantovani et al. 2015; Marron et al. 2018) and enriched (Danso et al. 1995; Marron et al. 2018; Menge et al. 2023) isotopes yielded similar ranges. Tissue type might play a small role. Whereas Mantovani et al. (2015) and Menge et al. (2023) used foliar tissue, and Danso et al. (1995) and Marron et al. (2018) used whole plant tissue, we used wood tissue. Danso et al. (1995) found somewhat higher estimates of $\%N_{dfa}$ from foliage only (93%) compared to all aboveground tissues (81%) or all tissues (79%).

The two major differences between our study and the others (Danso et al. 1995; Mantovani et al. 2015; Marron et al. 2018; Menge et al. 2023) that are most likely to explain the lower values we observed are tree age and the environmental setting. Whereas all the previous studies grew

Fig. 3. Effect of past crowding by *Robinia pseudoacacia* on the relative growth rate of neighboring trees. Each black dot is calculated from a coefficient value related to the response of relative growth rate to the proportion of crowding from N fixers at different times in the past, from 1 to 20 years. Specifically, the plotted coefficient values are N fixer effects, as in Staccone et al. (2021), which describe the percent change in relative growth rate in a scenario where all neighbors (at a given time in the past) were N fixers versus a scenario where all neighbors (at the same time) were all nonfixers. The black curve is the loess fit for the data points. The error bars are the standard errors predicted from the model. This figure shows that Robinia increases the future growth of its neighbors more than the concurrent growth. Whereas the Robinia effect was 18% for concurrent growth, it was 40% 14 years in the future. That is, if a tree's crowding from 14 years ago was exclusively from Robinia rather than exclusively from nonfixing trees, it would grow 40% faster today.



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trees 6 years old or younger in open fields or pots, we studied trees up to approximately 70 years old in a forest. Although we found no major effect of tree age on $\%N_{dfa}$ at the scale of decades, with about half of the trees' N coming from fixation from ~5 to 70 years old, tree rings are not well suited to studying the earliest ages of a tree's life. Tree rings measured at 1.3 m height only start when trees reach 1.3 m in height, which could take years. Nodule-based studies suggest that N fixation peaks early in *Robinia*'s lifespan (5 years or younger for $\%N_{dfa}$, 5–15 years for N fixation rates; Boring and Swank 1984*b*; Wurzburger et al. 2022), agreeing with the isotopic evidence of high $\%N_{dfa}$ in younger *Robinia* (Danso et al. 1995; Mantovani et al. 2015; Marron et al. 2018; Menge et al. 2023).

Delayed facilitation

We found that *Robinia* facilitates the concurrent growth of neighboring trees but inhibits the concurrent growth of surrounding *Robinia*, which is the average finding for *Robinia* across the USA (Staccone et al. 2021) and well within the range of effects for other N-fixing trees across the world (van Kessel et al. 1994; Forrester et al. 2006; Piotto 2008; Siddique et al. 2008; Hulvey et al. 2013; Hoogmoed et al. 2014; Chapin et al. 2016; Taylor et al. 2017; Lai et al. 2018; Xu et al. 2020). The main hypothesized mechanism of facilitation is the supply of N to the soil, which is expected to operate with a delay (Vitousek and Field 1999; Menge et al. 2009, 2015; Fisher et al. 2010). A delay is expected because, to reach neighboring trees, fixed N must return from the N fixer to the soil as organic matter, be decomposed, then be taken up by the neighboring nonfixing tree. Multiple such cycles might be required, given that litter-derived nutrients are more likely to return to the tree that dropped the litter than a neighbor. With our tree ring approach, we were able to test the possibility of delayed facilitation.

Consistent with our expectation, we found that the presence of N fixers facilitated the future growth of neighboring trees to a greater degree than it did current growth, a

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dynamic which to our knowledge has not been previously quantified in the literature. Specifically, we found that the degree to which *Robinia* facilitated their neighbors rose for 14 years. We did not quantify soil N fluxes under individual trees over the timespan of these trees' lives, so we cannot pinpoint the mechanism of delayed facilitation, but recent measurements are consistent with the mechanism of soil N supply. Specifically, net N mineralization and net nitrification measured in 2020, 1–1.5 years after we sampled tree cores, were higher in our UR site (61% *Robinia* by basal area) than in a nearby site without *Robinia* (Akana et al. 2023). Furthermore, within the UR site, the hotspots of net N mineralization were co-located with stems of the *Robinia* trees we studied (Akana et al. 2023). As further evidence of the soil N supply mechanism, the timescale of the delay we quantified is consistent with expectations from models that assume the mechanism of N supply through the soil, which show that N-fixing trees facilitate nonfixers within a few decades (Vitousek and Field 1999; Menge et al. 2009, 2015; Fisher et al. 2010).

Our detection of delayed facilitation helps make sense of disparate findings from previous studies. Longer term studies of plantations and regeneration on degraded lands (Schuster and Hutnik 1987; Piotto 2008; Hulvey et al. 2013) tend to find that N-fixing trees facilitate neighbors and forest growth, whereas inventory-based studies have found a range of results from facilitation to inhibition, with averages tending to no effect (Chapin et al. 2016; Taylor et al. 2017; Lai et al. 2018; Xu et al. 2020; Staccone et al. 2021). There are many differences between these studies that could help explain the differences, but a delayed facilitation effect would reconcile them, as plantation studies would be more likely to observe the delayed effects than inventory studies with remeasurement periods of a year to a decade. Delayed facilitation is also an important finding for forest restoration and management. Specifically, it suggests that efforts to plant N-fixing trees might not pay off in full for more than a decade.

Tales from N isotopes

Although N-fixation rates are the focus of our use of isotopes in this study, the N isotopes from tree rings provide two other useful pieces of information. In past work, tree ring $\delta^{15}N$ values of nonfixing trees have been used to document trends in the global nitrogen cycle, spanning from the 1850s–2010s, which has been interpreted to reflect both an increase (Hietz et al. 2011) and a decrease (Mason et al. 2022) in N availability, though others have called into question the use of N isotopes to infer N availability (Vitousek et al. 2024). Although we observed substantial variation across individuals and our two sites, our nonfixing trees did not show consistent temporal trends throughout their 50–70-year lifespans (Figs. 1*a* and 1*b*).

The use of natural abundances of nitrogen isotopes to estimate $\%N_{dfa}$ has well-known caveats (Shearer and Kohl 1986; Soper et al. 2015, 2021). Specifically, when the reference (nonfixing) plant isotopic values are too near the signal of pure fixation (i.e., there is a lack of "end member separation"), the calculation can yield estimates $\%N_{dfa}$ that are negative or over 100% or simply too uncertain to be meaningful. In our case, point estimates of the isotopic separation suggested that end member separation was adequate in both sites, and therefore, both sites gave reasonable (well within 0%–100%) mean estimates of N fixation. However, when we considered the variation in the end members and propagated error, the uncertainty bounds of $\%N_{dfa}$ estimates at the UR site went well beyond 0% and 100%, making it clear that this site did not meet the conditions for the method despite the reasonable mean values. There are many potential explanations for why the SH site met the conditions for the method but the UR site did not, such as potential differences in nitrification or denitrification, both of which fractionate against the heavier ^{15}N and thereby modify the isotopic composition of soil nitrogen (Robinson 2001). There may also be sources of fixed N aside from *Robinia*, such as free-living bacteria in leaf litter and soil or other cryptic sources (Cleveland et al. 2022), that might differ between our sites. These differences could result from localized differences in soil properties, even though our sites are within the same forest. However, we cannot draw any concrete conclusions about the causes of different reference plant values without a detailed isotopic accounting of the N cycle. Overall, our results highlight the importance of statistical rigor and luck in applying the natural abundance isotope method.

N fixation is notoriously difficult to measure with any method (Shearer and Kohl 1986; Unkovich and Pate 2000; Soper et al. 2015, 2021; Winbourne et al. 2018*a*), so it is useful to consider all options. While there are other methods to measure N fixation, it is rate to be able to use these methods to study decades worth of N fixation without resorting to chronosequences, which have problems associated with space-for-time substitution (Chazdon et al. 2007). Isotope pool dilution artificially enriches the soil N pool with ^{15}N-labelled compounds to compare tissue ^{15}N of fixers to reference plants (Soper et al. 2021), and has been successfully used to determine $\%N_{dfa}$ of *Robinia* at Black Rock Forest (Menge et al. 2023). Pool dilution is logistically infeasible for longer term studies, though, as enriched N, which is expensive, would need to be added for decades. Long-term isotope dilution typically means less than a decade (Mead and Preston 1992). Quantifying nodule abundance and activity is common and has advantages as well as disadvantages (Winbourne et al. 2018*a*; Soper et al. 2021). However, long-term measurements require multiple decades of painstaking field work and typically have large temporal gaps (Wurzburger et al. 2022). Natural abundance measurements of leaves (Shearer and Kohl 1986; Soper et al. 2021) have the issues already described herein for tree rings, except that they are limited to the lifespan of leaves, so decades of leaf sampling would be needed to extend a dataset to the same timespan we covered with a single time point of tree coring. The primary advantage of using tree core natural abundance is the multi-decade timespan it allows. Its drawbacks are the standard drawbacks for the natural abundance method (Soper et al. 2021). The primary challenge, which we encountered here, is finding a site where suitable reference plants exist and have isotopic signatures sufficiently distinct from pure N fixation (Shearer and Kohl 1986). Challenges in interpretation include ascribing the timing of N fixation. For example, a 30-year-old tree ring with $50\%N_{dfa}$ indicates that

half of the N in that ring has the isotopic signature of N fixation, but it does not specify whether that N was fixed that year and immediately put into wood or fixed multiple years ago and stored for years before being used to construct the ring.

Conclusion

This study makes two main contributions. It is the first to our knowledge to quantify N fixation (specifically, $\%N_{dfa}$) from tree core samples and the first to quantify delayed facilitation of N-fixing trees on their neighbors. It does so using *Robinia pseudoacacia*, the most common N-fixing tree in the USA (Staccone et al. 2020), at the northern edge of its range. *Robinia* fixed about half of its nitrogen throughout seven decades of its adult life, and helped its nonfixing neighbors grow faster, particularly after a delay of 14 years. Our hope is that the tree-core $\delta^{15}N$ method will become an additional tool in ecosystem ecologists' toolbox, and that others will delve into the details of delayed facilitation in other settings. Delayed facilitation of N-fixing trees has important implications for understanding fundamental forest ecology as well as for forest management.

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Data availability

Data generated or analyzed during this study are provided in full within the Supplementary Information provided with the published article.

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Visualization: AH
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Competing interests

There are no competing interests.

Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/cjfr-2024-0183.

References

Akana, P.R., Mifsud, I.E.J., and Menge, D.N.L. 2023. Soil nitrogen availability in a temperate forest exhibits large spatial variability at sub-tree spatial scales. Biogeochemistry, **164**: 537–553. doi:10.1007/s10533-023-01056-5.

Batterman, S.A., Hedin, L.O., van Breugel, M., Ransijn, J., Craven, D.J., and Hall, J.S. 2013. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. Nature, **502**(7470): 224–227. doi:10.1038/nature12525. PMID: 24037375.

Bauters, M., Mapenzi, N., Kearsley, E., Vanlauwe, B., and Boeckx, P. 2016. Facultative nitrogen fixation by legumes in the central Congo basin is downregulated during late successional stages. Biotropica, **48**(3): 281–284. doi:10.1111/btp.12312.

Beghin, R., Cherubini, P., Battipaglia, G., Siegwolf, R., Saurer, M., and Bovio, G. 2011. Tree ring growth and stable isotopes (^{13}C and ^{15}N) detect effects of wildfires on tree physiological processes in *Pinus sylvestris* L. Trees, **25**: 627–636. doi:10.1007/s00468-011-0539-9.

Binkley, D., Cromack, K., Jr., and Baker, D.D. 1994. Nitrogen fixation by red alder: biology, rates, and controls. The biology and management of red alder. Oregon State University Press, Corvallis. pp. 57–72.

Binkley, D., Sollins, P., Bell, R., Sachs, D., and Myrold, D. 1992. Biogeochemistry of adjacent conifer and alder-conifer stands. Ecology, **73**(6): 2022–2033. doi:10.2307/1941452.

Boddey, R.M., Peoples, M.B., Palmer, B., and Dart, P.J. 2000. Use of the ^{15}N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutr. Cycling Agroecosyst. **57**: 235–270. doi:10.1023/A:1009890514844.

Boring, L.R., and Swank, W.T. 1984a. Symbiotic nitrogen fixation in regenerating black locust (*Robinia pseudoacacia* L.) stands. For. Sci. **30**(2): 528–537.

Boring, L.R., and Swank, W.T. 1984b. The role of black locust (*Robinia pseudoacacia*) in forest succession. J. Ecol. **72**: 749–766. doi:10.2307/2259529.

Brookshire, E.N.J., Wurzburger, N., Currey, B., Menge, D.N.L., Oatham, M.P., and Roberts, C. 2019. Symbiotic N fixation is sufficient to support net aboveground biomass accumulation in a humid tropical forest. Sci. Rep. **9**(1):1–10. doi:10.1038/s41598-019-43962-5. PMID: 30626917.

Canham, C., LePage, P., and Coates, K. 2004. A neighborhood analysis of canopy tree competition: effects of shading versus crowding. Can. J. For. Res. **34**(4): 778–787. doi:10.1139/x03-232.

Chapin, F.S., Conway, A.J., Johnstone, J.F., Hollingsworth, T.N., and Hollingsworth, J. 2016. Absence of net long-term successional facilitation by alder in a boreal Alaska floodplain. Ecology, **97**(11): 2986–2997. doi:10.1002/ecy.1529. PMID: 27870053.

Chazdon, R.L., Letcher, S.G., Van Breugel, M., Martínez-Ramos, M., Bongers, F., and Finegan, B. 2007. Rates of change in tree communities of secondary neotropical forests following major disturbances.

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Philos. Trans. R. Soc. London Ser. B Philos. Trans. Biol. Sci. **362**: 273–289. doi:10.1098/rstb.2006.1990.

Cleveland, C.C., Reis, C.R.G., Perakis, S.S., Dynarski, K.A., Batterman, S.A., Crews, T.E., et al. 2022. Exploring the role of cryptic nitrogen fixers in terrestrial ecosystems: A frontier in nitrogen cycling research. Ecosystems, **25**: 1653–1669. doi:10.1007/s10021-022-00804-2.

Danso, S.K.A., Zapata, F., and Awonkaike, K.O. 1995. Measurement of biological N_2 fixation in field-grown *Robinia pseudoacacia* L. Soil Biol. Biochemistry, **27**(4–5): 415–419. doi:10.1016/0038-0717(95)98612-R.

Doughton, J.A., Vallis, I., and Saffigna, P.G. 1992. An indirect method for estimating ^{15}N isotope fractionation during nitrogen fixation by a legume under field conditions. Plant Soil, **144**:23–29. doi:10.1007/BF00018841.

Feldpausch, T.R., Prates-Clark, C., da, C., Fernandes, E.C.M., and Riha, S.J. 2007. Secondary forest growth deviation from chronosequence predictions in central Amazonia. Global Change Biol. **13**: 967–979. doi:10.1111/j.1365-2486.2007.01344.x.

Fisher, J.B., Sitch, S., Malhi, Y., Fisher, R.A., Huntingford, C., and Tan, S.Y. 2010. Carbon cost of plant nitrogen acquisition: a mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. Global Biogeochem. Cycles, **24**(1). doi:10.1029/2009GB003621.

Forrester, D.I., Bauhus, J., Cowie, A.L., and Vanclay, J.K. 2006. Mixed-species plantations of Eucalyptus with nitrogen-fixing trees: a review. For. Ecol. Manage. **233**(2–3): 211–230. doi:10.1016/j.foreco.2006.05.012.

Gerhart, L.M., and McLauchlan, K.K. 2014. Reconstructing terrestrial nutrient cycling using stable nitrogen isotopes in wood. Biogeochemistry, **120**(1–3): 1–21. doi:10.1007/s10533 014-9988-8.

Hietz, P., Turner, B.L., Wanek, W., Richter, A., Nock, C.A., and Wright, S.J. 2011. Long-term change in the nitrogen cycle of tropical forests. Science, **334**: 664–666. doi:10.1126/science.1211979. PMID: 22053047.

Holmes, R.L. 1997. The dendrochronology program library. *In* The international tree-ring data bank program library version 2.1 user's manual. *Edited by* H.D. Grission-Mayer, R.L. Holmes and H.C. Fritts. Laboratory of Tree-Ring Research, The University of Arizona, Tucson, AZ. pp. 40–71.

Hoogmoed, M., Cunningham, S.C., Baker, P., Beringer, J., and Cavagnaro, T.R. 2014. N-fixing trees in restoration plantings: effects on nitrogen supply and soil microbial communities. Soil Biol. Biochemistry, **77**: 203–212. doi:10.1016/j.soilbio.2014.06.008.

Hulvey, K.B., Hobbs, R.J., Standish, R.J., Lindenmayer, D.B., Lach, L., and Perring, M.P. 2013. Benefits of tree mixes in carbon plantings. Nat. Clim. Change, **3**(10): 869–874. doi:10.1038/nclimate1862.

Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y., and Field, C.B. 2003. Nitrogen and climate change. Science, **302**(5650): 1512–1513. doi:10.1126/science.1091390. PMID: 14645831.

Kohl, D.H., and Shearer, G. 1980. Isotopic fractionation associated with symbiotic N_2 fixation and uptake of NO_3^- by plants. Plant Physiol. **66**: 51–56. doi:10.1104/pp.66.1.51. PMID: 16661392.

Kou-Giesbrecht, S., Malyshev, S., Cano, I.M., Pacala, S.W., Shevliakova, E., Bytnerowicz, T.A., and Menge, D.N.L. 2021. A novel representation of biological nitrogen fixation and competitive dynamics between nitrogen-fixing and non-fixing plants in a land model (GFDL LM4.1-BNF). Biogeosciences, **18**: 4143–4183. doi:10.5194/bg-18-4143-2021.

Lai, H.R., Hall, J.S., Batterman, S.A., Turner, B.L., and van Breugel, M. 2018. Nitrogen fixer abundance has no effect on biomass recovery during tropical secondary forest succession. J. Ecol. **106**(4): 1415–1427. doi:10.1111/1365-2745.12979.

LeBauer, D.S., and Treseder, K.K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology, **89**(2): 371–379. doi:10.1890/06-2057.1. PMID: 18409427.

Levy-Varon, J.H., Batterman, S.A., Medvigy, D., Xu, X., Hall, J.S., van Breugel, M., and Hedin, L.O. 2019. Tropical carbon sink accelerated by symbiotic dinitrogen fixation. Nat. Commun. **10**(1): 5637. doi:10.1038/s41467-019-13656-7. PMID: 31822758.

Liao, W., and Menge, D.N.L. 2016. Demography of symbiotic nitrogen-fixing trees explains their rarity and successional decline in temperate forests in the United States. PLoS One, **11**(10): 1–13. doi:10.1371/journal.pone.0164522.

Mantovani, D., Veste, M., Boldt-Burisch, K., Fritsch, S., Koning, L.A., and Freese, D. 2015. Carbon allocation, nodulation, and biological nitrogen fixation of black locust (*Robinia pseudoacacia* L.) under soil water limitation. Annals For. Res. **58**: 1. doi:10.15287/afr.2015.420.

Marron, N., Gana, C., Gerant, D., Maillard, P., Priault, P., and Epron, D. 2018. Estimating symbiotic N_2 fixation in *Robinia pseudoacacia*. J. Plant Nutr. Soil Sci. **181**(2): 296–304. doi:10.1002/jpln.201700503.

Mason, R., Craine, J.M., Lany, N.K., Jonard, M., Ollinger, S.V., Groffman, P.M., et al. 2022. Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems. Science, **376**: eabh3767. doi:10.1126/science.abh3767. PMID: 35420945.

McLauchlan, K., Ferguson, C., Wilson, I., Ocheltree, T.W., and Craine, J.M. 2010. Thirteen decades of foliar isotopes indicate declining nitrogen availability in central North American grasslands. New Phytol. **187**(4): 1135–1145. doi:10.1111/j.1469-8137.2010.03322.x. PMID: 20553396.

Mead, D.J., and Preston, C.M. 1992. Nitrogen fixation in Sitka alter by ^{15}N isotope dilution after eight growing seasons in a lodgepole pine site. Can. J. For. Res. **22**: 1192–1194. doi:10.1139/x92-158.

Meerts, P. 2002. Mineral nutrient concentrations in sapwood and heartwood: a literature review. Annals For. Sci. **59**: 713–722. doi:10.1051/forest:2002059.

Menge, D.N.L., and Hedin, L.O. 2009. Nitrogen fixation in different biogeochemical niches along a 120, 000 year-chronosequence in New Zealand. Ecology, **90**: 2190–2201. doi:10.1890/08-0877.1. PMID: 19739381.

Menge, D.N.L., Levin, S.A., and Hedin, L.O. 2009. Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. Am. Nat. **174**(4): 465–477. doi:10.1086/605377. PMID: 19694561.

Menge, D.N.L., Wolf, A.A., and Funk, J.L. 2015. Diversity of nitrogen fixation strategies in Mediterranean legumes. Nat. Plants, **1**. doi:10.1038/nplants.2015.64. PMID: 27250004.

Menge, D.N.L., Wolf, A.A., Funk, J.L., Perakis, S.S., Akana, P.R., Arkebauer, R., et al. 2023. Tree symbioses sustain nitrogen fixation despite excess nitrogen supply. Ecol. Monogr. **e1562**.

Merrill, W., and Cowling, E.B. 1966. Role of nitrogen in wood deterioration: amounts and distribution of nitrogen in tree stems. Can. J. Bot. **44**: 1555–1580. doi:10.1139/b66-168.

Natural Resources Conservation Service. 2023. Web soil survey. United States Department of Agriculture. Available from http://websoilsurvey.sc.egov.usda.gov/ [accessed 14 April 2023].

Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., et al. 2011. A large and persistent carbon sink in the world's forests. Science, **333**(6045): 988–993. doi:10.1126/science.1201609. PMID: 21764754.

Pearson, H.L., and Vitousek, P.M. 2001. Stand dynamics, nitrogen accumulation, and symbiotic nitrogen fixation in regenerating stands of Acacia koa. Ecol. Appl. **11**(5): 1381–1394. doi:10.1890/1051-0761(2001)011[1381:SDNAAS]2.0.CO;2.

Phillips, D.L., and Gregg, J.W. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia, **127**: 171–179. doi:10.1007/s004420000578. PMID: 24577646.

Piotto, D. 2008. A meta-analysis comparing tree growth in monocultures and mixed plantations. For. Ecol. Manage. **255**(3–4): 781–786. doi:10.1016/j.foreco.2007.09.065.

R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/ [accessed 15 November 2018].

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. Trends Ecol. Evol. **16**: 153–162. doi:10.1016/S0169-5347(00)02098-X. PMID: 11179580.

Ruess, R.W., McFarland, J.M., Trummer, L.M., and Rohrs-Richey, J.K. 2009. Disease mediated declines in N-fixation inputs by *Alnus tenuifolia* to early-successional floodplains in interior and south-central Alaska. Ecosystems, **12**: 489–502. doi:10.1007/s10021-009 9237-5.

Saurer, M., Cherubini, P., Ammann, M., De Cinti, B., and Siegwolf, R. 2004. First detection of nitrogen from NO_x in tree rings: A $^{15}N/^{14}N$ study near a motorway. Atmos. Environ. **38**: 2779–2787. doi:10.1016/j.atmosenv.2004.02.037.

Schuster, W., and Hutnik, R. 1987. Community development on 35-year-old planted mine spoil banks in Pennsylvania. Reclam. Reveg. Res. **6**: 109–120.

Schuster, W.S.F., Griffin, K.L., Roth, H., Turnbull, M.H., Whitehead, D., and Tissue, D.T. 2008. Changes in composition, structure and aboveground biomass over seventy-six years (1930-2006) in the Black Rock

Forest, Hudson Highlands, southeastern New York State. Tree Physiol. **28**: 537–549. doi:10.1093/treephys/28.4.537. PMID: 18244941.

Shearer, G., and Kohl, D.H. 1986. N_2-fixation in field settings: estimations based on natural ^{15}N abundance. Funct. Plant Biol. **13**: 699–756.

Sheppard, P.R., and Thompson, T.L. 2000. Effect of extraction pretreatment on radial variation of nitrogen concentration in tree rings. J. Environ. Qual. **29**: 2037–2042. doi:10.2134/jeq2000.00472425002900060042x.

Siddique, I., Engel, V.L., Parrotta, J.A., Lamb, D., Nardoto, G.B., Ometto, J.P.H.B., et al. 2008. Dominance of legume trees alters nutrient relations in mixed species forest restoration plantings within seven years. Biogeochemistry, **88**(1): 89–101. doi:10.1007/s10533-008-9196-5.

Soper, F.M., Boutton, T.W., and Sparks, J.P. 2015. Investigating patterns of symbiotic nitrogen fixation during vegetation change from grassland to woodland using fine scale $\delta^{15}N$ abundance. Funct. Plant Biol. **13**: 699–756.

Soper, F.M., Taylor, B.N., Winbourne, J.B., Wong, M.Y., Dynarski, K.A., Reis, C.R., et al. 2021. A roadmap for sampling and scaling biological nitrogen fixation in terrestrial ecosystems. Methods Ecol. Evol. **12**(6): 1122–1137. doi:10.1111/2041-210X.13586.

Staccone, A., Liao, W., Perakis, S., Compton, J., Clark, C.M., and Menge, D.N.L. 2020. A spatially explicit, empirical estimate of tree-based biological nitrogen fixation in forests of the United States. Global Biogeochem. Cycles, **34**. doi:10.1029/2019GB006241. PMID: 32665747.

Staccone, A.P., Kou-Giesbrecht, S., Taylor, B.N., and Menge, D.N.L. 2021. Nitrogen-fixing trees have no net effect on forest growth across the coterminous United States. J. Ecol. **109**: 877–887. doi:10.1111/1365-2745.13513.

Stokes, M.A. 1996. An introduction to tree-ring dating. University of Arizona Press.

Sullivan, B.W., Smith, W.K., Townsend, A.R., Nasto, M.K., Reed, S.C., Chazdon, R.L., and Cleveland, C.C. 2014. Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical N cycle. Proc. Natl. Acad. Sci. U.S.A. **10**. doi:1320646111.

Sulman, B.N., Shevliakova, E., Brzostek, E.R., Kivlin, S.N., Malyshev, S., Menge, D.N.L., and Zhang, X. 2019. Diverse mycorrhizal associations enhance terrestrial C storage in a global model. Global Biogeochem. Cycles, **33**(4): 501–523. doi:10.1029/2018GB005973.

Taylor, B.N., and Menge, D.N.L. 2018. Light regulates tropical symbiotic nitrogen fixation more strongly than soil nitrogen. Nat. Plants, **4**(9): 655–661. doi:10.1038/s41477-018-0231-9. PMID: 30127409.

Taylor, B.N., Chazdon, R.L., and Menge, D.N.L. 2019. Successional dynamics of nitrogen fixation and forest growth in regenerating Costa Rican rainforests. Ecology, **100**(4): e02637. doi:10.1002/ecy.2637. PMID: 30698284.

Taylor, B.N., Chazdon, R.L., Bachelot, B., and Menge, D.N.L. 2017. Nitrogen-fixing trees inhibit growth of regenerating Costa Rican rainforests. Proc. Natl. Acad. Sci. U.S.A. **114**(33): 8817–8822. doi:10.1073/pnas.1707094114.

Unkovich, M.J., and Pate, J.S. 2000. An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. Field Crops Res. **65**(2–3): 211–228. doi:10.1016/S0378-4290(99)00088-X.

van Kessel, C., Farrell, R.E., Roskoski, J.P., and Keane, K.M. 1994. Recycling of the naturally-occurring ^{15}N in an established stand of *Leucaena leucocephala*. Soil Biol. Biochemistry, **26**(6): 757–762. doi:10.1016/0038-0717(94)90269-0.

Vitousek, P.M., and Field, C.B. 1999. Ecosystem constraints to symbiotic nitrogen fixers: a simple model and its implications. Biogeochemistry, **46**: 179–202. doi:10.1007/BF01007579.

Vitousek, P.M., Cen, X., and Groffman, P.M. 2024. Has nitrogen availability decreased over much of the land surface in the past century? A model-based analysis. Biogeochemistry, **167**: 793–806. doi:10.1007/s10533-024-01146-y.

Vitousek, P.M., Menge, D.N.L., Reed, S.C., and Cleveland, C.C. 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos. Trans. R. Soc. B, **368**(1621): 20130119. doi:10.1098/rstb.2013.0119.

Waring, B.G., Álvarez-Cansino, L., Barry, K.E., Becklund, K.K., Dale, S., Gei, M.G., et al. 2015. Pervasive and strong effects of plants on soil chemistry: a meta-analysis of individual plant "Zinke" effects. Proc. Biol. Sci. **282**(1812): 20151001. doi:10.1098/rspb.2015.1001.

Wieder, W.R., Cleveland, C.C., Smith, W.K., and Todd-Brown, K. 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. Nat. Geosci. **8**(6): 441–444. doi:10.1038/ngeo2413.

Winbourne, J.B., Feng, A., Reynolds, L., Piotto, D., Hastings, M.G., and Porder, S. 2018b. Nitrogen cycling during secondary succession in Atlantic Forest of Bahia, Brazil. Sci. Rep. **8**: 1377. doi:10.1038/s41598-018-19403-0.

Winbourne, J.B., Harrison, M.T., Sullivan, B.W., Alvarez-Clare, S., Lins, S.R., Martinelli, L., et al. 2018a. A new framework for evaluating estimates of symbiotic nitrogen fixation in forests. Am. Nat. **192**(5): 618–629. doi:10.1086/699828. PMID: 30332582.

Wurzburger, N., Motes, J.I., and Miniat, C.F. 2022. A framework for scaling symbiotic nitrogen fixation using the most widespread nitrogen fixer in eastern deciduous forests of the United States. J. Ecol. **110**(3): 569–581. doi:10.1111/1365-2745.13819.

Xu, H., Detto, M., Fang, S., Chazdon, R.L., Li, Y., Hau, B.C., et al. 2020. Soil nitrogen concentration mediates the relationship between leguminous trees and neighbor diversity in tropical forests. Commun. Biol. **3**(1): 317. doi:10.1038/s42003-020-1041-y. PMID: 32561898.

Zinke, P.J. 1962. The pattern of influence of individual forest trees on soil properties. Ecology, **43**(1): 130–133. doi:10.2307/1932049.

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