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RESEARCH ARTICLE

Leaf nitrogen affects photosynthesis and water use efficiency similarly in nitrogen-fixing and non-fixing trees

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Abstract

1. Nitrogen (N)-fixing trees are thought to break a basic rule of leaf economics: higher leaf N concentrations do not translate into higher rates of carbon assimilation. Understanding how leaf N affects photosynthesis and water use efficiency (WUE) in this ecologically important group is critical.
2. We grew six N-fixing and four non-fixing tree species for 4–5 years at four fertilization treatments in field experiments in temperate and tropical regions to assess how functional type (N fixer vs. non-fixer) and N limitation affected leaf N and how leaf N affected light-saturated photosynthesis (A_{sat}), stomatal conductance (g_{sw}) and WUE (WUE_i and $\delta^{13}C$).
3. A_{sat}, WUE_i and $\delta^{13}C$, but not g_{sw}, increased with higher leaf N. Surprisingly, N-fixing and non-fixing trees displayed similar scaling between leaf N and these physiological variables, and this finding was supported by reanalysis of a global dataset. N fixers generally had higher leaf N than non-fixers, even when non-fixers were not N-limited at the leaf level. Leaf-level N limitation did not alter the relationship of A_{sat}, g_{sw}, WUE_i and $\delta^{13}C$ with leaf N, although it did affect the photosynthetic N use efficiency. Higher WUE was associated with higher productivity, whereas higher A_{sat} was not.
4. *Synthesis*: The ecological success of N-fixing trees depends on the effect of leaf N on carbon gain and water loss. Using a field fertilization experiment and reanalysis of a global dataset, we show that high leaf-level photosynthesis and WUE in N fixers stems from their higher average leaf N, rather than a difference between N fixers and non-fixers in the scaling of photosynthesis and WUE with leaf N. By clarifying the mechanism by which N fixers achieve and benefit from high WUE, our results further the understanding of global N fixer distributions.

KEYWORDS
actinorhizal, leaf nitrogen, legume, nitrogen fixation, nitrogen limitation, plant–soil interactions, rhizobial, water use efficiency

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1 | INTRODUCTION

Nitrogen (N)-fixing trees, which can access atmospheric N_2 through symbiotic bacteria, play a critical role in the functioning of the terrestrial biosphere. Through their capacity to bring new N into ecosystems, at rates occasionally exceeding 100 kg N ha^{-1} $year^{-1}$ (Binkley et al., 1994; Cleveland et al., 1999), they can have pronounced effects on productivity and carbon storage (Batterman et al., 2013; Wieder, Cleveland, Lawrence, et al., 2015). Unlike other N inputs, biological N fixation has the capacity to respond to biotic N demand (Vitousek et al., 2002), which is predicted to increase with CO_2 fertilization (Terrer et al., 2019; Wieder, Cleveland, Smith, et al., 2015). The effects of N fixers on biogeochemical cycling at ecosystem to global scales depend on aggregated individual-level N fixation inputs (Wurzburger et al., 2022). At a mechanistic level, the effect that N fixation has on leaf N and how leaf N affects carbon assimilation and water use are critical for understanding individual N fixation inputs.

Once N is acquired by a plant, much of it is invested in leaves for capturing carbon. Decades of research have established that photosynthesis increases with leaf N (Evans, 1989; Field & Mooney, 1986; Osnas et al., 2013; Reich et al., 1997; Wright et al., 2004). Higher leaf N can also benefit plants in arid settings, as N invested into photosynthetic enzymes and pigments allows carbon assimilation to be maintained at reduced stomatal conductance (g_{sw}; Prentice et al., 2014; Wright et al., 2003). The ratio of carbon assimilation to water loss through transpiration during photosynthesis defines a plant's water use efficiency (WUE). Over time, greater WUE increases the carbon isotope ratio of leaf tissue ($\delta^{13}C$) due to a larger drawdown of intracellular CO_2, which reduces discrimination against $^{13}CO_2$ during photosynthesis (Farquhar et al., 1989; Seibt et al., 2008).

N fixers typically have higher leaf N than non-fixers (Adams et al., 2016; Fyllas et al., 2009; Nasto et al., 2014; Vergutz et al., 2012; Wright et al., 2005), though both leaf N and whole-plant N can be quite plastic in N fixers (Dovrat et al., 2020; Wolf et al., 2017). In plants generally, high leaf N is typically associated with high rates of photosynthesis (Reich et al., 1991; Wright et al., 2004), so one might expect N fixers to have higher rates of photosynthesis than non-fixers based on higher leaf N alone. However, this expectation assumes that N fixers have the same relationship between leaf N and photosynthesis as other plants, which has been called into question. Adams et al. (2016) reported that in at least one way—the log–log slope—N fixers are fundamentally different from non-fixers in how leaf N influences photosynthesis. Through interspecific global analyses of N fixers and non-fixers, they found that light-saturated photosynthesis (A_{sat}) did not increase with leaf N in N fixers (Adams et al., 2016). In the same study, they found that WUE (both intrinsic WUE [WUE_i; calculated as A_{sat}/g_{sw}] and $\delta^{13}C$) increased with leaf N in N fixers as well as in non-fixers (also see Cernusak et al. (2007) and Prentice et al. (2014) for the non-fixer relationship). Greater WUE would likely enhance survival in arid conditions, but a globally different relationship between A_{sat} and leaf N in N fixers versus non-fixers is surprising given that N fixers also play important ecological roles where rapid growth is favoured (e.g. early succession and light gaps in closed-canopy forests).

One possible explanation for why the leaf N-A_{sat} relationship might differ in N fixers is the degree of N limitation. Given their potential access to an inexhaustible amount of N, N fixers are likely to be less N-limited than non-fixers. If the leaf N-A_{sat} relationship holds only in plants that are N-limited, then a lack of N limitation in N fixers could explain the lack of a leaf N-A_{sat} relationship. Given that photosynthesis, and thus the relationship between N and photosynthesis, occurs at the scale of the leaf rather than whole plant, we distinguish here between N limitation to biomass growth (the classic definition; Chapin et al., 1986) and N limitation to leaf N concentrations. These two metrics of limitation are not necessarily the same, as N may continue to accumulate in leaf tissues beyond the N supply level at which N limits biomass growth (Chapin, 1980). As leaf N concentrations increase, the degree to which leaf N affects photosynthesis is likely to depend on the degree to which leaf N is invested into photosynthetic versus non-photosynthetic compounds (e.g. structure components or defence from herbivory).

Aside from differences between N fixers and non-fixers in the degree of N limitation, there are other possible explanations for the leaf N-A_{sat} difference observed by Adams et al. (2016). Some of these explanations are statistical, such as differences in sample size or the specific form of a statistical model. For example, there are more N-fixing species than there are non-fixing species in the Adams et al. (2016) dataset, and a lower sample size is less likely to show a significant trend. Other explanations are biological, such as the level of organization at which analyses are done. For example, if N fixers all have N-rich leaves, then a within-functional-group analysis might reveal no trend even if a cross-functional-group analysis reveals a strong trend. Furthermore, within-species comparisons may yield different relationships between functional traits than interspecific comparisons (Anderegg et al., 2018; Osnas et al., 2018). Yet, another possibility is that N fixers may be different than non-fixers in how they use leaf N (Adams et al., 2016). For example, a leaf N-A_{sat} relationship would not exist in N fixers if, in response to changing leaf N, N fixers adjusted their WUE through a change in stomatal conductance while keeping photosynthesis constant. Concurrent examination of how N supply influences leaf N, photosynthesis, WUE and plant growth in N fixers and non-fixers is needed to resolve these possibilities.

Our main questions are whether N fixers differ from non-fixers with respect to their leaf-level physiological traits (photosynthesis, stomatal conductance and WUE) and how their leaf-level physiological traits vary with leaf N. If so, to what degree does this depend on differences in N limitation, level of organization and analysis choice? We used a field fertilization experiment to determine within-species relationships and to evaluate the role of N limitation. In conjunction with our field experiment, we used a global dataset (Adams et al., 2016) to make cross-functional group comparisons of N fixers and non-fixers in their interspecific trait relationships. Our field fertilization experiment was conducted

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with six N-fixing tree species and four non-fixing tree species across sites in Hawaii, Oregon and New York. To focus on how leaf N concentrations affect leaf physiology, we delineated trees that had attained maximum leaf N concentrations (not N-limited at the leaf level) from those that had not, but would have with greater N supply (N-limited at the leaf level). We specifically asked (1) Do N fixers differ from non-fixers in leaf N, and to what degree does this depend on whether trees are N-limited at the leaf level? (2) Do the relationships between leaf N and leaf-level physiological variables (A_{sat}, g_{sw}, WUE_i, $\delta^{13}C$ and dark respiration [R_{dark}]) differ (a) between N fixers and non-fixers or (b) between trees that are versus are not N-limited at the leaf level? (3) How do A_{sat}, WUE_i and $\delta^{13}C$ affect whole-tree leaf and total above-ground biomass? Together these questions link how N is invested in leaves, the effect of leaf N on photosynthesis and WUE and the productivity benefits of higher photosynthesis and WUE.

2 | METHODS

2.1 | General description of field fertilization experiment

We grew six N-fixing and four non-fixing tree species in field fertilization experiments at two temperate and two tropical sites (Table 1). N-fixing tree species represented both tropical and temperate origins and rhizobial (legume plants with *Rhizobia*-type bacteria) and actinorhizal (nonlegume plants with *Frankia*-type bacteria) symbioses. Non-fixing tree species were chosen because they co-occur with our N-fixing species and share some characteristics with them. Below we provide a basic description of the field experiments, but see Menge et al. (2023) for additional information. Our temperate sites were at Black Rock Forest in New York and Starker Forest in the Oregon Coast Range. Our tropical sites were both on the Big Island of Hawai'i, with a higher elevation site at Volcano Research Station and a lower elevation site at Waiakea Research Station. At Starker Forest, we were granted research permits from Starker Forests, Inc. for 57-Shotpouch (permit numbers: 6015-8139, 6015-11035, 6015-13848 and 6015-17283). Other sites did not require formal use permits. At Black Rock Forest, we grew *Robinia pseudoacacia* (rhizobial fixer) and *Betula nigra* (non-fixer). At Starker Forest, we grew *Alnus rubra* (actinorhizal fixer) and *Pseudotsuga menziesii* (non-fixer). At Volcano Research Station, we grew *Acacia koa* (rhizobial fixer), *Morella faya* (actinorhizal fixer) and *Dodonaea viscosa* (non-fixer). At Waiakea Research Station, we grew *Gliricidia sepium* (rhizobial fixer), *Casuarina equisetifolia* (actinorhizal fixer) and *Psidium cattleianum* (non-fixer). Depending on growing conditions, *Morella* and *Dodonaea* can be classified as shrubs or small trees, and in our open-field sites, these species occurred as large shrubs. For the purposes of our manuscript, we refer to all species as trees. Our temperate species are generally considered native to NY or OR, with the possible exception of *Robinia* (NY has been contested as being north of its traditional native range). All tropical species, except for *Acacia* and *Dodonaea*, are non-native, and in some cases, invasive in HI.

Trees were grown under four fertilization treatments: a control treatment ('C'), $+10\,g\,N\,m^{-2}\,year^{-1}$ ('+10'); $+15\,g\,N\,m^{-2}\,year^{-1}$ ('+15'); and $+15\,g\,N\,m^{-2}\,year^{-1}+15\,g\,P\,m^{-2}\,year^{-1}$ ('+15+P'). N was applied as ammonium nitrate or urea (a switch to urea occurred mid-experiment at the New York site because we were no longer able to purchase ammonium nitrate) and P as monosodium phosphate three to four times per growing season (March–October in NY and OR and throughout the year in HI) around each tree in a 5 m radius.

Trees were arranged as pairs of N-fixing and non-fixing trees (NY and OR) or as triads with two N fixers and one non-fixer (HI; Figure S1). Trees within pairs or triads were 5 m apart and a minimum of 12 m from trees in other pairs or triads. Fertilizer was applied at the same per-ground-area rates listed above, with no doubling of fertilization in the small regions of plot overlap. Trees were planted as seedlings (basal diameter ranged from 1.2 to 6.7 mm and height ranged from 4.7 to 52 cm), although *Pseudotsuga* was planted 2 years prior and had a mean initial basal diameter of 25.4 mm and height of 120.3 cm when *Alnus* was planted. Seedling planting began spring 2015 in NY and spring 2016 in OR and HI. We had an initial sample size of eight trees per species and fertilization treatment (except Waiakea, which initially had nine trees per species and fertilization treatment), for a total of 332 trees. There was some mortality during the experiment which lowered our final sample size (Table S1). Trees were harvested after 5 (NY, OR) or 4 (HI) years (Carreras Pereira et al., 2023).

TABLE 1 Site characteristics. This information is also given in tab. 1 in Menge et al. (2023).

Site	Latitude	Longitude	Elevation (m)	MAT (°C)	MAP ($mm\,year^{-1}$)	N-fixing tree	Reference non-fixing tree
New York	41.42°	−74.02°	195	9.5	1248	*Robinia pseudoacacia*^{R}	*Betula nigra*
Oregon	44.56°	−123.60°	373	10.5	2166	*Alnus rubra*^{A}	*Pseudotsuga menziesii*
Waiakea, HI	19.64°	−155.08°	196	23.3	4318	*Gliricidia sepium*^{R}, *Casuarina equisetifolia*^{A}	*Psidium cattleianum*
Volcano, HI	19.47°	−155.26°	1249	16.3	3048	*Acacia koa*^{R}, *Morella faya*^{A}	*Dodonaea viscosa*

Note: Superscripts 'A' and 'R' indicate actinorhizal and rhizobial symbioses, respectively. Mean annual temperature (MAT) and mean annual precipitation (MAP) for New York and Oregon are averages from 1961 to 1990 from PRISM. MAT and MAP for Hawaii are University of Hawaii field station data.

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2.2 | Leaf measurements

We measured light-saturated leaf photosynthesis (A_{sat}; per leaf area, μmol CO_2 m^{-2} s^{-1} and per leaf mass, nmol CO_2 g^{-1} s^{-1}), stomatal conductance (g_{sw}, mol H_2O m^{-2} s^{-1}), leaf mass per area (LMA, g m^{-2}), leaf N content (g N m^{-2} and %N by mass) and leaf $\delta^{13}C$ (‰) for each individual tree. Intrinsic water use efficiency (WUE_i) was calculated as A_{sat}/g_{sw}. One recently matured leaf per individual was measured and subsequently collected from full-sun leaves at the top of the canopy or at *c*. 2 m for trees >2 m (the maximum height at which we could measure gas exchange). For *Pseudotsuga*, our only conifer, measurements were made on the end of a branch with recently matured needles. Leaf measurements occurred in July 2017 and August–September 2018 in NY, June 2019 in OR and July 2019 in HI.

Gas exchange (i.e. A_{sat} and g_{sw}) was measured with a LI-6400 (HI and 2017 NY) and LI-6800 (OR and 2018 NY) photosynthesis system (LI-COR). Measurements were conducted between 08:00 and 14:00 local time with light levels at 1800 (OR and NY) or 2000 (HI) μmol photon m^{-2} s^{-1}. These light levels approximately match the peak ambient light levels at the time of sampling: *c*. 1800 and 1900 μmol photon m^{-2} s^{-1} for NY (for 2018 and 2017, respectively), *c*. 1900 μmol photon m^{-2} s^{-1} for OR and *c*. 2100 μmol photon m^{-2} s^{-1} for HI. Gas exchange measurements were made in a random order across fertilization blocks to avoid potential bias due to time of sampling. Reference [CO_2] was maintained at 400 ppm, and block (LI-6400) and leaf (LI-6800) temperatures were maintained at 25°C. Water was scrubbed from the air entering the chamber as needed to maintain a relative humidity (RH) between 50%–80% for the LI-6400. For the LI-6800, vapour pressure deficit (VPD) was controlled (at 1.2 kPa) instead of RH (a VPD of 1.2 kPa corresponded to a RH between 50% and 80%). LI-6400 leaf temperature and g_{sw} were corrected following Garen et al. (2022). LI-6400 A_{sat} data were then corrected to 25°C with an A_{sat} temperature response function from Bytnerowicz et al. (2022), which is based on four tree genera (*Alnus*, *Morella*, *Gliricidia* and *Robinia*) represented here (see Note S1). LI-6800 data did not require additional temperature corrections, as leaf temperature was maintained at 25°C and thermal gradients in the LI-6800 are negligible (Garen et al., 2022). Rates were area-corrected to account for leaves that were too small to fill the chamber. *Pseudotsuga* rates were area-corrected using the needle area visible and illuminated from the top of the chamber.

Dark respiration (R_{dark}) at 25°C was measured in OR and NY, but not HI. R_{dark} measurements were made at night, between 23:00 and 05:00 local time. Due to an inability to maintain leaf temperature at 25°C in NY in 2017, 2017 R_{dark} measurements were made at 20°C and then corrected to 25°C by making R_{dark} temperature response curves. Temperature response curves were made by measuring R_{dark} on three *Robinia* and three *Betula* from 17 to 28°C and fitting a 2nd-order polynomial to the log-transformed R_{dark} data (following Heskel et al., 2016). R_{dark} temperature response measurements were made in 2018, using a LI-6800.

Following gas exchange measurements, the measured leaf was harvested and scanned for leaf area, dried at 60°C for 3 days, weighed to calculate LMA (g m^{-2}) and ground for leaf N content and $\delta^{13}C$. Leaf N content was determined using a Costech 4010 Elemental Combustion System (2018 NY, OR and HI; Costech Analytical Technologies) or by sending leaf samples to the UC Davis Stable Isotope Facility (SIF; 2017 NY). $\delta^{13}C$ was determined at SIF. Leaf N and $\delta^{13}C$ analyses at SIF used a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd.). LMA was used to convert traits between per area and per mass. In HI, LMA measurements were made on the whole leaf or leaflet (for *Gliricidia*; *Acacia* had phyllodes, not compound leaves) while LMA measurements in NY and OR only included the portion of the leaf or leaflet (for *Robinia*) that fit inside the LI-COR cuvette. For *Pseudotsuga* metrics (excluding R_{dark}), leaf area and mass in the chamber were calculated using only the needles (excluding the stem) for the part of the branch that was within the LI-6800 cuvette, because the stem's contribution to A_{sat} was likely negligible. For *Pseudotsuga* R_{dark} analyses, the stem in the LI-6800 cuvette was included in calculating area and mass in the chamber.

2.3 | Whole-tree leaf and above-ground biomass

Whole-tree leaf and above-ground biomass at the time of harvest was measured or calculated from allometric equations with tree height, basal diameter, diameter at 1.3 m (for large *Alnus* trees) and canopy area as input variables. We used allometric equations developed previously for *Alnus* and *Pseudotsuga* (Cooke, 1987; Helgerson, 1981; Tang, 1997) and developed our own allometric equations for the other eight species (Carreras Pereira et al., 2023; Menge et al., 2023) by felling a subset of trees and measuring whole-tree biomass components.

2.4 | Statistics

We leveraged our field fertilization experiment to make within-species comparisons for N fixers and non-fixers, while controlling for interspecific differences, and to test whether relationships between physiological traits were different for these functional groups. Specifically, we designed our statistical analyses to determine general trends for N fixers, non-fixers, trees that were N-limited at the leaf level and trees that were not N-limited at the leaf level. Data were analysed with linear mixed effects models using the 'lmer' package (Bates et al., 2015) in R (R Core Team, 2018). Random effects were determined following Zuur et al. (2009). For each model, we tested all combinations (both random slopes and intercepts or just random intercepts) on the maximal model from which we chose the random effect structure with the lowest AIC_c (Table S1), calculated using the 'bbmle' package (Bolker & R Core Team, 2014). That random effect structure was then used when determining the fixed effect structure

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(separate slopes and intercepts, identical slopes but separate intercepts, or identical slopes and intercepts for N fixers, non-fixers, and trees that were N-limited at the leaf level, or trees that were not N-limited at the leaf level). These random effects allowed us to control for interspecific differences while analysing within-species relationships between traits. Potential random effects were site (the NY portion of the experiment was split between two nearby sites in Black Rock Forest) and species (with measurement year nested within species because *Betula* and *Robinia* were measured in both 2017 and 2018). Individuals that were diseased and waterlogged or that sustained major damage (e.g. from herbivory) were not included in the analyses. All data except $\delta^{13}C$ were $\log_{10}$ transformed prior to analysis.

In our first set of analyses, corresponding to Question 1, we started by testing for the effect of fertilization treatment (a categorical fixed effect) on leaf N concentrations separately for N fixers and non-fixers. We used the likelihood ratio test to calculate p values and determine model significance. For post hoc comparisons, we used the 'emmeans' package (Lenth, 2021; using Tukey's HSD test). Trees in the control treatment were considered N-limited at the leaf level if mean leaf N concentrations were significantly lower than in N-fertilized treatments. Leaf-level N limitation was then used as a fixed effect in our subsequent analyses (described below).

After controlling for the effect of LMA on leaf N, we tested for differences between N fixers and non-fixers or between plants that were versus were not limited by N at the leaf level in leaf N (also Question 1) and A_{sat}, g_{sw}, WUE_i, $\delta^{13}C$ and R_{dark} to leaf N (Question 2). For these analyses, we used an ANCOVA in which we included a fixed effect for whether a species was or was not an N fixer (i.e. '1' for N fixer and '0' for non-fixer) and a fixed effect for leaf-level N limitation (i.e. '1' if N-limited at the leaf level and '0' if not N-limited at the leaf level). With this approach, we tested for differences in slopes and intercepts of the mixed-model mean regressions (N fixers vs. non-fixers and trees that were N-limited at the leaf level vs. those that were not). Fixed effect significance was determined at the 95% confidence level by profiling the likelihood. We used the 'MuMIn' package (Barton, 2020) to calculate the marginal R^2 (R_m^2; the proportion of variance explained by the fixed factors) and the conditional R^2 (R_c^2; the proportion of variance explained by the fixed and random factors together; Nakagawa & Schielzeth, 2013). Where slopes were different, we tested for statistical differences between mixed-model mean regression lines across values of the independent variable using the 'emmeans' R package.

For Question 3, we tested for the effect of A_{sat}, WUE_i and $\delta^{13}C$ on whole-tree leaf and above-ground biomass at harvest. This analysis used mixed models and calculated *p* values, R_m^2 and R_c^2 as described above. Because we had only one biomass value per individual tree, we only used 2018 A_{sat}, WUE_i and $\delta^{13}C$ measurements when making these calculations for *Robinia* and *Betula*.

For our interspecific analyses, we reanalysed the entire global dataset of Adams et al. (2016), which includes data for both woody and nonwoody plants primarily given at the interspecific level of organization (Table S1). Our analysis used an ANCOVA to explicitly test for differences between N fixers and non-fixers in the slopes and intercepts of A_{sat}, g_{sw}, WUE_i and $\delta^{13}C$ with leaf N (Question 2 at the interspecific level of organization). This differs from the analysis conducted by Adams et al. (2016), which focussed on interspecific trait relationships within each functional group (separately for N fixers and non-fixers).

Additionally, we used both our field fertilization experimental data and the entire Adams et al. (2016) dataset to test for a difference between N fixers and non-fixers in A_{sat}, g_{sw}, WUE_i and $\delta^{13}C$, independent of leaf N (see Note S3).

3 | RESULTS

3.1 | Do N fixers differ from non-fixers in leaf N, and to what degree does this depend on whether trees are N-limited at the leaf level? (Q1)

Non-fixers were N-limited at the leaf level in the control treatment, but not in the fertilized treatments ($p < 0.001$; Figure 1a; Figure S2a). On the contrary, N fixers never experienced leaf-level N limitation (Figure 1b; Figure S2b). Leaf-level N limitation was generally stronger than N limitation of biomass growth for non-fixers: there was only a marginal increase in whole-tree leaf and above-ground biomass from the control to the +15 treatment (Tukey's HSD $p = 0.056$ and 0.058, respectively; Figure S3a,c). For N fixers, though, biomass growth and leaf-level responses were similar: neither responded to fertilization (Figure S3b,d). For all response variables, the +10 treatment satisfied N demand, based on no difference between the +10 and +15 treatments in leaf N concentrations or biomass. There was no evidence that P fertilization affected leaf N concentrations or whole-tree leaf and above-ground biomass (determined by comparing the +15 vs. +15 +P treatments). Thus, we considered non-fixers from the control treatment to be N-limited at the leaf level and considered all other plants (non-fixers from fertilized treatments and N fixers in all treatments) to be not N-limited at the leaf level. Two alternative scenarios for assigning leaf-level N limitation were also considered and produced qualitatively similar results in the subsequent analyses (see Note S2; Figures S4 and S5).

After accounting for variation in LMA, leaf N was generally greater in N fixers than in non-fixers, regardless of whether non-fixers experienced leaf-level N limitation (Figure 2; Figures S6 and S7). Based on our statistical results, N fixer leaf N was higher than non-fixer leaf N with one exception: at low LMA ($<97\,g\,m^{-2}$), the leaf N of non-fixers that were not N-limited at the leaf level was not different from that of N fixers (Figure 2). Leaf-level N limitation was associated with lower leaf N in non-fixers; however, the statistical significance of this difference also depended on LMA (not significant above an LMA of $157\,g\,m^{-2}$; Figure 2). Together, LMA, whether a plant was an N fixer or a non-fixer, and leaf-level N limitation status, explained 70% of the variation in leaf N per area (84% when including random effects).

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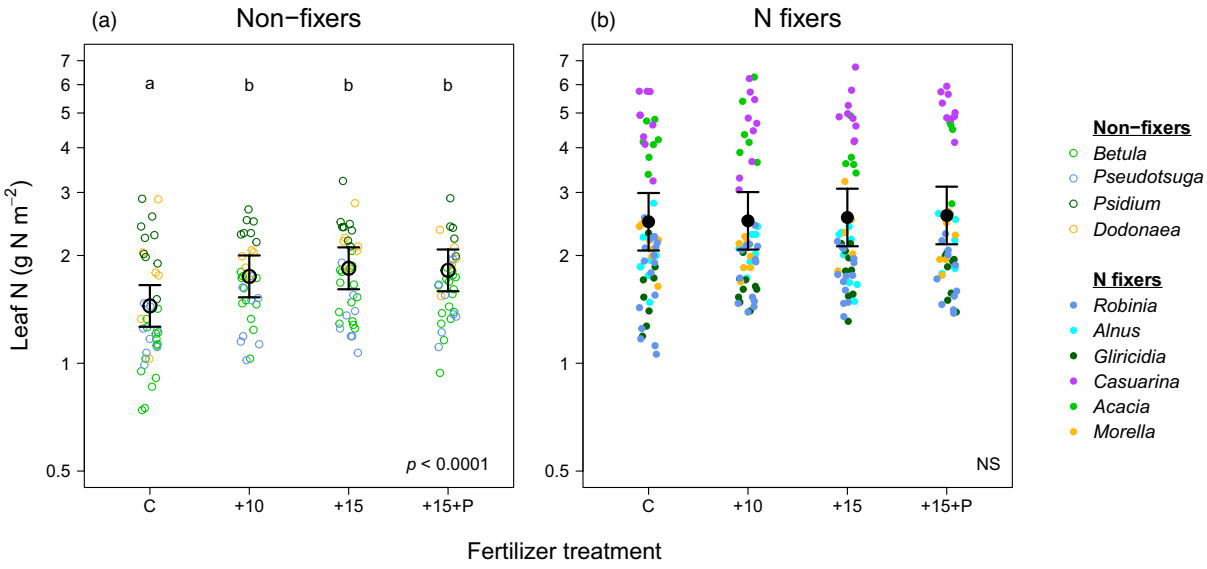


FIGURE 1 Effect of fertilization treatment on N per leaf area for non-fixers (a) and N fixers (b). Black symbols and bars represent mixed-model means ± 1 SE. Coloured symbols represent individual trees and are grouped by species (see legend). Model significance is in the bottom right, and letters represent significant (at 95% confidence level) post hoc differences between fertilization treatment levels. Random effects are given in Table S1.

3.2 | Do the relationships between leaf N and leaf-level physiological variables (A_{sat}, g_{sw}, WUE_i, $\delta^{13}C$ and dark respiration [R_{dark}]) differ (a) between N fixers and non-fixers or (b) between trees that are versus are not N-limited at the leaf level? (Q2)

Light-saturated photosynthesis (A_{sat}), intrinsic WUE (WUE_i) and the carbon isotope ratio of leaf tissue ($\delta^{13}C$) increased with leaf N per area for both N fixers and non-fixers ($p < 0.001$ for all relationships; Figure 3a,c,d), whereas stomatal conductance (g_{sw}) did not (Figure 3b; Figure S8). There was no difference in $\log_{10}$-$\log_{10}$ (for A_{sat}, g_{sw} and WUE_i as functions of leaf N per area) and $\log_{10}$-linear (for $\delta^{13}C$ as a function of leaf N per area) slopes between N fixers and non-fixers, regardless of whether non-fixers experienced leaf-level N limitation (Figure 3). Compared with trees that were not N-limited at the leaf level (N fixers and non-fixers together), non-fixers that were N-limited at the leaf level had significantly higher A_{sat} ($p = 0.02$) and g_{sw} ($p = 0.01$ or 0.03, depending on whether leaf N was (Figure S8) or was not (Figure 3b), respectively, included in the model; $\Delta AIC_c = 1.5$, which is <2, making both models reasonable) for a given leaf N. That is, trees that were N-limited at the leaf level had a higher photosynthetic N use efficiency. There was no difference in WUE_i (calculated as A_{sat}/g_{sw}) or $\delta^{13}C$ due to leaf-level N limitation. Whether A_{sat} as a function of leaf N was analysed on a per mass basis or per area basis did not matter qualitatively (Figure S9).

Relationships between A_{sat}, WUE_i and $\delta^{13}C$ and leaf N per area were concave down when back-transformed to a linear–linear scale (Figure S10). Because the relationship of A_{sat} with leaf N was analysed on a log–log scale, A_{sat} increased with leaf N faster on a linear–linear scale in trees that were N-limited at the leaf level than in trees that were not (due to differences in the intercepts in log–log space; Menge et al., 2018). We also analysed the relationship of A_{sat} and g_{sw} with leaf N per area by omitting the leaf-level N limitation status of trees from our models (Figure S11). This approach showed no difference in the $\log_{10}$-$\log_{10}$ slopes and intercepts between N fixers and non-fixers. A_{sat} increased with leaf N per area ($p < 0.001$; Figure S11a) whereas g_{sw} did not ($p = 0.30$; Figure S11b).

A reanalysis of a global dataset (Adams et al., 2016), where we directly compared N fixer and non-fixer slopes and intercepts, also showed no difference between N fixers and non-fixers with respect to the effect of leaf N on A_{sat}, g_{sw}, WUE_i and $\delta^{13}C$ ($p = 0.25$, 0.16, 0.40 and 0.51, respectively; Figure 4). When we tested for different slopes for N fixers and non-fixers, Δ slopes (i.e. N fixer slope minus non-fixer slope) and their 95% confidence intervals overlapped zero in all cases and were as follows: −0.23 (−0.50, 0.04), −0.42 (−0.87, 0.02), 0.19 (−0.14, 0.52) and −0.9 (−2.6, 0.7) for Figure 4a–d, respectively. In the global dataset, it was unclear which plants were N-limited at the leaf level or not. As in our field fertilization experiment, the global dataset showed that leaf N had a positive effect on A_{sat}, WUE_i and $\delta^{13}C$ ($p < 0.001$ for all), but no effect on g_{sw} ($p = 0.77$).

Additionally, we assessed whether A_{sat}, g_{sw}, WUE_i and $\delta^{13}C$ were higher in woody N-fixing plants than woody non-fixing plants independent of leaf N (Note S3). A_{sat} was marginally higher in N fixers ($p = 0.05$), g_{sw} and WUE_i were not different between N fixers and non-fixers ($p = 0.33$ and 0.73), and $\delta^{13}C$ was higher in N fixers ($p < 0.001$; Figure S12).

We also measured how R_{dark} varied with leaf N in the field fertilization experiment. R_{dark} increased with leaf N, but this relationship was only significant at a 95% CI level on a per mass basis ($p = 0.11$ and <0.001 on a per area and per mass basis, respectively;

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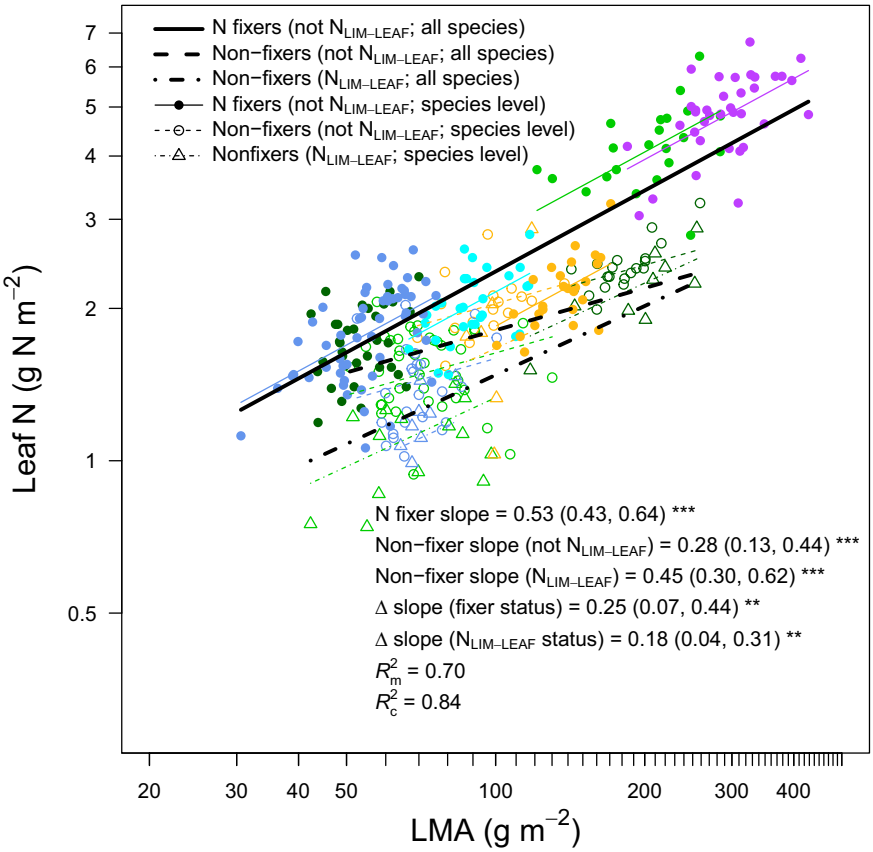


FIGURE 2 Leaf N per area in N fixers versus non-fixers after accounting for leaf mass per area (LMA) and N limitation. Log_{10}-log_{10} slopes (with their 95% CI in parentheses) and marginal and conditional R^2 are displayed. Asterisks (**, ***) denote level of significance ($p < 0.01$, 0.001, respectively). At the 95% confidence level, N fixers and non-fixers that were N-limited at the leaf level ($N_{LIM-LEAF}$) had significantly different slopes than non-fixers that were not N-limited at the leaf level ('Δ slope' on legend). Solid black lines represent N fixers, dashed black lines represent non-fixers that were not N-limited at the leaf level, and dashed-dotted lines represent non-fixers that were N-limited at the leaf level. Coloured lines are at the species level (random effects) and are solid for N fixers, dashed for non-fixers that were not N-limited at the leaf level, and dashed-dotted for non-fixers that were N-limited at the leaf level. Symbols represent individual trees, with solid circles for N fixers, open circles for non-fixers that were not N-limited at the leaf level, open triangles for non-fixers that were N-limited at the leaf level and different colours for different species (see legend in Figure 1). N fixers were never N-limited at the leaf level. Random effects are given in Table S1.

Figure S13). Neither leaf-level N limitation nor whether a plant was an N fixer versus a non-fixer affected R_{dark} and its relationship with leaf N.

3.3 | How do A_{sat}, WUE_i and $\delta^{13}C$ affect whole-tree leaf and total above-ground biomass? (Q3)

Productivity (measured both as whole-tree leaf biomass and above-ground biomass at harvest) generally increased with WUE (measured both as WUE_i and $\delta^{13}C$), but not A_{sat} (Figure 5; Figure S14). $\delta^{13}C$ was the best predictor of whole-tree leaf and above-ground biomass ($p < 0.001$). WUE_i was also a good predictor of whole-tree leaf biomass ($p = 0.006$), but less so for above-ground biomass ($p = 0.08$). Whole-tree leaf and above-ground biomass did not increase significantly with A_{sat} ($p = 0.23$ and 0.16, respectively).

4 | DISCUSSION

Our field fertilization experiment and reanalysis of a global dataset suggest that N fixers are not different than non-fixers in how light-saturated photosynthesis (A_{sat}) and WUE scale with leaf N. Rather, high photosynthesis and WUE in N-fixing trees are due to high leaf N concentrations in N fixers. Leaf N was generally higher in N fixers compared with non-fixers, even when non-fixers were not N-limited at the leaf level. However, leaf-level N limitation increased the photosynthetic N use efficiency (A_{sat} per unit of leaf N), which may be due to a concurrent increase in stomatal conductance (g_{sw}). Higher photosynthetic N use efficiency in non-fixers which were limited by N at the leaf level may contribute to photosynthesis being only marginally higher in woody N fixers, in spite of N fixers having higher leaf N. Leaf-level N limitation did not change WUE_i, $\delta^{13}C$, nor the rate at which A_{sat} and g_{sw} scale with leaf N. Furthermore, when all non-fixers were grouped together (i.e. when leaf-level N limitation was

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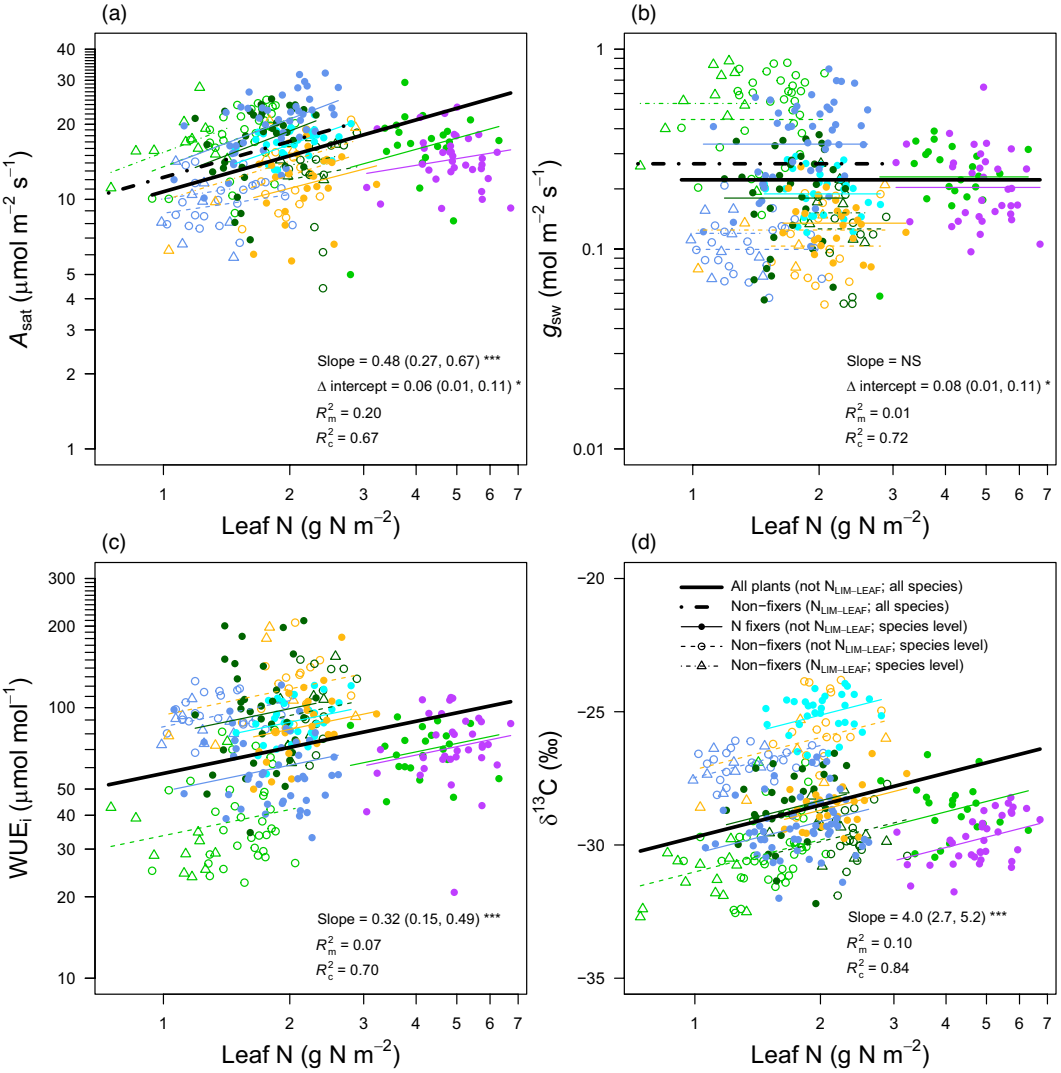


FIGURE 3 Light-saturated photosynthesis (A_{sat}; a), stomatal conductance (g_{sw}; b), intrinsic WUE (WUE$_i$; c) and the carbon isotope ratio of leaf tissue (δ^{13}C; d) as a function of leaf N per area. ANCOVA results showed no difference in the slopes and intercepts of N fixers and non-fixers. For g_{sw} (b), the best model is plotted, where leaf N per area is not included as a covariate. See Figure S4 for an alternative model ($\Delta AIC_c = 1.5$) which includes leaf N as a covariate for predicting g_{sw} ($p = 0.09$ for the slope). Log_{10}-log_{10} (a, c) and log_{10}-linear (d) slopes (with their 95% CI in parentheses) and marginal and conditional R^2 are displayed on all panels. Asterisks (*, ***) denote levels of significance ($p < 0.05$, 0.001, respectively). Solid black lines represent all trees that were not N-limited at the leaf level (N fixers and non-fixers), and dashed-dotted lines represent non-fixers that were N-limited at the leaf level ($N_{LIM-LEAF}$). Coloured lines are at the species level (random effects). Species-level lines are solid for N fixers. For non-fixers, species-level lines are different for different panels; in (a, b) they are dashed for non-fixers that were not N-limited at the leaf level and dashed-dotted for non-fixers that were N-limited at the leaf level, whereas in (c, d) they are dashed for all non-fixers (due to no difference in mixed-model mean slopes and intercepts due to leaf-level N limitation). Symbols and other details as in Figure 2. Random effects are given in Table S1.

not considered), changes in A_{sat}, g_{sw}, WUE$_i$ and δ^{13}C with leaf N were best explained by the same regressions for N fixers and non-fixers. The N fixers in our experiment were all fixing N, acquiring 19%–95% of their N from fixation (depending on species and fertilization treatment; Menge et al., 2023). Thus, our experiment suggests that N fixers are not different from non-fixers in how A_{sat}, g_{sw}, WUE$_i$ and δ^{13}C scale with leaf N, even when they are fixing N.

Our experimental results expand upon a global analysis that showed that A_{sat} scales with leaf N in non-fixers, but not in N fixers (Adams et al., 2016). We show that the discrepancy between the results of Adams et al. (2016) and our field fertilization experiment is due to a difference in analysis. Our analysis asked whether N fixers and non-fixers differed from each other, as opposed to whether relationships for N fixers and non-fixers separately were significantly different from zero. Upon reanalysing the global dataset (Adams et al., 2016) with the same approach we used for our experimental data, we found no difference between N fixers and non-fixers with respect to how A_{sat} changed with leaf N (and with respect to how g_{sw}, WUE$_i$ and δ^{13}C changed with leaf N). The direction of the relationships in the global dataset (Adams et al., 2016) was the same as

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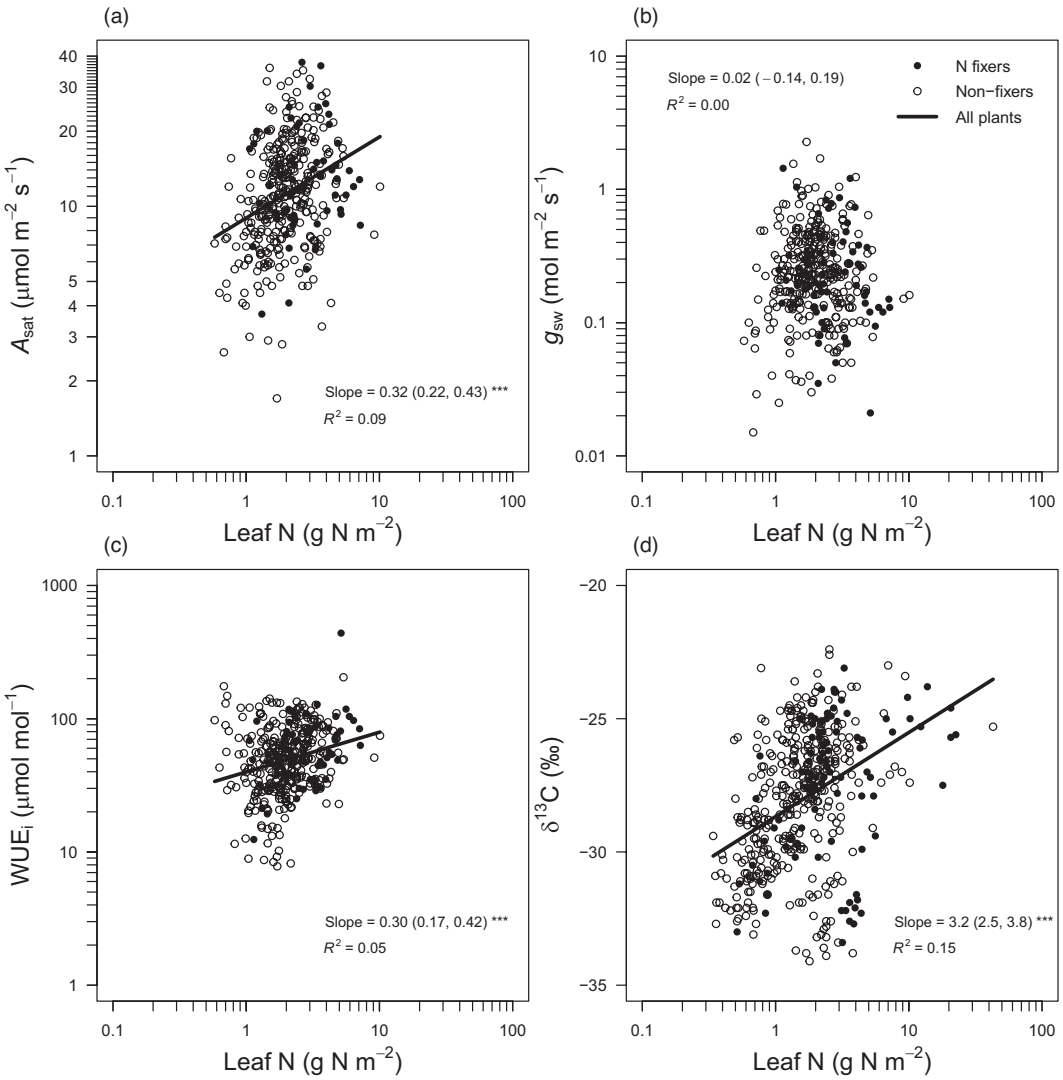


FIGURE 4 Reanalysis of data from Adams et al. (2016), showing light-saturated photosynthesis (A_{sat}; a), stomatal conductance (g_{sw}; b), intrinsic WUE (WUE$_i$; c) and the carbon isotope ratio of leaf tissue (δ^{13}C; d) as a function of the mass of leaf N per area. Lines are plotted where slopes are significantly different from zero at the 95% confidence level for N fixers and non-fixers. Asterisks (***) denote the level of significance ($p < 0.001$). ANCOVA results showed no difference in the slopes and intercepts of N fixers and non-fixers.

in our field experiment: leaf N had a positive effect on A_{sat}, WUE$_i$ and δ^{13}C, but g_{sw} was independent of leaf N. This also suggests that the scaling of these leaf-level physiological traits with leaf N is similar within species and across species. Below we discuss the details of how leaf-level N limitation and whether a plant was an N fixer affected leaf N, the benefits and causes of higher WUE and the implications for N fixer abundance patterns.

4.1 | Leaf N and LMA

Although N fixers and non-fixers did not differ in how A_{sat} and WUE scaled with leaf N, N fixers generally had more N in their leaves than non-fixers (not only for all non-fixers that were N-limited at the leaf level but also for high LMA leaves in non-fixers that were not N-limited at the leaf level). The general pattern of leaf N being higher in N fixers is well known (Adams et al., 2016; Fyllas et al., 2009; Nasto et al., 2014; Vergutz et al., 2012; Wright et al., 2005), but we clarify how the relationship depends on LMA and how it is modified by leaf-level N limitation. Leaf N is strongly correlated with LMA (Dong et al., 2022; Reich et al., 1991; Wright et al., 2004), making LMA critical to consider when making comparisons of leaf N across groups. Considering LMA is also likely to resolve reports of leaf N being higher in N fixers on a per mass or per area basis, but not both (Adams et al., 2016; Querejeta et al., 2022; although Querejeta et al., 2022 compared legumes, some of which were non-fixers, to nonlegumes). Our results suggest that in the global analysis by Dong et al. (2022), the greater difference between N fixer and non-fixer leaf N at high LMA was likely due to some but not all non-fixers being N-limited at the leaf level. However, we also showed that when leaf-level N limitation was alleviated under high soil N supply, non-fixers still tended to have lower leaf N than N fixers. High LMA leaves, which

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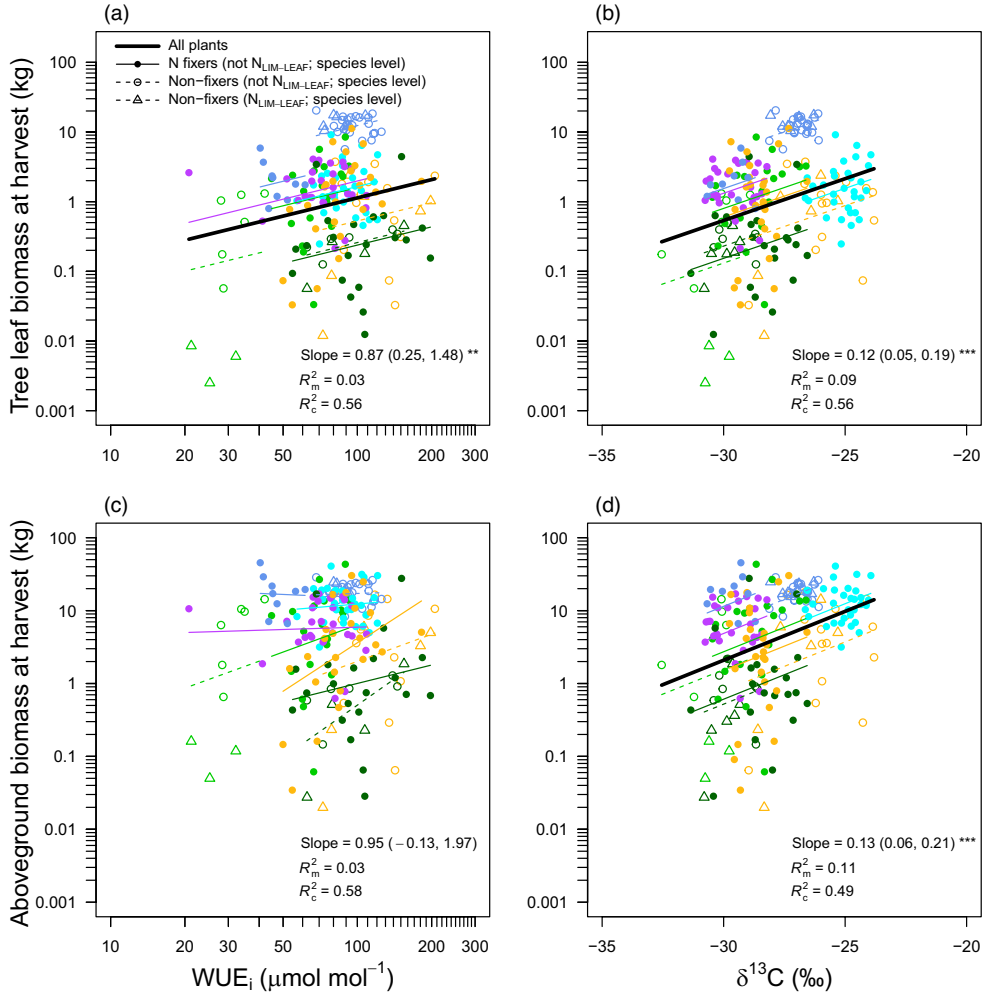


FIGURE 5 Effect of WUE$_i$ (a, c) and δ^{13}C (b, d) on whole-tree leaf (a, b) and above-ground (c, d) biomass. Log_{10}-log_{10} (a, c) and linear-log_{10} (b, d) slopes (with their 95% CI in parentheses) and marginal and conditional R^2 are displayed on all panels. Asterisks (**, ***) denote levels of significance ($p < 0.01$, 0.001, respectively). Solid black lines represent all plants. Coloured lines are at the species level (random effects) and are solid for N fixers and dashed for non-fixers. Symbols and other details as in Figures 2 and 3. Random effects are given in Table S1.

were less plastic in leaf N content, are known to have a greater proportion of N in use for nonphotosynthetic purposes (e.g. cell walls; Onoda et al., 2017; Poorter & Evans, 1998) that promote leaf longevity (Poorter et al., 2009; Wright et al., 2004). A greater investment of N into nonphotosynthetic purposes may also explain why A_{sat}, WUE$_i$ and δ^{13}C in *Acacia* and *Casuarina* (solid purple and light-green points in Figure 3, respectively), which had high LMA and leaf N per area, were lower than predicted by the mixed-model mean regression. A study that included some of our experimental species (*Acacia koa*, *Dodonaea viscosa* and *Psidium cattleianum*) also observed a decline of leaf N associated with photosynthesis with LMA (Funk et al., 2013). However, Funk et al. (2013) also observed that N fixers tended to have a greater proportion of leaf N associated with photosynthesis than non-fixers. This may be due to N fixers specializing in high-light environments (McCulloch & Porder, 2021; Schmidt et al., 2023; Taylor & Menge, 2018; and therefore having lower shade tolerance than non-fixers; Menge et al., 2010), where N associated with photosynthesis is likely to be more beneficial.

4.2 | Benefits and causes of high WUE

Higher leaf N resulted in higher A_{sat} and WUE (both WUE$_i$ and δ^{13}C), both within and across species. Our field fertilization experiment showed that leaf N concentrations varied even among trees that were not N-limited, and this leaf N variation influenced A_{sat} and WUE. The relationships of leaf N with A_{sat} and WUE were concave down on a linear scale, indicating diminishing returns on investing in high leaf N. Following the least-cost economic theory of photosynthesis (Prentice et al., 2014; Wang et al., 2017; Wright et al., 2001, 2003), where water and N are considered mutually substitutable resources to achieve a given rate of carbon assimilation, the optimal level of leaf N is predicted to depend on the degree of aridity. Following this logic, the cost of acquiring water can be alleviated by a greater supply of N. N fixers, which consistently have more leaf N, frequently peak in abundance in more arid biomes (Bytnerowicz & Menge, 2021; Gei et al., 2018; Liao et al., 2017; Pellegrini et al., 2016; Steidinger et al., 2019).

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As seen in our results, leaf N increased the maximum photosynthetic rate (A_{sat}) that can be achieved for a given rate of stomatal conductance (g_{sw}). The change in WUE_i (and $\delta^{13}C$ over longer timescales) with leaf N was due to an increase in A_{sat}, coupled with little to no change in g_{sw} (a marginally significant slope). Querejeta et al. (2022) observed that within the arid Sahel region, the relationship between leaf N per area and WUE in tree species (many of which were N-fixing legumes) was primarily driven by tighter stomatal regulation of transpiration (inferred from leaf $\delta^{18}O$) with increasing leaf N per area. Here, and in other arid sites, survival (enhanced through tighter stomatal regulation) is likely to be more important than maximum photosynthetic rates. Within the Sahel, legumes (most of which were N fixers) had higher leaf $\delta^{18}O$, and therefore lower transpiration rates than nonlegumes. Additionally, many of these legumes may be able to sustain lower transpiration rates than non-fixers because their ability to access atmospheric N makes them less reliant on transpiration-driven mass flow of soil N to roots (Cramer et al., 2009; Matimati et al., 2014; Salazar-Tortosa et al., 2018). Although sites in our field fertilization experiment were not arid overall (Black Rock Forest in NY was our driest site at *c*. 1250 mm precipitation per year), WUE (both WUE_i and leaf $\delta^{13}C$) was a good predictor of productivity, while A_{sat} was not. This may be due to trees in our sites experiencing periods of water limitation, both during the day and during the year, and a disproportionate importance of these periods of water stress. More generally, water deficits uncouple tree growth from photosynthesis, as turgor limitation strongly controls cambial activity (Muller et al., 2011; Peters et al., 2020). This matches the widespread decoupling of tree growth and photosynthesis that has been observed across temperate biomes (Cabon et al., 2022).

4.3 | Implications for N fixer abundance patterns

We found that N fixer leaves differ from non-fixer leaves primarily by having high N concentrations across different levels of soil fertility. Higher leaf N results in higher A_{sat} and, as a result, higher WUE, which benefits productivity. However, independent of leaf N, A_{sat} is only marginally higher for N-fixing trees and the evidence for higher WUE in N fixers is supported by higher $\delta^{13}C$, but not WUE_i.

Our results highlight that high WUE, which may promote greater N fixer survival and relative abundance in arid regions, is a better predictor of productivity than A_{sat} in nonarid sites. This may reflect turgor-driven controls of tree growth (Muller et al., 2011; Peters et al., 2020) and water limitation due to seasonality (e.g. in sites where precipitation is decoupled from the growing season) or high evapotranspiration (e.g. in tropical sites or during warm periods in temperate sites). However, survival is also important in highly productive systems, such as moist Costa Rican tropical forests where N fixers persist through succession due to their ability to survive better than non-fixing trees (Menge & Chazdon, 2016).

Although high leaf N can be beneficial across a range of environments, it can be costly to sustain in certain environments, for example in shade (which limits photosynthesis) or at cold temperatures (which limits N fixation; Bytnerowicz et al., 2022). N fixers are common in warm and dry environments (Steidinger et al., 2019), both in early and late successional forests (Bytnerowicz & Menge, 2021; Gei et al., 2018). Warm and dry environments are projected to increase in frequency with climate change (due to warming, shifts in precipitation and higher VPD), which may also increase the relative abundance of N fixers (Liao et al., 2017). It is less clear how N fixation inputs in individual plants will be affected by climate change. On one hand, N-fixing tree species are expected to have higher rates of N fixation at warmer temperatures (Bytnerowicz et al., 2022). On the other hand, low water availability can also limit N fixation rates in individual plants (Dovrat & Sheffer, 2019). In addition to N fixation, WUE can also be different under well-watered conditions than under drought; that is, divergent responses in ecosystem-level WUE have been observed across ecosystems exposed to drought (Wolf et al., 2013; Xu et al., 2019; Yang et al., 2016). Future work should address how WUE varies across N fixers and non-fixers with drought vs. sufficient water availability.

Overall, our results suggest that N fixers are able to benefit from high leaf N in a similar manner to non-fixers, but, unlike non-fixers, they are able to sustain high leaf N across a range of soil N supply rates. This clarifies how N fixers can achieve high WUE, which we show is associated with higher productivity. These results give us a better understanding of how N fixers will respond to global change, with important implications for nutrient-carbon-climate feedbacks.

AUTHOR CONTRIBUTIONS

All authors designed the study. Thomas A. Bytnerowicz and Jennifer L. Funk collected physiological data. All authors collected biomass data. Thomas A. Bytnerowicz analysed data and wrote the first draft. All authors edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data and R code for this paper are available on Zenodo: https://doi.org/10.5281/zenodo.8277641 (Bytnerowicz et al., 2023) and on GitHub: https://github.com/tbytnero/Bytnerowicz-Funk-Menge-Perakis-Wolf-Fixer-Leaf-Traits.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Note S1. Photosynthesis temperature correction.

Note S2. Alternative scenarios for assigning leaf-level N limitation.

Note S3. Are A_{sat}, g_{sw}, WUE_i, and $\delta^{13}C$ higher in N-fixing woody plants than in non-fixing woody plants independent of leaf N?

Table S1. Random effects and sample sizes used in Figures 1–5.

Figure S1. Experimental setup. Figure from Menge et al. (2023).

Figure S2. Effect of fertilization treatment on N per leaf mass. Details as in Figure 1. Although N fixer % leaf N (b) was higher in the +15+P treatment than the control treatment, we do not interpret this as leaf-level N limitation because % leaf N in the +10 and +15 treatments was not different than in the control. We also do not interpret this as leaf-level limitation by P because leaf N concentrations in the +15 and +15+P treatments were not different. Random effects were random intercepts for species (a) and species and site (b).

Figure S3. Effect of fertilization treatment on whole tree leaf (a, b) and aboveground biomass (c, d) for non-fixers (a, c) and N fixers (b, d). Details as in Figure 1. Random effects were random intercepts for species in all panels.

Figure S4. Leaf N per area (a) and per mass (b) as a function of leaf mass per area (LMA) and A_{sat} (c) and g_{sw} (d) as a function of leaf N per area where only *Betula* control trees are considered N limited at the leaf-level. Details as in Figure 2 (for a, b; except random effects are different intercepts for species, not different intercepts for species and site) and Figure 3a,b (for c, d).

Figure S5. Leaf N per area (a) and per mass (b) as a function of leaf mass per area (LMA) and A_{sat} (c) and g_{sw} (d) as a function of leaf N per area where *Betula*, *Psidium*, and *Dodonaea*, but not *Pseudotsuga*, control trees are considered N limited at the leaf-level. Details for (a, b) as in Figure 2 (except random effects are different intercepts for species, not different intercepts for species and site). Details for (c, d) as in Figure 3a,b except solid black line in c is for N fixers and the dashed black line is for non-fixers that are not N limited at the leaf-level (dashed-dotted black line is for non-fixers that are N limited at the leaf-level, as in Figure 3a).

Figure S6. Relationship between leaf mass per area (LMA) and leaf N mass per leaf mass. Details as in Figure 2. Random effects were random intercepts for species (a) and species and site (b). N fixers always had higher leaf N than non-fixers that were not N limited at the leaf-level. N fixers had significantly higher leaf N than non-fixers that did not experience leaf-level N limitation above an LMA of $97\,g\,m^{-2}$. Below an LMA of $156\,g\,m^{-2}$, non-fixers that were not N limited at the leaf-level had significantly higher leaf N than non-fixers that were N limited at the leaf-level.

Figure S7. Back-transformed relationship between leaf mass per area (LMA) and mass of leaf N per area (a) and per mass (b) from Figure 2 and Figure S6. Details as in Figure 2.

Figure S8. Stomatal conductance (g_{sw}) per area as a function of the mass of N per leaf area for an alternative model to Figure 3b ($\Delta AICc = 1.5$). Here, leaf N is included as a covariate in predicting g_{sw}, even though it is only marginally significant ($p = 0.09$). g_{sw} increased with leaf N, at the same rate for N fixers and non-fixers, regardless of leaf-level N limitation. Non-fixers that were N limited at the leaf-level had a significantly higher intercept than trees that were not N

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limited at the leaf-level (N fixers and fertilized non-fixers). Details (including random effects) as in Figure 3b.

Figure S9. Light-saturated photosynthesis (A_{sat}) per mass as a function of the mass of N per leaf mass. A_{sat} increased with leaf N, at the same rate for N fixers and non-fixers, regardless of leaf-level N limitation. Non-fixers that were N limited at the leaf-level had a significantly higher intercept than trees that were not N limited at the leaf-level (N fixers and fertilized non-fixers). Details as in Figure 3a. Random effects were: species (random slope and intercept).

Figure S10. Back-transformed relationships from Figure 3 of A_{sat}, g_{sw}, WUE_i, and $\delta^{13}C$ as a function of leaf N per area. A_{sat}, WUE_i, and $\delta^{13}C$ relationships with leaf N are concave down on a linear-linear scale. Details as in Figure 3.

Figure S11. Relationships of A_{sat} and g_{sw} as a function of leaf N per area where leaf-level N limitation is omitted from each model. Details as in Figure 3, except that the solid black line represents all trees. Random effects as in Figure 3a,b (see Table S1).

Figure S12. A_{sat}, g_{sw}, WUE_i, and $\delta^{13}C$ for N-fixing and non-fixing woody plants from a combination mean trait values from the control treatment in our experimental dataset with woody interspecific trait values from the global dataset of Adams et al. (2016). Solid points are from the field fertilization experiment and open points are from the Adams et al. global dataset. Black circles and bars represent mixed model mean ± 1 SE. Boxes show the quartiles of all data, with the center line denoting the median. Whiskers indicate the lesser of the most extreme value or 1.5 times the interquartile range. *p* values denote differences between N fixers and non-fixers.

Figure S13. Dark respiration (R_{dark}) per area (a) and per mass (b) as a function of leaf N per area and per mass, respectively. R_{dark} was only measured in Oregon and New York. In all cases N fixers and non-fixers were not significantly different from each other, regardless of leaf-level N limitation. Log10-log10 slopes (with their 95% CI in parentheses; a, b), marginal and conditional R^2 (a), and R^2 (b) are displayed in the legend. Solid black line represents all trees (N fixers and non-fixers). Solid symbols represent N fixers, open symbols represent non-fixers, and different colors represent different species. Colored lines are at the species level (random effects) and are solid for N fixers and dashed for non-fixers. Random effects were: species with measurement year nested within species (random intercept) and site (random intercept) for (a) and no random effects for (b).

Figure S14. Effect of A_{sat} on whole-tree leaf (a) and aboveground (b) biomass. Log_{10}-log_{10} slopes (with their 95% CI in parentheses) and marginal and conditional R^2 are displayed on all panels. Colored lines are at the species level (random effects) and are solid for N fixers and dashed for non-fixers. Symbols and other details as in Figure 5. Random effects were separate slope and intercept for each species (in both panels).

Figure S15. A_{sat} for N-fixing and non-fixing woody plants from the control plants from our field fertilization experiment (a), all plants in Adams et al. (2016) (b), woody plants in Adams et al. (2016) (c), herbaceous plants in Adams et al. (2016) (d). Black circles and bars represent mixed model mean ± 1 SE. Boxes show the quartiles of all data, with the center line denoting the median. Whiskers indicate the lesser of the most extreme value or 1.5 times the interquartile range. *p* values denote differences between N fixers and non-fixers.

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