Legumes are different: Leaf nitrogen, photosynthesis, and water use efficiency

Mark Andrew Adams[a,1], **Tarryn L. Turnbull**[a], **Janet I. Sprent**[b], **and Nina Buchmann**[c]

[a]Centre for Carbon Water and Food, Faculty of Agriculture and Environment, University of Sydney, Brownlow Hill, 2570 NSW, Australia; [b]Plant Sciences, University of Dundee at James Hutton Institute, Dundee DD2 5DA, Scotland; and [c]Department of Environmental Systems Science, ETH Zürich, 8092 Zurich, Switzerland

Edited by Peter M. Vitousek, Stanford University, Stanford, CA, and approved February 23, 2016 (received for review December 4, 2015)

Using robust, pairwise comparisons and a global dataset, we show that nitrogen concentration per unit leaf mass for nitrogen-fixing plants (N_2FP; mainly legumes plus some actinorhizal species) in nonagricultural ecosystems is universally greater (43–100%) than that for other plants (OP). This difference is maintained across Koppen climate zones and growth forms and strongest in the wet tropics and within deciduous angiosperms. N_2FP mostly show a similar advantage over OP in nitrogen per leaf area (N_{area}), even in arid climates, despite diazotrophy being sensitive to drought. We also show that, for most N_2FP, carbon fixation by photosynthesis (A_{sat}) and stomatal conductance (g_s) are not related to N_{area}—in distinct challenge to current theories that place the leaf nitrogen–A_{sat} relationship at the center of explanations of plant fitness and competitive ability. Among N_2FP, only forbs displayed an N_{area}–g_s relationship similar to that for OP, whereas intrinsic water use efficiency (WUE_i; A_{sat}/g_s) was positively related to N_{area} for woody N_2FP. Enhanced foliar nitrogen (relative to OP) contributes strongly to other evolutionarily advantageous attributes of legumes, such as seed nitrogen and herbivore defense. These alternate explanations of clear differences in leaf N between N_2FP and OP have significant implications (e.g., for global models of carbon fluxes based on relationships between leaf N and A_{sat}). Combined, greater WUE and leaf nitrogen—in a variety of forms—enhance fitness and survival of genomes of N_2FP, particularly in arid and semiarid climates.

legume | actinorhizal species | nitrogen | photosynthesis | water use efficiency

Through symbioses with diazotrophic bacteria, legumes and other N_2-fixing plants (N_2FP) acquire atmospheric dinitrogen (N_2) and are widely expected to maintain greater leaf nitrogen than nonfixing or other plants (OP) (1). N_2FP can profoundly influence both ecosystem development and responses to changing climate by alleviating nitrogen shortages that limit capacity of ecosystems to fix and sequester CO_2 (2–4). A central tenet of trait-based ecology (5, 6) is that carbon fixation and transpiration are directly related to leaf nitrogen; in turn, leaf nitrogen is used to drive global models of carbon (and water) exchanges between plants and the atmosphere (7).

The distribution, abundance, and activity of N_2FP in terrestrial ecosystems have remained unexplained, even "paradoxical" (8, 9), especially in relation to local and global nitrogen cycles. For the northern hemisphere, one recent explanation of the distribution of N_2FP (2) and their dominance in wet tropical forests relied on their greater ability to acquire phosphorus from old tropical soils and temperature maxima for N_2 fixation of around 25 °C (i.e., similar to prevailing temperatures in the tropics). Menge et al. (8) subsequently noted that the diazotrophic symbioses are typically rhizobial and facultative toward the tropics but actinorhizal and obligate north of about 35° N. Facultative symbioses in the tropics make evolutionary sense inasmuch as soil nitrogen availability is typically greater there than at the poles and nitrogen fixation carries a carbon cost for the plant. In support, concurrent research suggested that rates of nitrogen fixation may be less in N-rich tropical forests than previously thought (10).

N_2FP differ in their distribution in northern and southern hemispheres, albeit that N_2FP are common in the tropics in both hemispheres. By comparison with the north, beyond 35° S, there is relatively little land at all. Bryophyte–cyanobacteria associations again contribute significant nitrogen (11), albeit to much smaller areas than in the northern hemisphere, and actinorhizal plants (e.g., *Morella*/*Myrica* spp. in Africa and South America and *Casuarina* spp. in Australia) are as likely found in the tropics as closer to the southern pole (12). A distinctive feature of all three major continents in the southern hemisphere is the large areas of arid, semiarid, and Mediterranean (summer drought) climates between the equator and 35° S. In divergence from the "view from the north" (13), the "southern paradox" of the distribution of N_2FP is that woody legumes, notably of the genus *Acacia* (sensu lato) but also, from numerous other genera, dominate much of the large arid and semiarid areas, despite an abundance of other drought-tolerant woody species. For Australia, the paradox is exemplified by the dominance of *Acacia aneura* and *Acacia harpophylla* over large areas, whereas nominally drought-adapted species from the genus *Eucalyptus* are restricted to drainage lines or where groundwater is accessible.

Analysis of plant traits is now routinely used (14–18) to seek explanations for distributions of plant species and growth forms as well as their functional attributes. Leaf nitrogen is among the most significant and widely explored of plant traits. For example, it is frequently observed that leaf nitrogen is greater per unit mass or area for N_2FP than for OP (1). Leaf nitrogen has been a

Significance

Leaf traits are used to drive models of global carbon fluxes and understand plant evolution. Many syntheses have highlighted relationships between plant leaf nitrogen and photosynthesis as evidence of a strong evolutionary drive to "intercept light and capture CO_2." Different from previous studies, we compiled a global dataset constrained to sites and studies where nitrogen-fixing plants (N_2FP) and nonfixing species [other plants (OP)] could be directly compared. We show that photosynthesis is not related to leaf nitrogen for N_2FP, irrespective of climate or growth form. N_2FP have clear advantages in water use efficiency over OP. These findings contribute to a more complete explanation of global distributions of N_2FP and can help improve models of global carbon and nitrogen cycles.

Author contributions: M.A.A. and T.L.T. designed research; M.A.A. and T.L.T. performed research; M.A.A., T.L.T., J.I.S., and N.B. analyzed data; and M.A.A., T.L.T., J.I.S., and N.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: mark.adams@sydney.edu.au.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1523936113/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1523936113

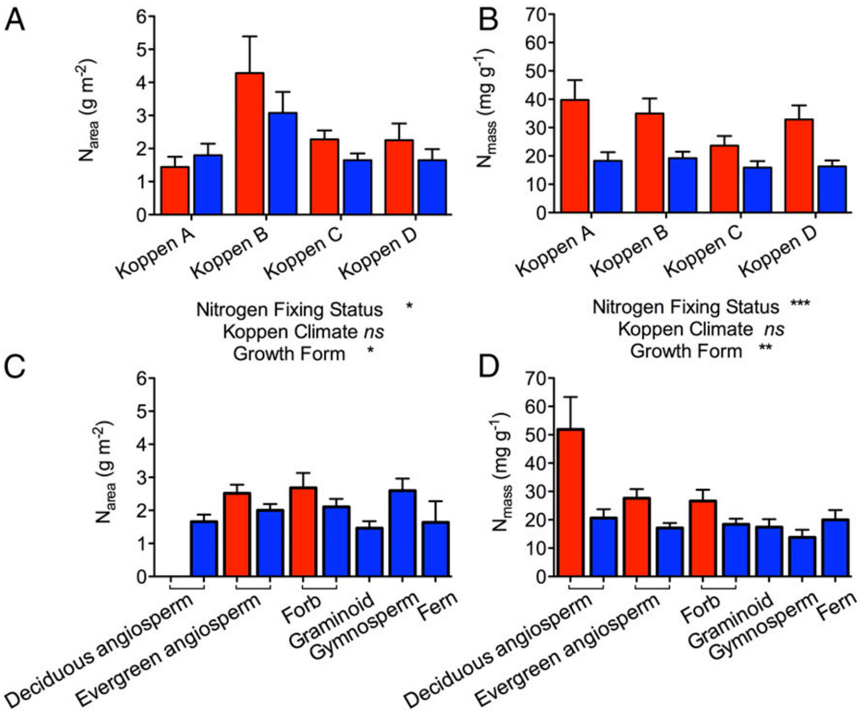

Fig. 1. Leaf nitrogen (either mass- or area-based) for N_2FP (red bars) and OP (blue bars) across Koppen climate classifications and growth forms. Koppen A is tropical, Koppen B is arid and semiarid, Koppen C is temperate, and Koppen D is continental. Linear mixed models were completed on $\log_{10}$-transformed data. Data shown are estimated marginal means and 1 SEs that were back-transformed from $\log_{10}$. Only main effects are shown; interaction terms are given in Table S1. *ns*, not significant. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

focus for trait-based studies of plants owing in part to strong positive relationships between leaf nitrogen and photosynthetic rate (19) and the implications for stomatal conductance (g_s) and transpiration (20, 21). Increased leaf nitrogen (especially increased abundance of the principal nitrogen-rich enzyme involved in carbon fixation; RubisCo) can increase consumption of intercellular CO_2, such that g_s is reduced (and rates of water loss are reduced), because a strengthened CO_2 diffusion gradient helps maintain supply of CO_2. A corollary is that maintaining rates of photosynthesis (A_{sat}) with reduced leaf nitrogen may require increased g_s and water loss. Recently, Prentice et al. (22) built on earlier analysis by Wright et al. (5) and proposed a new theoretical framework for plant ecology based on leaf traits, such as nitrogen per leaf area (N_{area}), A_{sat}, g_s, and the ratio of internal to external concentration of carbon dioxide (c_i/c_a). Prentice et al. (22) focused on the relative constancy of c_i/c_a over a wide range of conditions, tested their theory using sites in Australia, including *Acacia* spp. and other N_2FP, and argued that N_{area} should increase with aridity and that high N_{area} is an adaptation to drought. Despite some recent studies (23), that theory lacks testing for N_2FP across the globe.

To test "paradoxes" associated with the global distribution of N_2FP, we formalized hypotheses in accordance with the literature. Leaf nitrogen should reflect rates of A_{sat} (hypothesis A)—irrespective of whether the plant species can fix nitrogen. Increases in leaf N should, thus, result in reduced g_s and loss of water (hypothesis B) and as a result of either or both, increase water use efficiency [WUE; as indicated by intrinsic water use efficiency (WUE_i) or carbon isotope ratio of leaf tissue ($\delta^{13}C$); hypothesis C].

We tested our hypotheses using a climate-stratified dataset constrained to sites where both N_2FP and OP (paired dataset) were measured for either (*i*) N_{area}, A_{sat}, g_s, and WUE_i or (*ii*) N_{area} and $\delta^{13}C$ (that is, sites where N_2FP and OP were both growing and measured in situ). We complemented this parsimonious, albeit more limited dataset (81 sites) with a larger dataset, in which either N_2FP or OP were studied (nonpaired dataset) for WUE_i (including A_{sat} and g_s) and N_{area} (63 sites) or $\delta^{13}C$ and nitrogen concentration per unit leaf mass (N_{mass}; 351 sites). We adopted the Koppen system—the most frequently used and robust method for climate classification and related analyses (24, 25).

Results

Based on our paired dataset (direct comparison of N_2FP and OP) and with the exception of Koppen A climates, N_2FP maintained a significant advantage over OP in N_{area} (Fig. 1*A* and Table S1). All plants in arid and semiarid Koppen B climates produce foliage distinctly enriched in N relative to other climate zones (Fig. 1*A* and Table S1), an advantage that was also revealed by the nonpaired dataset (Table S2). On average, foliage of N_2FP in arid and semiarid regions (Koppen B) (Fig. 1*A*) has N_{area} around threefold that of N_2FP in the tropics (Koppen A climate), whereas OP show a more modest N enrichment in Koppen B relative to Koppen A zones. Advantages of N_2FP over OP in N_{area} were retained in nontropical climate zones (i.e., Koppen B–D climates), despite wide variation in lifeforms (Fig. 1*C* and Tables S1 and S2).

Differences in N_{mass} and N_{area} between Koppen A and Koppen B zones reflect differences in specific leaf area. Consequently and as expected, N_{mass} was consistently greater in N_2FP than OP growing on the same site (Fig. 1*B* and Table S1) across all climate zones. In the Koppen A zone, foliage of N_2FP was, on average, twice as rich in N as that of OP, and the advantage in terms of leaf N was never less than 40% across climate zones. Effects of N-fixing status on N_{mass} were strongest at low and relatively high latitudes and in deciduous angiosperms (Fig. 1*D*). This pattern was replicated when we included indirect comparisons of N_2FP and OP (nonpaired dataset) (Table S2).

Multivariate analysis showed that N_{area} dominated predictions of A_{sat} (model of best fit) for OP of all growth forms (Table 1). This pattern can be readily seen (Fig. 2) in the large proportion of variance in A_{sat} that was attributed to N_{area} (accept hypothesis A for OP). In contrast, N_{area} had no influence on predicted A_{sat} for N_2FP (Fig. 2 and Table 1) (reject hypothesis A for N_2FP). N_{area} contributed to the model of best fit for predicting g_s in N_2FP forbs but played no role for N_2FP evergreen, woody angiosperms (Table 1) (reject hypothesis B). For OP, N_{area} was

Table 1. Stepwise multiple regressions between A_{sat}, g_s, WUE_i, and $\delta^{13}C$ and predictive variables: N_{area}, latitude, mean annual precipitation, mean annual temperature, dryness index, and elevation

Growth form	Equation	R^2	*P* value
$Log_{10}\ A_{sat}$			
N_2FP evergreen angiosperm	$Log_{10}A_{sat} = 1.253 - 0.0002MAP + 0.003Lat - 0.024DI$	0.52	0.000
N_2FP forb	$Log_{10}A_{sat} = 1.330 - 0.24DI$	0.34	0.015
OP deciduous angiosperm	$Log_{10}A_{sat} = 0.902 + 0.602log_{10}N_{area}$	0.39	0.000
OP evergreen angiosperm	$Log_{10}A_{sat} = 0.909 + 0.419log_{10}N_{area} + 0.002Lat - 0.018DI$	0.26	0.000
OP forb	$Log_{10}A_{sat} = 1.015 + 0.568log_{10}N_{area} - 0.0001Elev$	0.25	0.001
OP graminoid	$Log_{10}A_{sat} = 1.116 + 0.720log_{10}N_{area}$	0.35	0.035
$Log_{10}\ g_s$			
N_2FP evergreen angiosperm	$Log_{10}g_s = -0.694 + 0.006Lat$	0.35	0.000
N_2FP forb	$Log_{10}g_s = -0.40 + 1.186log_{10}N_{area} - 0.0004Elev - 0.006Lat$	0.70	0.001
OP deciduous angiosperm	$Log_{10}g_s = -0.833 + 1.067log_{10}N_{area} + 0.0003MAP - 0.020MAT$	0.64	0.000
OP evergreen angiosperm	$Log_{10}g_s = -1.034 + 0.0002MAP + 0.005Lat + 0.293log_{10}N_{area}$	0.46	0.000
OP forb	$Log_{10}g_s = -0.597 + 0.401log_{10}N_{area}$	0.08	0.014
$Log_{10}\ WUE_i$			
N_2FP evergreen angiosperm	$Log_{10}WUE_i = 1.816 + 0.394log_{10}N_{area} - 0.003Lat - 0.014MAT$	0.47	0.000
N_2FP forb	$Log_{10}WUE_i = 1.642 - 0.722log_{10}N_{area} + 0.005Lat$	0.67	0.000
OP deciduous angiosperm	$Log_{10}WUE_i = 0.891 - 0.002MAP + 0.036MAT - 0.452log_{10}N_{area} + 0.14Lat$	0.74	0.000
OP evergreen angiosperm	$Log_{10}WUE_i = 2.103 - 0.002MAP - 0.003Lat - 0.008MAT - 0.011DI$	0.70	0.000
OP forb	$Log_{10}WUE_i = 1.426 + 0.016MAT$	0.12	0.002
$\delta^{13}C$			
N_2FP evergreen angiosperm	$\delta^{13}C = -25.537 - 0.003MAP + 0.233DI$	0.52	0.000
N_2FP forb	$\delta^{13}C = -31.809 + 5.328DI - 0.229MAT - 0.063Lat$	0.72	0.000
OP deciduous angiosperm	$\delta^{13}C = -27.020 - 0.003MAP + 3.809log_{10}N_{area} - 0.001Elev$	0.43	0.000
OP evergreen angiosperm	$\delta^{13}C = -29.883 + 2.003log_{10}N_{area} + 0.002Elev + 0.125MAT - 0.002MAP$	0.60	0.000
OP forb	$\delta^{13}C = -25.746 - 0.008MAP + 0.001Elev + 2.739log_{10}N_{area}$	0.83	0.000
OP graminoid	$\delta^{13}C = -22.809 - 0.009MAP + 2.352log_{10}N_{area}$	0.66	0.000
OP gymnosperm	$\delta^{13}C = -24.547 - 0.012Elev$	0.87	0.021

Equations were developed for growth forms within N_2FP and OP using log_{10}-transformed data for A_{sat}, g_s, WUE_i, and N_{area} and untransformed data for other variables. Absence of an equation for a specific combination of growth form and nitrogen-fixing status signifies either insufficient data or a statistically insignificant regression. Predictive variables were N_{area}, latitude (Lat), mean annual precipitation (MAP), mean annual temperature (MAT), dryness index (DI), and elevation (Elev).

again a key driver of g_s (Table 1). It is noteworthy that N_{area} had a positive relationship with g_s for all OP and forbs within N_2FP (reject hypothesis B).

Patterns for WUE_i and $\delta^{13}C$ were very different to those for A_{sat} and g_s. N_{area} was particularly important to predicting WUE_i (Fig. 2 and Table 1) for all growth forms of N_2FP and of much lesser significance for OP; $\delta^{13}C$ was best predicted using a variety of combinations of precipitation, latitude, temperature, elevation, and dryness index.

Bivariate analyses of the data mostly lend support to multivariate analyses showing N_{area} of N_2FP unrelated to A_{sat} (reject hypothesis A) (Fig. 3*A* and Table 2) or g_s (reject hypothesis B) (Fig. 3*C*). For OP, N_{area} was significantly related to A_{sat} (accept hypothesis A) (Fig. 3*B*) but not g_s (Fig. 3*D*). Instantaneous WUE

Table 2. Bivariate relationships among A_{sat}, g_s, WUE_i, $\delta^{13}C$, and climate-related variables for N_2FP and OP

Independent variable and nitrogen-fixing status	$Log_{10}A_{sat}$			$Log_{10}g_s$			$Log_{10}WUE_i$			$\delta^{13}C$		
	R^2	*P* value	Slope	R^2	*P* value	Slope	R^2	*P* value	Slope	R^2	*P* value	Slope
Latitude												
N_2FP	0.55	0.000	0.003	0.64	0.000	0.006	0.48	0.000	−0.003	0.10	0.276	
OP	0.29	0.000	0.002	0.43	0.000	0.004	0.31	0.000	−0.002	0.08	0.105	
MAP (mm)												
N_2FP	0.40	0.001	0.0002	0.03	0.822		0.30	0.016	−0.0001	0.70	0.000	−0.005
OP	0.22	0.000	0.0006	0.30	0.000	0.0001	0.57	0.000	−0.0002	0.57	0.000	−0.004
MAT (°C)												
N_2FP	0.41	0.001	−0.015	0.19	0.123		0.06	0.647		0.07	0.464	
OP	0.09	0.127		0.01	0.849		0.09	0.128		0.05	0.333	
Dryness index												
N_2FP	0.15	0.235		0.01	0.963		0.12	0.351		0.49	0.000	0.685
OP	0.02	0.758		0.08	0.183		0.08	0.144		0.39	0.000	0.547
Elevation (meters above sea level)												
N_2FP	0.19	0.112		0.19	0.143		0.11	0.390		0.06	0.486	
OP	0.17	0.003	−0.004	0.14	0.012	−0.0006	0.04	0.464		0.03	0.584	

Pearson correlations were completed on log-transformed data for all variables, with the exception of $\delta^{13}C$. Slopes are shown for significant relationships only. MAP, mean annual precipitation; MAT, mean annual temperature.

was related to N_{area} for both N_2FP and OP but more significantly and tightly so for the former (accept hypothesis C) (Fig. 3 *E* and *F*). Relative to OP, N_2FP showed marginally faster rates of both photosynthetic carbon fixation and g_s in Koppen zones B–D, irrespective of whether data were constrained to sites where direct comparisons could be made (Table S1) or not so constrained (Table S2). Both OP and N_2FP show clearly significant relationships between $\delta^{13}C$ and N_{area} (Fig. 3 *G* and *H*). Additional bivariate analysis (Table 2) helped elucidate specific non-N influences on physiological properties. For both N_2FP and OP, latitude was a surprisingly strong predictor of A_{sat}, g_s, and WUE_i; $\delta^{13}C$, however, was much better predicted by precipitation (Table 2) and was not significantly related to latitude. Our larger, nonpaired dataset produced similar results, albeit that the relationships were generally weaker than those of the paired data (Table S3).

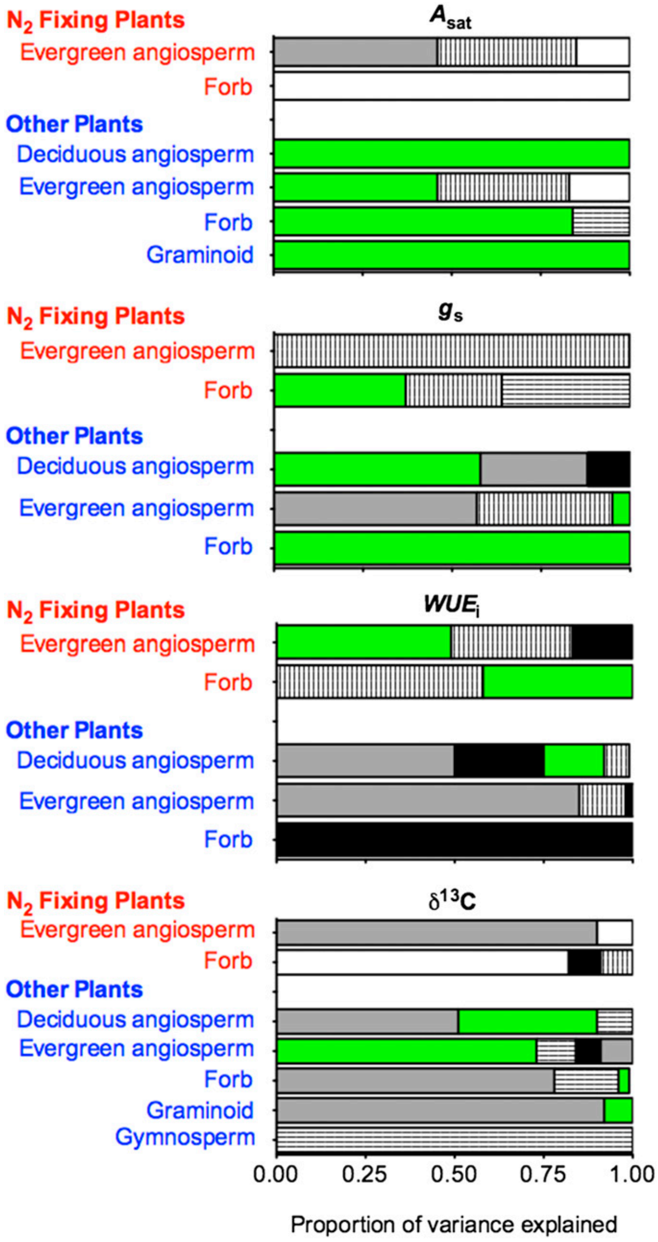

Fig. 2. Proportional contributions to explain variance in multivariate relationships describing physiological parameters (shown in Table 1). Contributions are shown for N_{area} (green bars), latitude (vertical line bars), precipitation (gray bars), temperature (black bars), dryness index (white bars), and elevation (horizontal line bars).

Discussion

Positive relationships between leaf N and A_{sat} have been widely reported at scales ranging from individual plant species to the globe. For example, our independent analysis for OP (Fig. 3*B*) is qualitatively similar to those in the works by Evans (19) and Wright et al. (5). However, our analysis also shows that this is not the case for N_2FP in nonagricultural ecosystems (Fig. 3*A*), and the literature shows that it is not true for agricultural systems (26). Our results also challenge the prevailing theory that additional leaf N will increase A_{sat} or reduce g_s (20). We found that additional leaf N was only ever a positive influence on both A_{sat} and g_s.

Osnas et al. (6) and many others draw on the broad observation that leaves have evolved primarily to intercept light and capture CO_2 to propose that photosynthetic capabilities are mostly proportional to leaf area. There are, however, other evolutionary forces at work. Given the lack of support among N_2FP for either greater carbon gain (hypothesis A) or reduced leaf water loss (hypothesis B) but good evidence for enhanced WUE (hypothesis C), can these other forces help explain leaf N and the dominance of many arid and semiarid zones by woody legumes?

Rates of leaf and plant growth are only part of evolutionary success and must be considered alongside a plant's ability to survive and reproduce. Relative to photosynthetic needs, overinvestment of nitrogen in leaves in harsh semiarid to arid regions has remained unexplained (22). In these areas, there is little selection pressure for light, to create a large canopy, or to grow quickly. A potent selective force is the ability to survive (as either plant or seed) periods of drought that might last weeks to months or even a decade or more.

For annual agricultural legumes, Hardwick (27) noted that canopy A_{sat} varies according to the rate of growth of the seed—not the other way around. There is also abundant evidence that remobilization of nitrogen from foliage and other plant tissues may account for 70–90% of seed nitrogen in annual agricultural legumes (28). Prolific flowering and generation of seedpods and seeds are features of many N_2FP (Fig. S1). Although it is not known how much nitrogen is remobilized from leaves to seeds for the thousands of species of N_2FP in nonagricultural ecosystems, current knowledge suggests that leaf N is an investment in the ability of N_2FP to produce seed and the "survival of the genome" (27). Furthermore, the competitive ability of N_2FP is enhanced by their ability to take up other forms of N available in the soil (29) or when diazotrophy is restricted by water availability (30, 31). N_2FP also make efficient use of N temporarily stored in foliage. For example, in the forms of amines, polyamines, alkaloids, cyanogenic glucosides, and many others, N-rich molecules help N_2FP cope with drought (by osmotic adjustment) as well as freezing conditions (32) and also, help deter herbivores in both tropical and nontropical forests (33, 34).

Despite relatively recent evolution (~60 MyBP) (35, 36), possibly from a "single cryptic evolutionary innovation" (36), symbioses with diazotrophic bacteria ensure access of N_2FP to nitrogen—one of the most limiting resources for plant growth, survival, and reproduction. That insurance and other nitrogen-related advantages have facilitated the spread of N_2FP throughout the globe and their contributions to global N cycles (37, 38). The facultative nature of the symbiosis with respect to soil nitrogen (4, 8–10) is augmented by its flexibility in relation to soil water—N_2FP seldom fix nitrogen under drought conditions (29–31), although their ability to nodulate may be unimpeded (39) and help restore fixation after drought is relieved. These features facilitate the dominant role played by N_2FP in both wet and dry tropics as well as large areas of temperate and Mediterranean climates. WUE contributes further to the evolutionary advantages enjoyed by legumes and other N_2FP. In their recent synthesis of the now large body of work that informs our

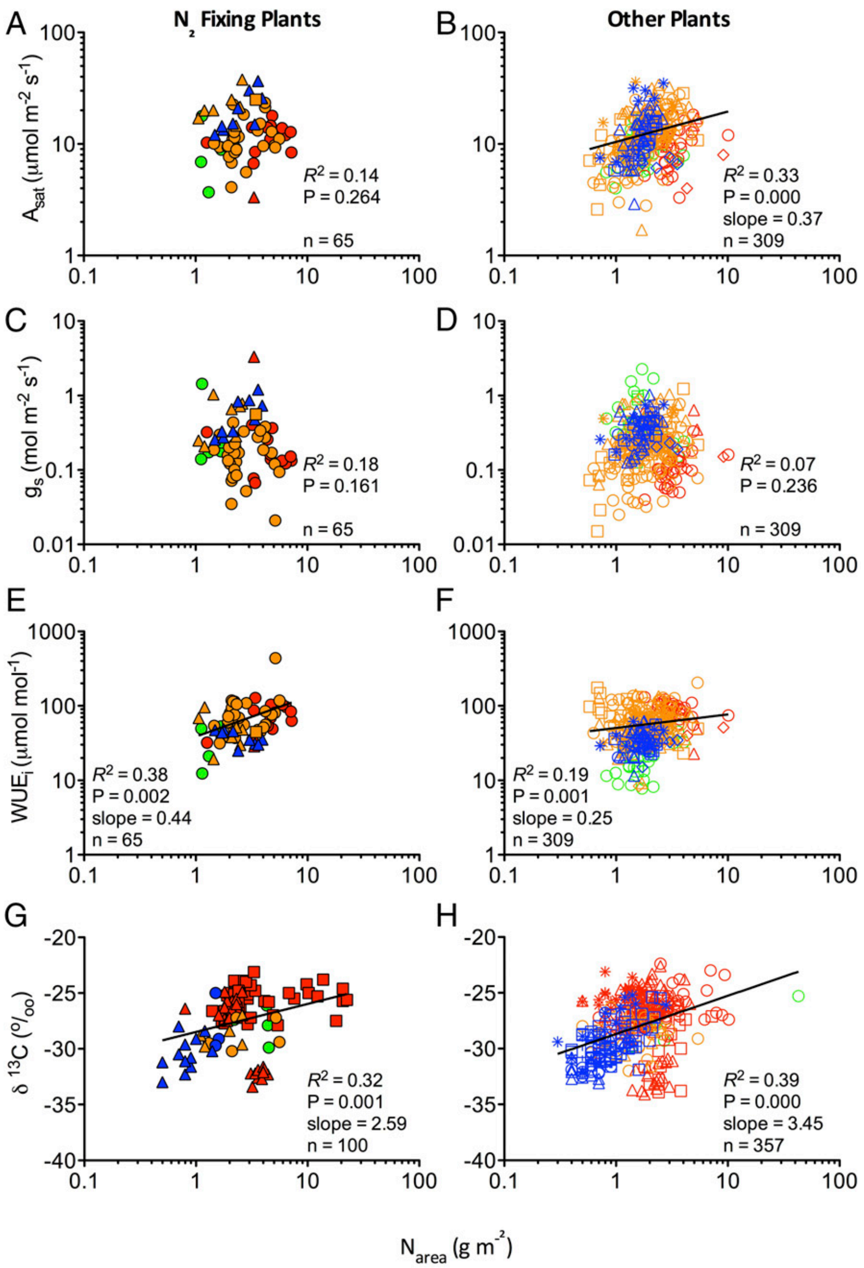

Fig. 3. Relationships between N_{area} (grams meter^{-2}) and light-saturated A_{sat} (micromoles meter^{-2} second^{-1}), light-saturated rate of g_s (moles meter^{-2} second^{-1}), WUE_i (micromoles CO_2 moles^{-1} H_2O), and $\delta^{13}C$ (percentage) for (*A*, *C*, *E*, and *G*) N_2FP and (*B*, *D*, *F*, and *H*) OP. Symbol shape corresponds to growth form: evergreen angiosperm (circle), deciduous angiosperm (square), forb (triangle), fern (dash), gymnosperm (diamond), and graminoid (asterisk). Symbol color denotes Koppen climate classification: A (green; tropical), B (red; arid and semiarid), C (orange; temperate), and D (blue; continental). Pearson correlations completed on $\log_{10}$-transformed data for all variables. Slopes are shown for significant relationships only.

understanding of δ-values in plants, Cernusak et al. (21) noted that, for C_3 plants, the range in δ-values (Cernusak used Δ in place of δ) was constrained by coordination of g_s and A_{sat}. A more sophisticated and complex relationship between δ and WUE than what was once recognized does not detract from the evidence presented here that the latter contributes to our knowledge of the benefits enjoyed by legumes and why they are different from OP (40).

If trait-based models of regional and global carbon cycles (7) are to achieve promised predictive capabilities, they will need to incorporate WUE as well as traits, such as the ability of N_2FP to store and use N in leaves for other survival-related functions. Increasingly dry conditions in many areas of the globe reinforce this point. In similar fashion, the absence of significant predictive power of leaf nitrogen for rates of carbon fixation by N_2FP will pose ongoing challenges given their dominance of so many wet tropical forests that collectively are critical to global C cycles.

Methods

Data Acquisition. We developed a database from a global meta-analysis of published literature (Table S4). Our database was targeted to our hypotheses; studies included from natural systems had to contain a measure of leaf nitrogen content and a measure of leaf WUE for N_2FP and OP. We identified relevant literature by screening the Web of Science and Google Scholar search engines for keywords: carbon isotope discrimination, ^{13}C, WUE, water use efficiency, leaf nitrogen, legume*, n-fix*, and nodulation; it also included relevant citations documented in these literature. We included targeted searches for each of the major actinorhizal genera.

We constructed two datasets: one based on studies with concurrent data that were collected from the same site for both N_2FP and OP (paired dataset) and one that included studies with data for either N_2FP or OP presented (nonpaired dataset). For each of the paired and nonpaired datasets, we had two subsets: one comprised of data of N_{mass} (milligrams gram^{-1}) and δ^{13}C (percentage) recorded concurrently and one comprised of data for studies of N_{area} (grams meter^{-2}) reported concurrently with WUE$_i$ (micromoles CO_2 moles^{-1} H_2O) or both A_{sat} (micromoles CO_2 meter^{-2} second^{-1}) and g_s to water vapor (moles meter^{-2} second^{-1}), such that we could calculate WUE$_i$. The paired dataset includes 22 sites across the globe for studies that presented data in a form from which we could record or calculate N_{area} together with WUE$_i$ and 81 sites containing data in a form from which we could record or calculate N_{mass} and δ^{13}C, with 57 of those sites also presenting data for specific leaf area (meters^2 kilogram^{-1}) or leaf mass per unit area (grams centimeter^{-2}), which enabled calculation of N_{area}. The nonpaired dataset contains 63 sites across the globe for N_{area} and WUE$_i$ and 351 sites for N_{mass} and δ^{13}C. For studies where a treatment was applied, only data from the control were used. Species were identified as N_2FP (including actinorhizal and nodulating plants) or OP and classified by their growth form: fern, forb, graminoid, gymnosperm, woody evergreen angiosperm, or woody deciduous angiosperm. In total, 11 actinorhizal species were included, the majority of which are from the families Rosaceae or Casuarinaceae (Fig. S2). Digital latitude and longitude of each site were recorded and used to identify site mean annual temperature (degrees Celsius), mean annual precipitation (millimeters), dryness index (mean annual precipitation/potential evaporation), and elevation (meters a.s.l.). We also identified sites according to their Koppen classification (A, tropical/megathermal; B, dry/arid/semiarid; C, temperate/mesothermal; and D, continental/microthermal).

Data Analysis. Shapiro–Wilk tests showed that data for all variables were significantly nonnormal (skewed to the right). Log$_{10}$ transformations improved normality distributions of data for all variables except δ^{13}C, which had distribution that did not improve with either log$_{10}$ or square root transformation; hence, all analyses were performed on nontransformed δ^{13}C data.

We used multivariate analyses (linear mixed models and maximum likelihood) to quantify the combined influence of N-fixing status, climate variables, and growth form on leaf nitrogen. Site and author were treated as random factors for all analyses to counter nonindependence. We used bivariate analyses (Pearson correlations) to assess simple relationships between measures of WUE and leaf nitrogen content or measures of WUE and climate-related variables. Multivariate stepwise multiple regressions better explained relationships in toto among leaf nitrogen, climate, and leaf WUE. The large range in data for bivariate analyses was conserved between N_2FP and OP groups. All analyses were performed with SPSS. Unless denoted otherwise, data and analyses refer to the paired dataset.

ACKNOWLEDGMENTS. We thank Alexandra Barlow for helping us screen the literature. We also thank the numerous authors who provided additional data on request and the two reviewers for their suggestions that significantly improved this article. We thank the Australian Research Council for support. ETH Zurich is thanked for its support to M.A.A. as a visiting professor.

1. Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations or carbon and nutrients in leaves of terrestrial plants. *Ecol Monogr* 82(2):205–220.
2. Houlton BZ, Wang Y-P, Vitousek PM, Field CB (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature* 454(7202):327–330.
3. Batterman SA, et al. (2013) Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* 502(7470):224–227.
4. Menge DNL, Levin SA, Hedin LO (2008) Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. *Proc Natl Acad Sci USA* 105(5):1573–1578.
5. Wright IJ, et al. (2004) The worldwide leaf economics spectrum. *Nature* 428(6985): 821–827.
6. Osnas JLD, Lichstein JW, Reich PB, Pacala SW (2013) Global leaf trait relationships: Mass, area, and the leaf economics spectrum. *Science* 340(6133):741–744.
7. Krinner G, et al. (2005) A dynamic global vegetation model for studies of the coupled atmosphere-biosphere system. *Global Biogeochem Cycles* 19:GB1015.
8. Menge DNL, Lichstein JW, Angeles-Pérez G (2014) Nitrogen fixation strategies can explain the latitudinal shift in nitrogen-fixing tree abundance. *Ecology* 95(8): 2236–2245.
9. Hedin LO, Brookshire ENJ, Menge DNL, Barron AR (2009) The nitrogen paradox in tropical forest ecosystems. *Annu Rev Ecol Evol Syst* 40:613–635.
10. Sullivan BW, et al. (2014) Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical N cycle. *Proc Natl Acad Sci USA* 111(22):8101–8106.
11. Arróniz-Crespo M, et al. (2014) Bryophyte-cyanobacteria associations during primary succession in recently Deglaciated areas of Tierra del Fuego (Chile). *PLoS One* 9(5): e96081.
12. Gtari M, Dawson JO (2011) An overview of actinorhizal plants in Africa. *Funct Plant Biol* 38(9):653–661.
13. Adams MA, Simon J, Pfautsch S (2010) Woody legumes: A (re)view from the South. *Tree Physiol* 30(9):1072–1082.
14. Thuiller W, Lavorel S, Midgley G, Lavergne S, Rebelo T (2004) Relating plant traits and species distributions along bioclimatic gradients for 88 *Leucadendron* taxa. *Ecology* 85(6):1688–1699.
15. Ordonez JC, et al. (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Glob Ecol Biogeogr* 18(2):137–149.
16. Pollock LJ, Morris WK, Vesk PA (2012) The role of functional traits in species distributions revealed through a hierarchical model. *Ecography (Cop.)* 35(8):716–725.
17. Stahl U, Reu B, Wirth C (2014) Predicting species' range limits from functional traits for the tree flora of North America. *Proc Natl Acad Sci USA* 111(38):13739–13744.
18. Violle C, Reich PB, Pacala SW, Enquist BJ, Kattge J (2014) The emergence and promise of functional biogeography. *Proc Natl Acad Sci USA* 111(38):13690–13696.
19. Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78(1):9–19.
20. Farquhar GD, Buckley TN, Miller JM (2002) Optimal stomatal control in relation to leaf area and nitrogen content. *Silva Fenn* 36(3):625–637.
21. Cernusak LA, et al. (2013) Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytol* 200(4):950–965.
22. Prentice IC, Dong N, Gleason SM, Maire V, Wright IJ (2014) Balancing the costs of carbon gain and water transport: Testing a new theoretical framework for plant functional ecology. *Ecol Lett* 17(1):82–91.
23. Song M, Djagbletey G, Nkrumah EE, Huang M (2016) Patterns in leaf traits of leguminous and non-leguminous dominant trees along a rainfall gradient in Ghana. *J Plant Ecol* 9(1):69–76.
24. Kottek M, Grieser J, Beck C, Rudolf B, Rubel F (2006) World map of the Köppen-Geiger climate classification updated. *Meteorol Z* 15(3):259–263.
25. Mahlstein I, Daniel JS, Solomon S (2013) Pace of shifts in climate regions increases with global temperature. *Nat Clim Chang* 3(8):739–743.
26. Del Pozo A, Garnier E, Aronson J (2000) Contrasted nitrogen utilization in annual C_3 grass and legume crops: Physiological explorations and ecological considerations. *Acta Oecol* 21(1):79–89.
27. Hardwick RC (1988) Critical physiological traits in pulse crops. *World Crops: Cool Season Food Legumes*, ed Summerfield RJ (Kluwer, Dordrecht, The Netherlands), pp 885–896.
28. Schiltz S, Munier-Jolain N, Jeudy C, Burstin J, Salon C (2005) Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by 15N in vivo labeling throughout seed filling. *Plant Physiol* 137(4): 1463–1473.
29. Erskine PD, et al. (1996) Water availability—a physiological constraint on nitrate utilization in plants of Australian semi-arid mulga woodlands. *Plant Cell Environ* 19(10):1149–1159.
30. Serraj R, Sinclair TR, Purcell LT (1999) Symbiotic N_2 fixation response to drought. *J Exp Bot* 50(331):143–155.
31. Valentine AJ, Benedito VA, Kang Y (2010) Abiotic stress in legume N2 fixation: From physiology to genomics and beyond. *Annual Plant Reviews Volume 42: Nitrogen Metabolism in Plants in the Post-Genomic Era*, eds Foyer C, Zhao M (Wiley-Blackwell, Oxford), pp 207–248.
32. Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. *Plant Cell* 7(7):1099–1111.
33. Wink M (2013) Evolution of secondary metabolites in legumes (Fabaceae). *S Afr J Bot* 89:164–175.
34. Kursar TA, et al. (2009) The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus Inga. *Proc Natl Acad Sci USA* 106(43):18073–18078.
35. Lavin M, Herendeen PS, Wojciechowski MF (2005) Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the tertiary. *Syst Biol* 54(4):575–594.
36. Werner GDA, Cornwell WK, Sprent JI, Kattge J, Kiers ET (2014) A single evolutionary innovation drives the deep evolution of symbiotic N_2-fixation in angiosperms. *Nat Commun* 5:4087.
37. Vitousek PM, Menge DNL, Reed SC, Cleveland CC (2013) Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Philos Trans R Soc Lond B Biol Sci* 368(1621):20130119.
38. Powers JS, Tiffin P (2010) Plant functional type classifications in tropical dry forests in Costa Rica: Leaf habit versus taxonomic approaches. *Funct Ecol* 24(4):927–936.
39. Wurzburger N, Miniat CF (2014) Drought enhances symbiotic dinitrogen fixation and competitive ability of a temperate forest tree. *Oecologia* 174(4):1117–1126.
40. McKey D (1994) Legumes and nitrogen: The evolutionary ecology of a nitrogen-demanding lifestyle. *Advances in Legume Systematics, Vol 5: The Nitrogen Factor*, eds Sprent JJ, McKey D (Royal Botanic Gardens, Burlington, ON, Canada), pp 211–228.