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**Abstract**

Terrestrial biosphere models commonly predict leaf photosynthesis based on positive relationships between soil nitrogen, leaf nitrogen, and photosynthetic capacity. While positive empirical relationships between soil nitrogen and leaf nitrogen are common, recent work leveraging photosynthetic least-cost theory indicates that interactions between aboveground climate and soil nitrogen may be a more reliable predictor of leaf nitrogen allocation than soil nitrogen alone. Specifically, the theory predicts that increasing aridity will increase leaf nitrogen allocation, which will allow individuals to maintain photosynthesis at lower water use. It also predicts that soil nitrogen availability should increase the positive effect of aridity on leaf nitrogen allocation and water-use efficiency. However, few direct tests of this theory exist, and the timescale that plants respond to is unknown. To test the theory, we measured leaf nitrogen and water-use efficiency at 25 sites scattered across a precipitation and soil nitrogen availability gradient in Texan grasslands. We found that soil nitrogen availability increased the positive effect of aridity on leaf nitrogen per leaf mass, but only in C3 graminoid and herbaceous species. We also observed that soil nitrogen availability increased the positive effect of aridity on water use efficiency, a pattern also only observed in C3 graminoid and herbaceous species. These results support patterns expected from photosynthetic least-cost theory and support a mechanism explaining increased leaf nitrogen allocation in arid or dryland systems.

**Introduction**

Terrestrial biosphere models, which comprise the land surface component of Earth system models, are sensitive to the formulation of photosynthetic processes (). This is because photosynthesis is the largest carbon flux between the atmosphere and terrestrial biosphere, and is constrained by ecosystem carbon and nutrient biogeochemical cycles (Hungate *et al.*, 2003; LeBauer & Treseder, 2008; IPCC, 2013; Fay *et al.*, 2015). Many terrestrial biosphere models formulate photosynthesis by parameterizing photosynthetic capacity within plant functional types through positive relationships between soil nitrogen availability, leaf nitrogen allocation, and photosynthetic capacity (Rogers, 2014; Rogers *et al.*, 2017). While empirical support for these relationships is abundant (Brix, 1971; Evans & Seemann, 1989; Evans, 1989; Walker *et al.*, 2014; Firn *et al.*, 2019), plant acclimation responses to changing environments (e.g., increasing CO2, light availability, temperature, precipitation variability, etc.) can alter leaf nitrogen allocation and photosynthetic capacity independent of soil nutrient availability (Poorter *et al.*, 2019, 2022; Luo *et al.*, 2021). The inability of terrestrial biosphere models to capture such acclimation responses to environmental change casts uncertainty in the ability of these models to accurately simulate accurate and robust photosynthetic responses to global change (Smith & Dukes, 2013; Harrison *et al.*, 2021).

[why leaf nitrogen allocation? What does it tell us and what do we need to understand about its plasticity in response to environmental change across time and space?]

[existing frameworks for predicting leaf nitrogen allocation across time and space]

Contemporary analyses using photosynthetic least-cost theory suggest that leaf nutrient allocation, photosynthetic capacity, and relationships between leaf nutrient allocation and photosynthetic capacity may be better predicted through factors that influence leaf nutrient demand to build and maintain photosynthetic machinery (Dong *et al.*, 2017, 2020, 2022; Smith *et al.*, 2019; Paillassa *et al.*, 2020). Indeed, studies show that leaf nitrogen and photosynthetic capacity can be reliably predicted through mean growing season irradiance, growing season temperature, growing season vapor pressure deficit, or edaphic characteristics such as soil pH (Dong *et al.*, 2017, 2020; Paillassa *et al.*, 2020). However, relationships between leaf nitrogen and photosynthetic capacity may also be determined through costs of leaf construction, commonly evidenced through leaf mass per area, or other species identity traits, such as whether a species associated with nitrogen-fixing bacteria (Dong *et al.*, 2017)

Photosynthetic least-cost theory provides a useful framework for understanding when and where factors that influence leaf nutrient demand to build and maintain photosynthesis modify leaf nitrogen allocation and photosynthetic capacity. The theory predicts that plants acclimate to their environment by maximizing photosynthetic carbon gain at the lowest summed cost of nitrogen and water usage (Wright *et al.*, 2003; Prentice *et al.*, 2014). Nitrogen and water use can be substituted for each other to maintain the lowest summed cost of nitrogen and water use, such that less efficient use of a relatively more abundant resource should be traded for more efficient use of a relatively less abundant resource. For example, plants growing in arid or semiarid systems should acclimate to their growing conditions by increasing leaf nitrogen allocation and decreasing stomatal conductance, allowing a given photosynthetic rate to be maintained with less efficient nitrogen use and more efficient water use. This strategy is particularly useful in arid or semiarid systems because it allows for plants to save water without needing to sacrifice productivity (Paillassa *et al.*, 2020).

While there is a clear need to understand when and where factors influence leaf nutrient demand to build and maintain photosynthetic machinery, it is also imperative that we understand the relevant timescales plants use to acclimate to their environment. Smith & Dukes (2017) used model selection techniques to show that photosynthetic biochemical process rates were best predicted by the mean temperature of the seven days leading up to a measurement. This was done across temperate and tropical forest sites spanning a large latitudinal gradient, and their results are limited to said ecosystem types. Understanding whether these timescales differ across other ecosystem types is also important, especially in grassland systems because they occupy approximately XX% of land in the coterminous United States and XX% of land globally.

In this study, we measured leaf traits in 554 individuals spanning XX species across 25 Texan grassland sites in summer 2020 and 2021. Texas is home to a diverse climatic gradient, with mean annual precipitation ranging from XX to XX, mean annual temperature ranging from XX to XX, and mean annual vapor pressure deficit ranging from XX to XX (Table 1; Fig. 1). Texas is also home to diverse soils and nutrient availability thresholds. Following the approach explained in Smith & Dukes (2018), we used model selection to determine whether mean 15-year climatic factors, iterations of short-term climatic factors (one day leading up to thirty days pre-measurement), edaphic characteristics such as soil nutrient availability, cation exchange capacity, or soil pH, or leaf and species identity traits such as leaf mass per area or nitrogen-fixing potential were . Given the best model fits, we hypothesized that increasing aridity would increase leaf nitrogen allocation, which would allow individuals to maintain photosynthesis at lower water usage. We also hypothesized that soil nutrient availability would increase the positive effect of aridity on leaf nitrogen allocation and water use efficiency.

**Methods**

*Property selection and sampling methodology*

This environmental gradient experiment was conducted through the Texas Ecological Laboratory program, which grants researchers access to privately owned properties in the state of Texas. We collected leaf and soil samples from 23 properties scattered across central and eastern Texas in summer 2020 and summer 2021 (Fig. 1). Twelve properties were visited between June and July 2020, and 15 properties (11 unique from 2020) were visited between May and June 2021 (Table 1). We explicitly chose properties that maximized between-site climatic and edaphic diversity and contained a dominant open savanna or grass land component (Table 1). Any property with anecdotal or written evidence of grazing or disturbance (e.g., mowing, feral hog activity, etc.) were excluded from our analysis.

*Field collection methods and functional type assignments*

We identified and collected leaf material of the five most dominant species at each property. Leaf material was collected from three individuals of each species at random locations in the property. All leaves were fully expanded and free from shading by nearby shrubs or trees. Composite soil samples were also collected at random locations in each property. In the 2021 field season, we attached a MultispeQ photosynthesis device (PhotosynQ Inc., East Lansing, MI, USA) to fully expanded leaves prior to leaf collection to obtain chlorophyll fluorescence data and gather snapshot relative chlorophyll content and PAM fluorescence parameters.

All collected and identified species were assigned a plant functional type based on their photosynthetic pathway (C3, C4) and growth habit (graminoid, forb, etc.) according to the classifications listed in the USDA PLANTS database ([https://plants.usda.gov](https://plants.usda.gov/home)). This created four distinct plant functional types within our dataset: C3 legumes (n=56), C3 forbs (n=315), C3 graminoids (n=31), and C4 graminoids (n=123). We used plant functional type as the primary descriptor of species identity to replicate major vegetation classes used in many terrestrial biosphere and Earth system models.

*Leaf trait measurements*

Images of each leaf were taken immediately following each property visit using a flat-bed scanner. Wet leaf area was determined from each image using the 'LeafArea' R package (Katabuchi, 2015), which automates leaf area calculations using ImageJ software (Schneider *et al.*, 2012). Each leaf was then dried at 65C for at least 48 hours, weighed for dry biomass, and ground in a mortar and pestle until homogenized. Subsamples of dried and homogenized leaf tissue were used to measure leaf nitrogen content (*N*mass; gN g-1) through elemental combustion analysis (Costech-4010, Costech Instruments, Valencia, CA) and leaf δ13C through isotope ratio mass spectroscopy (PDZ Europa 20-20 Isotope Ratio Mass Spectrometer, Sercon Ltd., Chestshire, UK). Leaf δ13C values were processed through services offered by the University of California-Davis Stable Isotope Facility. We calculated leaf mass per area (*M*area; g m -2) as the ratio of wet leaf area to dry leaf biomass, and leaf nitrogen per leaf area (*N*area; gN m-2) as the product of *N*mass and *M*area

We used leaf δ13C values to estimate the ratio of intercellular (*C*i) to extracellular (*C*a) CO2 (χ; Pa Pa-1) following the approach of Farquhar *et al.* (1989) described in Cernusak *et al.* (2013). We derived χ as:

(Eqn. 1)

Δ13C represents the relative difference between leaf δ13C (‰) and air δ13C (‰), and is calculated from the following equation:

(Eqn. 2)

where δ13Cair is assumed to be -8‰ (Keeling *et al.*, 1979; Farquhar *et al.*, 1989), *a* represents the fractionation between 12C and 13C due to diffusion in air, assumed to be 4.4‰, and *b* represents the fractionation caused by Rubisco carboxylation, assumed to be 27‰ (Farquhar *et al.*, 1989). For C4 species, *b* in Eqn. 1 was calculated as:

(Eqn. 3)

Where c was set to -5.7‰ and d was set to 30‰ (Farquhar 1989 cite). φ, which is the bundle sheath leakiness term, was set to 0.4. All χ values less than 0.2 and greater than 1.0 were removed.

*Edaphic characteristics*

Composite soil samples from all property visits were sent to the Texas A&M Soil, Water and Forage Laboratory to quantify soil pH, cation exchange capacity, and macronutrient concentrations. Soil pH was measured in a 1:2 soil: deionized water slurry after a 30-minute incubation period with a hydrogen selective electrode. Cation exchange capacity (μmho cm-1) was similarly measured in a 1:2 soil: deionized water slurry after a 30-minute incubation period with a conductivity probe. Soil phosphorus, potassium, calcium, magnesium, sodium, and sulfur concentrations (all ppm) were measured using the Mehlich III method. Soil nitrate-nitrogen (NO3-N; ppm) was extracted in 1 M KCl and measured spectrophotometrically at 520nm through the end product of a NO3-N to NO2-N cadmium reduction. We also determined soil texture using the simple jar method to determine percent silt, clay, and sand.

*Site climate data*

We used the Parameter-elevation Regressions on Independent Slopes Model (PRISM; Daly *et al.*, 2008) climate product to access gridded daily temperature and precipitation data for the coterminous United States at a 4-km grid resolution between January 1, 1991 and July 31, 2021 (PRISM Climate Group, Oregon State University, <https://prism.oregonstate.edu>, data created 4 Feb 2014, accessed 24 Mar 2022). Daily mean air temperature and total precipitation data were extracted from the grid cell that contained the latitude and longitude of each property using the ‘extract’ function in the ‘terra’ R package (Hijmans, 2022). PRISM data were used in lieu of local weather station data because several rural properties did not have a local weather station present within a 20-km radius of the property. Daily site climate data were used to estimate mean annual precipitation and mean annual temperature for each property between 1991 and 2020 (Table 1). We also iteratively calculated total precipitation and mean daily air temperature for the prior 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, and 30 days leading up to each site visit to later determine the relevant climatic timescale that best explained *N*mass, *M*area, *N*area, and χ using an information-theoretic model selection approach.

*Site aridity*

Site aridity was estimated as the ratio of total precipitation to total potential evapotranspiration. Potential evapotranspiration was estimated using the ‘splash’ R package , which is an R implementation of the SPLASH model explained in Davis *et al.* (2017). The SPLASH model calculates daily estimates of total solar radiation and three estimates of evapotranspiration (potential, actual, equilibrium) through Priestley-Taylor equations using daily mean temperature, precipitation, the number of daily sunlight hours, and latitude as model inputs (Davis *et al.*, 2017). Daily sunlight hours were estimated for each day at each site using the ‘getSunlightTimes’ function in the ‘suncalc’ R package, which estimated sunrise and sunset times of each property using date, property latitude, and property longitude (Thieurmel & Elmarhraoui, 2019). We used daily precipitation and potential evapotranspiration estimates to quantify property aridity across four timescales: (1) 30 days prior to each property visit, (2) 60 days prior to each property visit, (3) 90 days prior to each property visit, and (4) mean annual aridity between 2006 and 2020.

**Table 1** Descriptions of each property, including GPS coordinates, sampling year(s), visit type, 2006-2020 mean annual precipitation (MAP), 2006-2020 mean annual temperature (MAT), and 2006-2020 mean annual aridity (MAA)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Property** | **Latitude** | **Longitude** | **Sampling year** | **MAP (mm yr-1)** | **MAT (°C)** | **MAA (unitless)** |
| Austin\_2020\_03 | 29.78 | -96.24 | 2021 | 1110.1 | 20.6 | 0.734 |
| Bandera\_2020\_03 | 29.85 | -99.30 | 2021 | 789.7 | 18.9 | 0.527 |
| Bell\_2021\_08 | 31.06 | -97.55 | 2021 | 938.9 | 19.6 | 0.637 |
| Bexar\_2019\_13 | 29.24 | -98.43 | 2020 | 760.0 | 21.4 | 0.490 |
| Blanco\_2019\_16 | 29.99 | -98.43 | 2021 | 834.9 | 19.2 | 0.556 |
| Brazos\_2020\_16 | 30.93 | -96.23 | 2021 | 1079.5 | 20.1 | 0.731 |
| Brazos\_2020\_18 | 30.52 | -96.21 | 2020, 2021 | 1102.3 | 20.4 | 0.739 |
| Burnet\_2020\_12 | 30.82 | -98.06 | 2021 | 815.3 | 19.3 | 0.550 |
| Burnet\_2020\_14 | 30.84 | -98.34 | 2021 | 764.5 | 19.5 | 0.516 |
| Comal\_2020\_19 | 30.01 | -98.32 | 2021 | 845.4 | 19.3 | 0.564 |
| Comal\_2020\_21 | 29.79 | -98.43 | 2020 | 881.0 | 19.9 | 0.581 |
| Edwards\_2019\_17 | 29.95 | -100.36 | 2020 | 560.6 | 19.0 | 0.368 |
| Fayette\_2019\_04 | 30.09 | -96.78 | 2020 | 1018.4 | 20.6 | 0.675 |
| Fayette\_2020\_09 | 29.86 | -96.71 | 2021 | 1004.0 | 20.8 | 0.661 |
| Fayette\_2021\_12 | 29.86 | -97.21 | 2021 | 985.9 | 20.4 | 0.652 |
| Harris\_2020\_03 | 29.88 | -95.31 | 2020, 2021 | 1487.6 | 21.5 | 0.970 |
| Hays\_2021\_54 | 29.96 | -98.17 | 2021 | 861.2 | 20.0 | 0.570 |
| Kerr\_2020\_03 | 30.06 | -99.34 | 2021 | 674.1 | 18.3 | 0.453 |
| Menard\_2020\_01 | 30.91 | -99.57 | 2020 | 642.6 | 18.3 | 0.437 |
| Sansaba\_2020\_01 | 31.29 | -98.62 | 2020 | 735.0 | 18.9 | 0.504 |
| Uvalde\_2020\_02 | 29.59 | -100.09 | 2020, 2021 | 649.8 | 19.5 | 0.427 |
| Washington\_2020\_08 | 30.28 | -96.41 | 2021 | 1077.4 | 20.4 | 0.717 |
| Williamson\_2019\_09 | 30.71 | -97.86 | 2020 | 867.3 | 19.6 | 0.585 |
| Williamson\_2019\_10 | 30.54 | -97.77 | 2020 | 820.3 | 19.9 | 0.550 |

*Data analysis*

To address our first hypothesis on relevant timescales that drive leaf nitrogen allocation and water use efficiency, we used an information-theoretic model selection approach to determine whether 30-day, 60-day, or 90-day property aridity conferred the best model fit for *N*mass, *M*area, *N*area,χ, and *N*area:χ. To do this, we constructed three linear mixed effects models where 30-day, 60-day, or 90-day property aridity was included as a single continuous fixed effect, with species as a random intercept term. This was done iteratively for models where *N*mass, *M*area, *N*area,χ, and *N*area:χ were included as the response variable. We used corrected Akaike Information Criterion (AICc), RMSE, and marginal R2 to select the aridity index timescale that conferred the best model fit. We defined this as models that had the lowest AICc value, highest RMSE value, and highest marginal R2. We repeated this process to select the precipitation total pooled across 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 days prior to each property visit that conferred the best fit for *N*mass, *M*area, *N*area,χ, and *N*area:χ. The short-term aridity and precipitation timescale that conferred the best model fit were then fed into the second set of linear mixed effects models explained below.

To investigate effects of edaphic characteristics, climate characteristics, and interactions between edaphic and climate characteristics on *N*mass, *M*area, *N*area,χ, and *N*area:χ, we constructed three separate series of linear mixed effects models. The first set of linear mixed effects models investigated individual and interactive effects of edaphic characteristics on *N*mass, *M*area, *N*area,χ, and *N*area:χ. In this set of models, soil pH, NO3-N availability, phosphorus availability, potassium availability, CEC, and % clay were included as individual continuous fixed effects. We also included interactions between all fixed effects, sampling year as a random intercept term, and species ID as an additional random intercept term.

The second set of linear mixed effects models investigated individual and interactive effects of climatic characteristics on *N*mass, *M*area, *N*area,χ, and *N*area:χ. This set of models included the relevant short-term aridity timescale selected using information theory, the relevant short-term precipitation timescale also selected using information theory, mean annual precipitation between 2006 and 2020, and mean annual temperature between 2006 and 2020 as individual continuous fixed effects. We also included interaction terms between all fixed effects, sampling year as a random intercept term, and species ID (not plant functional type) as an additional random intercept term.

The third set of linear mixed effects models investigated individual and interactive effects of important edaphic and climatic predictors on *N*mass, *M*area, *N*area,χ, and *N*area:χ. This was done by constructing models with significant edaphic and climatic predictors from the first two sets of models. We set a benchmark for whether a given predictor would be fed into this third set of models by quantifying relative importance of each individual predictor (described below). Specifically, predictors that had relative importance values greater than 5% were fed into the third set of linear mixed effects models both as individual and interactive fixed effects. We also included plant functional type as an additional categorical fixed effect, with sampling year and species ID included as random intercept terms.

All response variables were natural-log transformed to satisfy residual normality assumptions. In all models, we used the 'lmer' function in the 'lme4' R package (Bates et al., 2015) to fit each model and the 'Anova' function in the 'car' R package (Fox & Weisberg, 2019) to calculate Type II Wald's χ2 and determine the significance level (α=0.05) of each fixed effect coefficient. We also used the ‘calc.relimp’ function in the ‘relaimpo’ R package to determine relative importance, averaged over orders, of the predictors included in the first two sets of models. Finally, we used the 'emmeans' R package (Lenth, 2019) to conduct post-hoc comparisons using Tukey's tests, where degrees of freedom were approximated using the Kenward-Roger approach (Kenward & Roger, 1997). All analyses and plots were conducted in R version 4.1.1 (R Core Team, 2021).

**Results**

*Leaf nitrogen per leaf area*

*N*area was driven by a two-way interaction between long-term aridity and plant functional type. This interaction indicated that *N*area generally increased with long-term aridity in C3 forbs (Tukey: p=), marginally increased with long-term aridity in C4 graminoids (Tukey: p=0.060), and decreased with long-term aridity in legumes (Tukey: p=0.011). Despite this, there was no overall effect of long-term aridity on *N*area when plant functional types were pooled (Table 1). There was also a two-way interaction between short-term aridity and plant functional type on *N*area, which revealed no effect of short-term aridity in any plant functional groups aside from an increase in *N*area with increasing aridity in legumes (Tukey: p=0.001). There was no overall effect of short-term aridity on *N*area when plant functional types were pooled (Table 1). Finally, there was a two-way interaction between plant functional type and soil nitrogen availability, which revealed a null effect of soil nitrogen availability in all plant functional types except for an increase in *N*area in legumes (Tukey: p=0.008). Nonetheless, soil nitrogen availability had an individual positive effect on *N*area when pooled across plant functional types (Table 1).

**Table 1** Analysis of variance results explaining short-term aridity, long-term aridity, soil nitrogen availability, and plant functional type on leaf nitrogen per leaf area (*N*area), leaf nitrogen content (*N*mass), and leaf mass per area (*M*area)\*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | ln *N*area | | ln *N*mass | | ln *M*area | |
|  | df | χ2 | p | χ2 | p | χ2 | p |
| AI90 | 1 | 0.04 | 0.839 | 21.89 | **<0.001** | 17.79 | **<0.001** |
| AI2006\_2020 | 1 | 0.26 | 0.612 | 20.78 | **<0.001** | 12.28 | **<0.001** |
| Soil NO3-N (N) | 1 | 9.26 | **0.002** | 73.84 | **<0.001** | 21.73 | **<0.001** |
| PFT | 3 | 60.85 | **<0.001** | 43.54 | **<0.001** | 3.04 | 0.385 |
| AI90 \* N | 1 | 0.14 | 0.705 | 15.27 | **<0.001** | 13.89 | **<0.001** |
| AI2006\_2020 \* N | 1 | 0.08 | 0.779 | 1.53 | 0.216 | 1.66 | 0.198 |
| AI90 \* PFT | 3 | 20.29 | **<0.001** | 6.01 | 0.111 | 13.74 | **0.003** |
| AI2006\_2020 \* PFT | 3 | 31.16 | **<0.001** | 14.02 | **0.003** | 10.83 | **0.013** |
| N \* PFT | 3 | 19.08 | **<0.001** | 5.36 | 0.147 | 31.38 | **<0.001** |
| AI90 \* N \* PFT | 3 | 6.79 | 0.079 | 5.01 | 0.171 | 1.76 | 0.624 |
| AI2006\_2020 \* N \* PFT | 3 | 5.06 | 0.167 | 2.84 | 0.417 | 5.68 | 0.128 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold. All response variables are natural log transformed. Key: AI90=aridity index 90 days prior to collection, AI2006\_2020=mean annual aridity between 2006 and 2020, PFT=plant functional type

**Figure 1**

Chart, scatter chart

Description automatically generated

**Figure 1**. Effects of short-term aridity (90 days prior to measurement; panel A), long-term aridity (15-year mean annual aridity; panel B), and soil NO3-N availability (panel C) on log-transformed leaf nitrogen per leaf area. Yellow points and trendlines indicate measurements and model results for C3 forbs, red points and trendlines indicate measurements and model results for C3 graminoids, blue points and trendlines indicate measurements and model results for C4 graminoids, and grey points and trendlines indicate points and measurements and model results for legumes. The black trendline indicates the relationship between short-term aridity (panel A), long-term aridity (panel B), and soil NO3-N availability (panel C) when averaged across plant functional types. Solid trendlines indicate slopes that are statistically different from zero (p<0.05), while dashed trendlines indicate slopes that are not statistically different from zero (p>0.05). Plant functional type trendlines are only included when there is a two-way interaction with short-term aridity, long-term aridity, or soil nitrogen availability.

*Components of leaf nitrogen per leaf area*

*N*mass was driven by a two-way interaction between 90-day aridity and soil nitrogen availability, indicating that increasing soil nitrogen availability decreased the negative effect of aridity on *N*mass (Table 1). Specifically, there was a negative effect of 90-day aridity on *N*mass when nitrogen availability was set to 0 ppm (Tukey: p=0.022) or 10 ppm NO3-N (Tukey: p=0.046), with no effect of 90-day aridity nitrogen availability was set to 20 ppm (Tukey: p=0.435), 40 ppm (Tukey: p=0.787), or 80 ppm NO3-N (Tukey: p=0.475). We also observed a two-way interaction between 15-year aridity and plant functional type (Table 1). This interaction indicated that *N*mass increased with increasing aridity in C3 forbs (Tukey: p<0.001) and C4 graminoids (Tukey: p=0.030), but did not change in C3 graminoids (Tukey: p=0.299) or legumes (Tukey: p=0.852). Finally, we observed an individual effect of 90-day aridity, 15-year aridity, and soil nitrogen availability on *N*mass (Table 1). These patterns indicated a general positive effect of 15-year aridity, a general negative effect of 90-day aridity, and a general positive effect of increasing soil nitrogen availability on *N*mass.

*M*area was driven by multiple two-way interactions. First, there was a two-way interaction between short-term aridity and soil nitrogen availability. This interaction indicated a positive effect of increasing short-term aridity on *M*area when soil nitrogen availability was set to 0 ppm NO3-N (Tukey: p=0.010) or 10 ppm NO3-N (Tukey: p=0.028), with no effect of short-term aridity on *M*area when soil nitrogen availability was set to 20 ppm NO3-N (Tukey: p=0.367), 40 ppm NO3-N (Tukey: p= 0.830), or 80 ppm NO3-N (Tukey: p=0.477). No such interactions were observed between long-term aridity and soil nitrogen availability (Table 1). We did, however, find a two-way interaction between short-term aridity and plant functional type, indicated by a positive effect of increasing short-term aridity on *M*area in legumes (Tukey: p<0.001) and C3 forbs (Tukey: p=0.028), with no effect in C3 graminoids (Tukey: p=0.775) or C4 graminoids (Tukey: p=0.748). We also observed a two-way interaction between long-term aridity and plant functional type, indicated by a negative effect of increasing long-term aridity on *M*area in legumes with no observable effect in any other plant functional type. Finally, we observed a two-way interaction between soil nitrogen availability and plant functional type, indicating that increasing soil nitrogen availability decreased *M*area in C3 forbs (Tukey: p<0.001), marginally decreased *M*area in C4 graminoids (Tukey: p=0.095), marginally increased *M*area in legumes (Tukey: p=0.050), and did not change *M*area in C3 graminoids (Tukey: p=0.914).

*Chi*

*Leaf nitrogen per leaf area : chi*

**Table 2** Analysis of variance results explaining short-term aridity, long-term aridity, soil nitrogen availability, and plant functional type on leaf nitrogen per leaf area (*N*area), leaf nitrogen content (*N*mass), and leaf mass per area (*M*area)\*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | χ | | *N*area: χ | |
|  | df | χ2 | p | χ2 | p |
| AI90 | 1 | 4.88 | **0.027** | 0.29 | 0.590 |
| AI2006\_2020 | 1 | 24.03 | **<0.001** | 3.21 | 0.073 |
| Soil NO3-N (N) | 1 | 0.01 | 0.924 | 3.18 | 0.074 |
| PFT | 3 | 76.34 | **<0.001** | 17.87 | **<0.001** |
| AI90 \* N | 1 | 0.46 | 0.500 | 0.90 | 0.342 |
| AI2006\_2020 \* N | 1 | 0.67 | 0.412 | 0.65 | 0.422 |
| AI90 \* PFT | 3 | 176.13 | **<0.001** | 11.47 | **0.009** |
| AI2006\_2020 \* PFT | 3 | 93.94 | **<0.001** | 16.06 | **0.001** |
| N \* PFT | 3 | 7.73 | 0.052 | 9.80 | **0.020** |
| AI90 \* N \* PFT | 3 | 23.98 | **<0.001** | 9.82 | **0.020** |
| AI2006\_2020 \* N \* PFT | 3 | 1.51 | 0.680 | 3.68 | 0.299 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold. All response variables are natural log transformed. Key: AI90=aridity index 90 days prior to collection, AI2006\_2020=mean annual aridity between 2006 and 2020, PFT=plant functional type

**Figure 2**

**Discussion**

**References**

**Bonham CD, Mergen DE, Montoya S**. **2004**. Plant cover estimation: a contiguous Daubenmire frame. *Rangelands* **26**: 17–22.

**Brix H**. **1971**. Effects of nitrogen fertilization on photosynthesis and respiration in Douglas-fir. *Forest Science* **17**: 407–414.

**Cernusak LA, Ubierna N, Winter K, Holtum JAM, Marshall JD, Farquhar GD**. **2013**. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist* **200**: 950–965.

**Cramer W, Prentice IC**. **1988**. Simulation of regional soil moisture deficits on a European scale. *Norsk Geografisk Tidsskrift - Norwegian Journal of Geography* **42**: 149–151.

**Daly C, Halbleib M, Smith JI, Gibson WP, Doggett MK, Taylor GH, Curtis J, Pasteris PP**. **2008**. Physiographically sensitive mapping of climatological temperature and precipitation across the conterminous United States. *International Journal of Climatology* **28**: 2031–2064.

**Daubenmire RF**. **1959**. Canopy coverage method of vegetation analysis. *Northwest Science* **33**: 39–64.

**Davis TW, Prentice IC, Stocker BD, Thomas RT, Whitley RJ, Wang H, Evans BJ, Gallego-Sala A V, Sykes MT, Cramer W**. **2017**. Simple process-led algorithms for simulating habitats (SPLASH v.1.0): robust indices of radiation, evapotranspiration and plant-available moisture. *Geoscientific Model Development* **10**: 689–708.

**Dong N, Prentice IC, Evans BJ, Caddy-Retalic S, Lowe AJ, Wright IJ**. **2017**. Leaf nitrogen from first principles: field evidence for adaptive variation with climate. *Biogeosciences* **14**: 481–495.

**Dong N, Prentice IC, Wright IJ, Evans BJ, Togashi HF, Caddy-Retalic S, McInerney FA, Sparrow B, Leitch E, Lowe AJ**. **2020**. Components of leaf‐trait variation along environmental gradients. *New Phytologist* **228**: 82–94.

**Dong N, Wright IJ, Chen JM, Luo X, Wang H, Keenan TF, Smith NG, Prentice IC**. **2022**. Rising CO2 and warming reduce global canopy demand for nitrogen. *New Phytologist*.

**Evans JR**. **1989**. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* **78**: 9–19.

**Evans JR, Seemann JR**. **1989**. The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences, and control. *Photosynthesis* **8**: 183–205.

**Farquhar GD, Ehleringer JR, Hubick KT**. **1989**. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503–537.

**Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall AS, Seabloom EW, Wragg PD, *et al.*** **2015**. Grassland productivity limited by multiple nutrients. *Nature Plants* **1**: 15080.

**Firn J, McGree JM, Harvey E, Flores-Moreno H, Schütz M, Buckley YM, Borer ET, Seabloom EW, La Pierre KJ, MacDougall AS, *et al.*** **2019**. Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. *Nature Ecology & Evolution* **3**: 400–406.

**Harrison SP, Cramer W, Franklin O, Prentice IC, Wang H, Brännström Å, de Boer H, Dieckmann U, Joshi J, Keenan TF, *et al.*** **2021**. Eco-evolutionary optimality as a means to improve vegetation and land-surface models. *New Phytologist* **231**: 2125–2141.

**Hijmans RJ**. **2022**. terra: Spatial Data Analysis.

**Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB**. **2003**. Nitrogen and climate change. *Science* **302**: 1512–1513.

**IPCC**. **2013**. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*.

**Katabuchi M**. **2015**. LeafArea: An R package for rapid digital analysis of leaf area. *Ecological Research* **30**: 1073–1077.

**Keeling CD, Mook WG, Tans PP**. **1979**. Recent trends in the 13C/12C ratio of atmospheric carbon dioxide. *Nature* **277**: 121–123.

**LeBauer DS, Treseder K**. **2008**. Nitrogen limitation of net primary productivity. *Ecology* **89**: 371–379.

**Lhomme J-P**. **1997**. A theoretical basis for the Priestley-Taylor coefficient. *Boundary-Layer Meteorology* **82**: 179–191.

**Luo X, Keenan TF, Chen JM, Croft H, Prentice IC, Smith NG, Walker AP, Wang H, Wang R, Xu C, *et al.*** **2021**. Global variation in the fraction of leaf nitrogen allocated to photosynthesis. *Nature Communications* **12**: 4866.

**Paillassa J, Wright IJ, Prentice IC, Pepin S, Smith NG, Ethier G, Westerband AC, Lamarque LJ, Wang H, Cornwell WK, *et al.*** **2020**. When and where soil is important to modify the carbon and water economy of leaves. *New Phytologist* **228**: 121–135.

**Poorter H, Knopf O, Wright IJ, Temme AA, Hogewoning SW, Graf A, Cernusak LA, Pons TL**. **2022**. A meta-analysis of responses of C3 plants to atmospheric CO2: dose–response curves for 85 traits ranging from the molecular to the whole-plant level. *New Phytologist* **233**: 1560–1596.

**Poorter H, Niinemets Ü, Ntagkas N, Siebenkäs A, Mäenpää M, Matsubara S, Pons TL**. **2019**. A meta-analysis of plant responses to light intensity for 70 traits ranging from molecules to whole plant performance. *New Phytologist* **223**: 1073–1105.

**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. *Ecology Letters* **17**: 82–91.

**Priestley CHB, Taylor RJ**. **1972**. On the Assessment of Surface Heat Flux and Evaporation Using Large-Scale Parameters. *Monthly Weather Review* **100**: 81–92.

**Rogers A**. **2014**. The use and misuse of Vc,max in Earth System Models. *Photosynthesis Research* **119**: 15–29.

**Rogers A, Medlyn BE, Dukes JS, Bonan G, von Caemmerer S, Dietze MC, Kattge J, Leakey ADB, Mercado LM, Niinemets Ü, *et al.*** **2017**. A roadmap for improving the representation of photosynthesis in Earth system models. *New Phytologist* **213**: 22–42.

**Schneider CA, Rasband WS, Eliceiri KW**. **2012**. NIH Image to ImageJ: 25 years of image analysis. *Nature methods* **9**: 671–675.

**Smith NG, Dukes JS**. **2013**. Plant respiration and photosynthesis in global-scale models: Incorporating acclimation to temperature and CO2. *Global Change Biology* **19**: 45–63.

**Smith NG, Keenan TF, Prentice IC, Wang H, Wright IJ, Niinemets Ü, Crous KY, Domingues TF, Guerrieri R, Ishida F oko, *et al.*** **2019**. Global photosynthetic capacity is optimized to the environment (S Niu, Ed.). *Ecology Letters* **22**: 506–517.

**Thieurmel B, Elmarhraoui A**. **2019**. suncalc: Compute sun position, sunlight phases, moon position, and lunar phase.

**Walker AP, Beckerman AP, Gu L, Kattge J, Cernusak LA, Domingues TF, Scales JC, Wohlfahrt G, Wullschleger SD, Woodward FI**. **2014**. The relationship of leaf photosynthetic traits - Vcmax and Jmax - to leaf nitrogen, leaf phosphorus, and specific leaf area: a meta-analysis and modeling study. *Ecology and Evolution* **4**: 3218–3235.

**Wright IJ, Reich PB, Westoby M**. **2003**. Least-cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist* **161**: 98–111.