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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 nutrient availability, leaf nutrient 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 nutrient allocation and photosynthetic capacity independent of soil nutrient availability (Poorter *et al.*, 2019, 2022; Luo *et al.*, 2021). The inability of many terrestrial biosphere models to capture such acclimation responses to environmental change casts uncertainty in the ability of these models to accurately simulate robust photosynthetic responses to global change (Smith & Dukes, 2013; Harrison *et al.*, 2021).

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 typically 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 the summers of 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*

This experiment was conducted through the Texas Ecological Laboratory program, which grants researchers access to privately owned properties in the state of Texas with little to no recent history of farming or grazing. We collected leaf and soil samples from 25 properties scattered across central and eastern Texas in summer 2020 and summer 2021 (Fig. 1). Fourteen 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 contained a dominant savanna or grassland component for both sampling years and maximized between-site climatic and edaphic diversity (Table 1). Any property with anecdotal or written evidence of grazing or disturbance during the calendar year leading up to our site visits were excluded from our analysis.

*Property visits and sampling methodology*

Due to the uncertainty of the SARS-CoV-2 pandemic and high regional contagion risks in Texas at the time of the 2020 field season, we divided property visits into initial property visits and primary property visits to maximize data acquisition and safely minimize human-to-human contact for any given field excursion. We repeated this property visit schedule in 2021 to replicate our 2020 sampling effort. Initial property visits were conducted at each of 14 properties in 2020 between June 15 and June 21, and at each of 15 properties in 2021 between May 25 and June 1.

Initial property visits served as an initial survey of the property for later primary site selection. At each property, leaves of three individuals of the five most dominant species were collected at random locations in each property. Composite soil samples and leaf area per ground area measurements 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) prior to leaf collection to obtain chlorophyll fluorescence data and gather snapshot photochemical parameters that drive leaf photosynthesis.

Following initial property visits, five properties for each sampling year were selected for a second, more intensive sampling effort. The five properties were chosen based on site position along the climatic gradient in Texas, landowner cooperation and approval, and species similarity relative to the other four properties. Each primary property visit was completed by setting up 5, 1m x 1m square plots coupled with a 1m x 0.1m rectangular plot adjacent to each square plot. In each square plot, species composition was determined through percent cover estimates using the Daubenmire method (Daubenmire, 1959; Bonham *et al.*, 2004) and leaves of all species present in the plot were collected. Leaf area per ground area measurements were collected at 5 points in each square plot using a LI-COR 2200C (Li-COR Biosciences, Lincoln, NE, USA), and were used to estimate plot-level leaf area index. A composite soil sample was also collected down to 10 centimeters below the soil surface within each square plot. The rectangular 1m x 0.1m plot was clipped for aboveground biomass. In 2020, primary property visits were conducted between July 17 and July 21 and, in 2021, were conducted between June 25 and June 28.

*Leaf trait measurements, plant functional groups, and soil characteristics*

Images of each leaf were taken immediately following each property visit using a flat-bed scanner. Wet leaf area was then 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 (University of California-Davis Stable Isotope Facility; instrument). We calculated specific leaf area (*SLA*; cm2 g-1) as the ratio of wet leaf area to dry leaf biomass, and leaf nitrogen per leaf area (*N*area; gN m-2) as the ratio of leaf nitrogen content to specific leaf area multiplied by 10,000 to convert cm-2 to m-2.

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).

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. We also determined soil texture using the simple jar method to determine percent silt, clay, and sand (Table 1).

*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 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. This was done to determine the relevant climatic timescale that best explained *N*mass, *SLA*, *N*area,and χ using an information theoretic approach (see *Data analysis*).

*Site aridity*

Property 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 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 1991 and 2020. Thirty, 60, and 90 day aridity estimates were used to determine the most relevant short-term site aridity timescale for predicting *N*mass, *SLA*, *N*area,and χ, again using an information-theoretic approach (see *Data analysis*).

*Plant functional type assignment*

All collected and identified species were assigned a plant functional type based on their photosynthetic pathway (C3, C4), lifespan (annual, perennial), 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 annual forbs (n = ), C3 annual graminoids (n = ), C4 graminoids (n = ), and C3 shrubs (n = ). We used plant functional type as the primary descriptor of species to replicate major vegetation classes in many terrestrial biosphere models.

*Data analysis*

To address our first hypothesis regarding relevant timescales that drive leaf nitrogen allocation and water use efficiency, we used an information-theoretic approach using Akaike Information Criterion (AICc) to select the precipitation total and mean daily air temperature 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, *SLA*, *N*area,and χ. To determine the relevant precipitation total timescale that conferred the best fit, we constructed a series of linear mixed-effects models that contained each respective pooled precipitation total as the only fixed effect and species as a random intercept term. This was done for each of *N*mass, *SLA*, *N*area,and χ. We repeated this process using each respective pooled mean daily air temperature as the only fixed effect, again done for each of *N*mass, *SLA*, *N*area,and χ. The precipitation and temperature timescale that conferred the best model fit (i.e. the lowest AICc value) was fed into the general linear mixed-effects model explained below.

We also used an information-theoretic approach to determine whether property aridity 30, 60, or 90 days prior to each property visit conferred the best model fit for *N*mass, *SLA*, *N*area,and χ. As with precipitation and air temperature, we constructed a series of linear mixed effects models where 30 day, 60 day, or 90 day property aridity was the sole fixed effect and species was a random intercept term. This was done iteratively for models where *N*mass, *SLA*, *N*area,and χ were the response variable. We selected the aridity index timescale that conferred the best model fit (i.e. the lowest AICc value) and fed this timescale into the general linear mixed-effects model explained below.

After determining the relevant short-term precipitation, temperature, and aridity timescales that conferred the best model fits, we constructed a third series of linear mixed-effects models to investigate primary drivers of *N*mass, *SLA*, *N*area,and χ. In all models, we included soil nitrogen availability, soil pH, mean annual temperature, mean annual precipitation, the relevant short-term mean daily air temperature, the relevant short-term precipitation total, and the relevant short-term aridity index as separate continuous fixed effects. We also included plant functional type and sampling year as categorical fixed effects, with interaction terms between all fixed effect coefficients. To account for interspecies variation within each plant functional type, we included species as a random intercept term.

**Results**

**Discussion**

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