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**Title**:Soil nitrogen availability increases the positive effect of aridity on water use efficiency

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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**

Photosynthesis couples carbon and nitrogen cycles in terrestrial ecosystems (Hungate *et al.*, 2003). Specifically, plants fix carbon dioxide from the atmosphere into simple sugars using enzymes, such as Ribulose-1,5-bisphosphate carboxylase/oxygenase (“Rubisco”), that have large nitrogen requirements to build and maintain (Evans, 1989). Recent photosynthetically derived carbon can be accumulated as biomass (cite), lost as a substrate of plant respiration (Glover, 1973), or allocated belowground to acquire nutrients (). Belowground photosynthate can be used to acquire nutrients either directly from the soil (cite), indirectly through root exudates that prime soil microbial communities and organic matter decomposition (Bengtson *et al.*, 2012), or indirectly through symbioses with mycorrhizal fungi and/or symbiotic nitrogen-fixing bacteria (Smith & Read, 2008). Of these carbon fates, previous studies note that large percentages of recent photosynthate get allocated belowground for nutrient acquisition (greater than 30% in some cases; cite).

Given that most leaf nitrogen gets allocated to the construction and maintenance of photosynthetic machinery, and the large portion of recent photosynthate that gets allocated belowground for nutrient acquisition, many have found positive relationships between soil nitrogen availability, leaf nitrogen allocation, and photosynthetic capacity (Brix, 1971; Walker *et al.*, 2014). However, contemporary analyses using optimality and photosynthetic least-cost theoretical frameworks indicate that leaf nitrogen allocation can be reliably predicted independent of soil nitrogen availability (Dong *et al.*, 2017, 2020), and may instead be driven by leaf demand to build and maintain photosynthetic machinery (Dong *et al.*, 2022). These studies indicate that leaf nitrogen allocation can be predicted by growing season temperature, growing season irradiance, growing season vapor pressure deficit, leaf mass per area, or soil pH. Further, relationships between soil nitrogen availability and leaf nitrogen allocation likely depend on plant demand to acquire nutrients, which varies by species, species acquisition strategy, and phenology. Thus, there is a need to better understand primary drivers of leaf nitrogen allocation across different spatiotemporal scales.

Photosynthetic least-cost theory provides a possible explanation for the integrative role of soil nitrogen availability, aboveground climate, and leaf traits on leaf nitrogen allocation. The theory predicts that plants allocate nitrogen to photosynthetic leaf tissue to maximize photosynthetic carbon gain at the lowest summed cost of using nitrogen and water. The theory predicts that nitrogen and water use are substitutable, such that an increase in either nitrogen or water should induce a predictable acclimation response. For example, plants growing in arid or semiarid regions are predicted to have increased leaf nitrogen allocation, as this response allows a given photosynthetic rate to be achieved with decreased stomatal conductance and water use.

To date, few direct tests of photosynthetic least-cost theory exist, and the climatic timescale that leaf nitrogen allocation responds to remains unknown and untested. Understanding primary drivers of leaf nitrogen allocation and the mechanisms underlying these responses is crucial to inform the development of future terrestrial biosphere and Earth system models.

In summer 2020 and 2021, we measured leaf and soil traits in XX individuals spanning XX species at 25 total sites across a broad climatic gradient in central and eastern Texas. Texas is home to a diverse climatic gradient, with mean annual precipitation ranging from XX to XX and mean annual temperature ranging from XX to XX, and a wide range in soil characteristics and nutrient availability ranges. Following patterns expected from photosynthetic least-cost theory, 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 (Fig. 1). Any properties 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 at the time of the 2020 field season, we divided property visits into initial property visits and primary property visits. This was done 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 the 14 properties in 2020 between June 15 and June 21, and at each of the 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. In 2020, initial property visits were conducted at each of the 14 properties between June 15 and June 21 and, in 2021, were conducted at each of the 15 properties between May 25 and June 1.

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 consisted of 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.

*Site climatic data*

We acquired gridded 1991-2020 monthly temperature, precipitation, and vapor pressure deficit data from PRISM at a 4-km spatial resolution (PRISM Climate Group, Oregon State University, <https://prism.oregonstate.edu>, data created 4 Feb 2014, accessed 24 Mar 2022). PRISM climate normal data were downloaded using the ‘prism’ R package (Hart & Bell, 2015). We extracted monthly mean temperature, maximum temperature, minimum temperature, precipitation, maximum vapor pressure deficit, and minimum vapor pressure deficit from the grid cell that contained each field site. Mean annual precipitation was then calculated as the sum of precipitation for each month, and mean annual temperature as the average temperature per month. We also calculated mean normal growing season temperature, precipitation, and vapor pressure deficit using only months where the minimum temperature was greater than 0°C. Finally, we calculated normal growing season aridity by dividing precipitation by potential evapotranspiration.

We also acquired gridded daily temperature, precipitation, and vapor pressure deficit from PRISM, again at a 4-km spatial resolution, between June 01, 2019 and July 31, 2021 (PRISM Climate Group, Oregon State University, <https://prism.oregonstate.edu>, data created 4 Feb 2014, accessed 24 Mar 2022). PRISM data were downloaded using the ‘prism’ R package (Hart & Bell, 2015). We extracted daily mean temperature, maximum temperature, minimum temperature, precipitation, maximum vapor pressure deficit, and minimum vapor pressure deficit from the grid cell that contained each field site for the 30 days leading up to each property visit. We used the PRISM dataset in lieu of local weather station data because the closest weather station for several rural properties were greater than 20 kilometers away and at a different slope direction or elevation than the property itself.

Using daily mean temperature, precipitation, and number of daily sunlight hours, we estimated plant-available surface moisture using the ‘splash’ R package, which is an R implementation of the SPLASH model described in Davis *et al.* (2017). The SPLASH model estimates plant-available surface moisture using the Priestley-Taylor coefficient (α), which is calculated as the ratio of actual evapotranspiration to equilibrium evapotranspiration (Priestley & Taylor, 1972; Lhomme, 1997). Property growing season aridity was also calculated as a function of mean precipitation and potential evapotranspiration of the previous three months leading up to each property visit. We also estimated normal property aridity as a function of 1991-2020 mean annual precipitation divided by normal potential evapotranspiration.

*Leaf trait measurements and soil characteristics*

Images of each leaf were taken immediately following each property visit using a flat-bed scanner. Images were then used to determine wet leaf area using the 'LeafArea' R package (Katabuchi, 2015), which automates leaf area calculations using ImageJ software (Schneider *et al.*, 2012). Each leaf was dried at 65C for at least 48 hours, weighed, and ground until homogenized. Specific leaf area (*SLA*; cm2 g-1) was calculated as the ratio of wet leaf area to dry leaf biomass. Using subsamples of ground and homogenized leaf biomass, we determined leaf nitrogen content (*N*mass; g g-1) through elemental combustion analysis (Costech-4010, Costech Instruments, Valencia, CA) and leaf δ13C through isotope ratio mass spectroscopy. We sent leaf samples to the University of California-Davis Stable Isotope facility to acquire leaf δ13C. Leaf nitrogen mass per unit leaf area (*N*area; g m-2) was calculated by dividing *N*mass by *SLA*, then multiplying 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). While intercellular and extracellular CO2 concentrations were directly measured during each CO2 response curve, deriving χ from δ13C provides a more integrative estimate of the *C*i:*C*a over an individual leaf’s lifespan . 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, as percent clay has been shown in previous analyses to modify expected photosynthetic least-cost patterns.

*Plant functional type assignment*

Collected species were assigned a plant functional type based on their photosynthetic pathway (C3, C4), lifespan (annual, perennial), and growth habit (graminoid, forb, etc.). This created four distinct plant functional types within our dataset: C3 annual forbs (), C3 annual graminoids (), C4 graminoids, and C3 shrubs (). We used plant functional type as the primary descriptor of species as we were unable to identify several species down to a genus level.

*Statistical analysis*

We constructed a series of linear mixed-effects models to explore the effects of edaphic and climatic factors on leaf nitrogen allocation and water use efficiency. We included soil nitrogen availability, soil pH, mean annual temperature, mean annual precipitation, short-term temperature, and short-term precipitation as continuous fixed effects, with plant functional type as a categorical fixed effect. We also included interactions between all fixed effects as additional model coefficients. To account for interspecies variation within each plant functional type, we included species as a random intercept term.

Short-term temperature and precipitation were chosen using model selection. We constructed a series of linear mixed-effects models to determine appropriate short-term temperature and precipitation timescales to include in the models explained above. To determine the short-term temperature timescale to include in our models, constructed a series of models using a series o

we used Akaike Information Criterion with

We constructed a series of single factor mixed effects models where either short-term temperature or short-term precipitation was included as the only fixed effect. Species was included as a random intercept term. We used a series of time ranges

Species was included as a random intercept term to maintain the random effect structure.

Specifically, we constructed a series of single factor mixed effects models for both

we used Akaike Information Criterion and Bayesian Information Criterion to

Climatic timescales were chosen using Akaike Information Criterion and Bayesian Information Criterion to select

to select the daily climate threshold that best explained effects of edaphic characteristics on leaf nitrogen allocation and water use efficiency. This was done using a sequence of mean daily temperature and total precipitation values that ranged from five days before each site visit to thirty days before each site visit on a single day timestep.

**Results**

**Discussion**

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