Terrestrial biosphere models predict leaf photosynthesis based on positive relationships between soil nitrogen, leaf nitrogen, and photosynthetic capacity. While many have documented positive empirical relationships between soil nitrogen and leaf nitrogen, recent work leveraging photosynthetic least-cost theory suggests that leaf nitrogen allocation is better explained through aboveground local climate or interactions between aboveground climate and soil nutrients than by soil nutrient availability alone.

In summer 2020 and 2021, we measured leaf and soil traits in XX individuals spanning XX species 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**

*Field site selection*

This project was conducted at 26 privately owned properties in central and eastern Texas between summers 2020 and 2021. 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). For both sampling years, we explicitly chose properties that contained a dominant savanna/grassland component. We also chose properties to maximize climatic diversity across a broad precipitation gradient (Fig. 1). Properties were mostly unmanaged land plots, although a few were either actively managed or had a history of management/grazing. Land management details are noted in Table 1. Mean annual precipitation across all selected properties for both sampling years ranged from XX to XX, with mean annual temperature ranging between XX and XX.

*Field site visits and sampling methodology*

Due to the uncertainty of the COVID-19 pandemic and high regional contagion risks at the time of the 2020 sampling season, we divided property visits into initial property visits and primary property visits. We then repeated this property visit schedule in 2021 to replicate our 2020 sampling effort.

Initial property visits consisted of brief visits at each of the 14 properties in June 2020 and 15 properties in May 2021. At each initial property visit, we collected 3 random leaves of the five most dominant species and composite soil samples at random locations in the property. We also recorded leaf area per ground area measurements at random locations in each property to assess plant cover density. In 2021, 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. The five properties received a second visit to put mixed bed ion exchange resin bags into the soil ~10cm below the litter-cleared soil surface to accumulate soil nitrate-nitrite and ammonium concentrations over a month. Activated resin bags (see *Soil characteristics and soil nutrient availability*) were inserted at random locations on the property, but were within 1,000m of each other. About one month following initial property visits and resin bag insertion, primary properties were visited a final time for a more labor-intensive sampling effort. Sampling at 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. Plots were set up in the same location as resin bag insertion points, and resin bags were extracted. 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.

*Site climatic data*

We acquired gridded 1991-2020 normal 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 then 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, while mean annual temperature was calculated 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.

Additionally, we 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 >20-km away and at a different aspect slope or elevation than the property.

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). We also estimated property growing season aridity as a function of mean precipitation and potential evapotranspiration of the previous three months leading up to each property visit.

*Leaf trait measurements*

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‰ (Farquhar et al., 1989; Keeling et al., 1979), *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).

*Soil characteristics and soil nutrient availability*

Composite soil samples from each initial property visit and plot within each primary property visit 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, and were then classified based on percent silt, clay, and sand

We used mixed-bed resin bags to quantify ammonium-N and nitrate-N concentrations in each plot during our gas exchange measurement period. Lycra mesh bags were filled with 5 grams of Dowex Marathon MR-3 hydrogen and hydroxide form resin (MilliporeSigma, Burlington, MA USA) and sealed with a zip tie. Each bag was activated by soaking in 0.5 M HCl for 20 minutes, then in 2 M NaCl until pH of the saline solution stabilized, as described in Allison et al. (2008). Five mixed-bed resin bags were inserted about 10 cm below the litter cleared soil surface and remained in the soil until each primary property visit.

Prior to anion and cation extraction, each resin bag was rinsed with ultrapure water to remove surface soil residues. Anions and cations were extracted from surface-cleaned resin bags by individually soaking and shaking each bag in a 100mL of a 0.1 M HCl/2.0 M NaCl matrix for one hour. We quantified nitrate-N concentrations spectrophotometrically at 540 nm using the end product of a single reagent vanadium (III) chloride reaction (Doane & Horwáth, 2003) and ammonium-N concentrations at 650 nm using the end product of a modified phenol-hypochlorite reaction (Rhine et al., 1998; Weatherburn, 1967). Both nitrate-N and ammonium-N protocols were optimized for use with a microplate reader (Biotek Synergy H1; Biotek Instruments, Winooski, VT USA). We also used a series of negative and positive controls throughout each well plate to verify the accuracy and precision of our measurements. Soil inorganic nitrogen availability (mg L-1) was estimated at the plot level as the sum of the nitrate-N and ammonium-N concentrations within each plot. We then converted soil nitrogen availability values to μg N g-1 resin day-1, as in Allison et al. (2008), to better characterize the accumulation of nitrate and ammonium ions throughout our gas exchange measurement period.

*Statistical analysis*