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**Author List:** Evan A. Perkowski, Nicholas G. Smith

**Author Affiliations:** Department of Biological Sciences, Texas Tech University, Lubbock, TX

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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 soil nitrogen availability should decrease the summed unit cost of nutrient and water use, which should drive an increase in leaf nitrogen content at lower stomatal conductance. The theory suggests that these patterns may depend on soil moisture, as increasing soil moisture may increase the summed unit cost of nutrient and water use, which would decrease leaf nitrogen content at higher stomatal conductance. However, it is currently unknown whether increases in leaf nitrogen content are driven directly by changes in soil nutrient availability or indirectly through changes in the summed unit cost of nutrient and water use. Further, few direct tests of this theory exist across concurrent soil nutrient availability and soil moisture gradients. Here, we measured leaf nitrogen content and carbon isotope estimates of stomatal conductance at 25 sites scattered across a precipitation and soil nitrogen availability gradient in grassland ecosystems of Texas, USA. We found that variance in leaf nitrogen content across sites was positively driven by a reduction in the summed unit cost of nutrient and water use, despite an apparent marginal positive direct effect of soil nitrogen availability on leaf nitrogen content.

**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 groups through linear relationships between area-based leaf nitrogen content and estimates of photosynthetic capacity, namely the maximum carboxylation rate of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rogers, 2014; Rogers *et al.*, 2017). Some models are beginning to include coupled carbon-nitrogen cycles within their architecture (e.g., CLM5.0, Lawrence *et al.*, 2019; JULES-CN; Wiltshire *et al.*, 2021), which allows leaf photosynthesis to be predicted directly through changes in area-based leaf nitrogen content and indirectly through changes in soil nitrogen availability. Despite these model improvements, open questions remain regarding the generality of relationships between soil nitrogen availability and leaf nitrogen allocation across time and space.

Empirical support for relationships between soil nitrogen availability, leaf nitrogen content, and photosynthetic capacity is abundant (e.g., Brix, 1971; Evans & Seemann, 1989; Evans, 1989; Walker *et al.*, 2014; Firn *et al.*, 2019). However, plant responses to changing aboveground growing conditions can alter leaf nitrogen allocation and photosynthetic capacity in ways that are independent from changes in soil nitrogen availability (Poorter *et al.*, 2019, 2022; Luo *et al.*, 2021). Indeed, recent analyses indicate that variance in leaf nitrogen content and photosynthetic capacity across space and time is better explained through interactions between aboveground climatic and belowground edaphic factors than can be explained by variance in soil nitrogen availability alone (Dong *et al.*, 2017, 2020, 2022; Smith *et al.*, 2019; Paillassa *et al.*, 2020). Relationships between leaf nitrogen and photosynthetic capacity may also be determined through costs of leaf construction, commonly evidenced through leaf mass per unit leaf area, species identity traits such as whether a species associated with nitrogen-fixing bacteria (Dong *et al.*, 2017) or photosynthetic pathway ().

Alternatively, discrepancies in the relationship between soil nitrogen availability and leaf nitrogen content may be driven by plant costs of nitrogen acquisition and use (Perkowski *et al.*, 2021; Braghiere *et al.*, 2022). Costs of plant nitrogen acquisition and use have been previously shown to decrease with increasing soil nitrogen availability (Perkowski *et al.*, 2021), which, in the same study, corresponded with an increase in leaf nitrogen allocation and leaf mass per unit leaf area (Waring *et al.* in prep). However, costs of plant nitrogen acquisition and use may also depend on aboveground climatic factors that modify leaf or whole plant demand to acquire nitrogen, such as increasing CO2 (Terrer *et al.*, 2018) or light availability (Perkowski *et al.*, 2021), and may further be a product of interactions between soil nitrogen supply and plant nitrogen demand.

Photosynthetic least-cost theory provides a useful framework for detecting primary aboveground and belowground drivers of leaf nitrogen content, including costs associated with nitrogen acquisition and use. The theory predicts that plants acclimate to environments by maximizing photosynthetic carbon gain at the lowest summed cost of nitrogen and water use (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.

The theory predicts that costs to acquire and use nitrogen relative to water should decrease with increasing soil nitrogen availability.

[theoretical expectations of nutrient-water use tradeoffs in response to soil nutrient availability; brief review of empirical support from patterns expected from theory]

[theoretical expectations of nutrient-water use tradeoffs in response to soil nutrient availability; brief review of empirical support for patterns expected from theory]

[theoretical expectations for the possible interaction between soil nutrient availability and water availability on nutrient-water use tradeoffs]

In summer 2020 and 2021, we measured leaf nitrogen content and carbon isotopic signatures of 520 individuals spanning 57 species scattered across 24 grassland sites in Texas, USA. Texas contains a diverse climatic gradient, indicated by 2006-2020 mean annual precipitation totals ranging from 204 to 1803 mm, mean annual temperature ranging from 11.8° to 24.6°C, and mean annual vapor pressure deficit ranging from 0.59 to 2.29 kPa within the state boundary. Variability in soil nutrient availability was expected to be apparent across sites, owing to differences in soil texture and aboveground climate that would drive differential rates of nutrient transformations to plant-available forms in the soil. We leveraged the climatic and expected edaphic variability across Texas to test the following hypotheses related to eco-evolutionary optimality theory:

1. Increasing soil nutrient availability will decrease the unit cost of acquiring and using nutrients relative to water, which will increase water use efficiency and leaf nutrient content. However, species capable of forming associations with symbiotic nitrogen-fixing bacteria are expected to be less sensitive to changes in soil nutrient availability than species not capable of forming such associations and should therefore exhibit weaker stimulations in leaf nutrient content and water use efficiency in response to increasing soil nutrient availability. Species that photosynthesis via the C4 photosynthetic pathway should exhibit lower unit costs of acquiring and nutrients relative to water
2. Soil moisture will increase the unit cost of acquiring and using nutrients relative to water. This should result in a reduction in water use efficiency that corresponds with a reduction in leaf nutrient content with increasing soil moisture. However, we expect that species that undergo C4 photosynthesis to be less sensitive to changes in soil moisture availability and should demonstrate weaker nutrient-water use tradeoffs expected from the theory compared to species that undergo C3 photosynthesis. We also expect species that undergo C4 photosynthesis to have increased water-use efficiency compared to their C3 counterparts irrespective of soil moisture availability.
3. In drier sites (i.e., ones with lower soil moisture), soil nutrient availability will induce stronger reductions in the unit cost of acquiring and using nutrients relative to water. This will result in stronger increases in water use efficiency and leaf nutrient content at drier sites than wetter sites. We expect that these patterns will be context dependent on the ability of species to associate with symbiotic nitrogen-fixing bacteria or to perform C4 photosynthesis. Specifically, we expected the interaction between soil moisture and soil nutrient availability to be strongest in C3 species without capabilities to associate with symbiotic nitrogen-fixing bacteria.

**Methods**

*Site descriptions 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 24 sites scattered across central and eastern Texas in summer 2020 and summer 2021 (Fig. 1). Twelve sites were visited between June and July 2020 and 14 sites (11 unique from 2020) were visited between May and June 2021 (Table 1). We explicitly chose sites that maximized climatic and edaphic variability between sites and contained dominant open savanna or grassland components (Table 1). Any site with personally communicated or anecdotal evidence of grazing or disturbance (e.g., mowing, feral hog activity, etc.) was excluded from our analysis.

**Table 1** Site locality information, sampling year(s), 2006-2020 mean annual precipitation (MAP; mm), mean annual temperature (MAT; °C), and mean annual vapor pressure deficit (MAV). Rows are arranged by longitude to visualize precipitation variability across sites

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Property** | **Latitude** | **Longitude** | **Sampling year** | **MAP** | **MAT** | **MAV** |
| Edwards\_2019\_17 | 29.95 | -100.36 | 2020 | 563.5 | 19.0 | 1.21 |
| Uvalde\_2020\_02 | 29.59 | -100.09 | 2020, 2021 | 648.5 | 19.5 | 1.23 |
| Menard\_2020\_01 | 30.91 | -99.57 | 2020 | 641.9 | 18.3 | 1.29 |
| Kerr\_2020\_03 | 30.06 | -99.34 | 2021 | 672.4 | 18.3 | 1.19 |
| Bandera\_2020\_03 | 29.85 | -99.30 | 2021 | 789.4 | 18.8 | 1.22 |
| Sansaba\_2020\_01 | 31.29 | -98.62 | 2020 | 733.0 | 18.8 | 1.26 |
| Comal\_2020\_21 | 29.79 | -98.43 | 2020 | 878.5 | 19.9 | 1.21 |
| Blanco\_2019\_16 | 29.99 | -98.43 | 2020 | 833.0 | 19.2 | 1.15 |
| Bexar\_2019\_13 | 29.24 | -98.43 | 2020 | 759.3 | 21.5 | 1.33 |
| Burnet\_2020\_14 | 30.84 | -98.34 | 2021 | 763.3 | 19.5 | 1.24 |
| Comal\_2020\_19 | 30.01 | -98.32 | 2021 | 845.0 | 19.3 | 1.15 |
| Hays\_2021\_54 | 29.96 | -98.17 | 2021 | 861.3 | 20.0 | 1.17 |
| Burnet\_2020\_12 | 30.82 | -98.06 | 2021 | 815.1 | 19.4 | 1.17 |
| Williamson\_2019\_09 | 30.71 | -97.86 | 2020 | 867.7 | 19.7 | 1.18 |
| Williamson\_2019\_10 | 30.54 | -97.77 | 2020 | 819.5 | 19.9 | 1.20 |
| Bell\_2021\_08 | 31.06 | -97.55 | 2021 | 937.3 | 19.6 | 1.20 |
| Fayette\_2021\_12 | 29.86 | -97.21 | 2021 | 985.7 | 20.4 | 1.12 |
| Fayette\_2019\_04 | 30.09 | -96.78 | 2020 | 1017.4 | 20.6 | 1.10 |
| Fayette\_2020\_09 | 29.86 | -96.71 | 2021 | 1002.7 | 20.8 | 1.10 |
| Washington\_2020\_08 | 30.28 | -96.41 | 2021 | 1077.4 | 20.4 | 1.08 |
| Austin\_2020\_03 | 29.78 | -96.24 | 2021 | 1108.7 | 20.6 | 1.01 |
| Brazos\_2020\_16 | 30.93 | -96.23 | 2021 | 1078.0 | 20.1 | 1.09 |
| Brazos\_2020\_18 | 30.52 | -96.21 | 2020, 2021 | 1099.4 | 20.4 | 1.12 |
| Harris\_2020\_03 | 29.88 | -95.31 | 2020, 2021 | 1492.0 | 21.6 | 1.00 |

**Figure 1**

**Chart

Description automatically generated**

**Figure 1** Maps that detail site locations along 2006-2020 mean annual precipitation (panel A) and mean annual temperature (panel B) gradients in Texas, USA. Precipitation and temperature data were plotted at a 4-km grid resolution and are masked to include only grid cells that occur in the Texas state boundary in the United States. In both panels, open circles refer to sites visited in 2020, open triangles to sites visited in 2021, and closed circles to sites visited in 2020 and 2021. The scale bar in panel A applies to both panels.

*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, mean vapor pressure deficit, 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 sites 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, mean annual temperature, and mean annual vapor pressure deficit for each site between2006 and 2020 (Table 1). We also iteratively calculated total precipitation, mean daily air temperature, and mean daily vapor pressure deficit for the prior 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 60, and 90 days leading up to each site visit. This was done to determine relevant climatic timescales that best explained *β* and *χ*.

*Site edaphic characteristics*

Composite soil samples were sent to the Texas A&M Soil, Water and Forage Laboratory to quantify macronutrient concentrations, pH, and electrical conductivity. Soil nitrate-nitrogen (NO3-N; ppm) was extracted in 1 M KCl and measured spectrophotometrically at 520 nm through the end product of a NO3-N to NO2-N cadmium reduction reaction (Keeney & Nelson, 1983; Kachurina *et al.*, 2000). Soil phosphorus and potassium were extracted in a solution containing 0.2 M acetic acid, 0.25 M ammonium nitrate, 0.015 M ammonium fluoride, 0.013 M nitric acid, and 0.001 M EDTA (Mehlich, 1984). Soil pH was measured in a 1:2 soil: deionized water slurry after a 30-minute incubation period at room temperature with a hydrogen selective electrode. Electrical conductivity was similarly measured in a 1:2 soil: deionized water slurry after a 30-minute incubation period at room temperature with a conductivity probe (Rhoades, 1983). Soil NO3-N concentration was chosen as the primary indicator of site soil nutrient availability, as NO3-N concentrations had strong positive correlations with soil phosphorus concentration (Pearson’s r=0.44, p=0.006; Fig. S1) and electrical conductivity (Pearson’s r=0.74, p<0.001; Fig. S1). There was no correlation between soil NO3-N concentration and soil pH (Pearson’s r=-0.02, p=0.908; Fig. S1) or soil potassium concentration (Pearson’s r=0.04, p=0.819; Fig. S1).

Soil moisture was not measured in the field, but was instead estimated using the ‘Simple Process-Led Algorithms for Simulating Habitats’ model ('SPLASH'; Davis *et al.*, 2017). This model, derived from the STASH model (Cramer & Prentice, 1988), spins up a bucket model that calculates daily soil moisture (*W*n; mm) as a function of the previous day’s soil moisture (*W*n-1; mm), daily precipitation (*P*n; mm), condensation (*C*n; mm), actual evapotranspiration (; mm), and runoff (RO; mm):

(Eqn. 4)

This equation is solved on a daily timestep using a 150mm bucket depth (Cramer & Prentice, 1988) by first equilibrating the previous day’s soil moisture using successive model iterations with daily mean air temperature, precipitation, the number of daily sunlight hours, and latitude as model inputs to estimate daily solar radiation, condensation, evapotranspiration, and runoff using equations explained in Cramer & Prentice (1988) and 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 and site coordinates (Thieurmel & Elmarhraoui, 2019). We used daily soil moisture outputs from the SPLASH model for each site to iteratively calculate mean daily soil moisture for the prior 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 60, and 90 days leading up to each site visit. This was done to determine relevant climatic timescales that best explained *β* and *χ*. Soil moisture values are expressed in this study as a fraction of bucket depth, as done in Stocker *et al.* (2018).

*Sampling methodology and leaf trait measurements*

At each site, we collected leaf material from three individuals each of the five most abundant species at random locations in the property. All collected leaves were fully expanded, with no visible herbivory or damage, and free from shading by nearby shrubs or trees. Five soil samples were collected from 0-15cm soils at random locations in the property. Soil samples were then mixed together by hand to create one composite soil sample per site.

Images of each leaf were taken immediately following each site visit using a flat-bed scanner. Fresh 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 manually 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). We calculated leaf mass per area (*M*area; g m-2) as the ratio of fresh 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.

Subsamples of dried and homogenized leaf tissue were also sent to the University of California-Davis Stable Isotope Facility to determine leaf δ13C. Leaf δ13C values were determined using an elemental analyzer (PDZ Europa ANCA-GSL; Sercon Ltd., Chestshire, UK) interfaced to an isotope ratio mass spectrometer (PDZ Europa 20-20 Isotope Ratio Mass Spectrometer, Sercon Ltd., Chestshire, UK). We used leaf δ13C values to estimate the ratio of intercellular (*C*i) to extracellular (*C*a) CO2 (χ; unitless) 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 set to 6.3‰, and was derived from:

(Eqn. 3)

Where c was set to -5.7‰ and d was set to 30‰ (Farquhar *et al.*, 1989). 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.

We derived the unit cost of resource use (*β*) using leaf χ and site climatic data with equations described in Prentice et al. (2014) and simplified in Lavergne et al. (2020):

(Eqn. 4)

η\* is the viscosity of water relative to 25ºC, calculated using elevation and mean air temperature of the seven days leading up to each site visit following equations in Huber *et al.* (2009). D represents vapor pressure deficit (Pa), set to the mean vapor pressure deficit of the seven days leading up to each site visit, *C*a represents atmospheric CO2 concentration, set to 420 μmol mol-1 CO2. K (μmol mol-1) is the Michaelis-Menten coefficient for Rubisco affinity to CO2 and O2, calculated as:

(Eqn. 5)

where Kc (μmol mol-1) and *K*o (μmol mol-1) are the Michaelis-Menten coefficients for Rubisco affinity to CO2 and O2, respectively, and Oi is the intercellular O2 concentrations, assumed to be 210 μmol mol-1. Γ\* (μmol mol-1) is the CO2 compensation point in the absence of dark respiration. We determined *K*c, *K*o, and *Γ*\* using equations described in Medlyn *et al.* (2002) and derived in Bernacchi *et al.* (2001):

(Eqn. 6a)

and

(Eqn. 6b)

and

(Eqn. 6c)

In all three equations, *T*k is the leaf temperature (in Kelvin), which was approximated using the mean daily air temperature of the seven days leading up to each site visit. R is the universal gas constant (8.314 J mol-1 K-1).

*Plant functional group assignments*

Plant functional group were assigned to each species and used as the primary descriptor of species identity. Specifically, we assigned plant functional groups based on photosynthetic pathway (C3, C4) and ability to form associations with symbiotic nitrogen-fixing bacteria. The ability to form associations with symbiotic nitrogen-fixing bacteria was assigned based on whether species were in the *Fabaceae* family. This created three distinct plant functional groups within our dataset: C3 legumes (n=53), C3 non-legumes(n=353), and C4 non-legumes (n=114).

*Data analysis*

We constructed a series of separate linear mixed-effects models to investigate environmental drivers of *β*, *χ*, and *N*area.

To explore environmental drivers of *β*, we built a model that included soil moisture, soil nitrogen availability, and plant functional group as fixed effect coefficients. Species were designated as a random intercept term. Interaction coefficients between all possible combinations of the three fixed effect coefficients were also included. *β* was natural log transformed to linearize data and satisfy residual normality assumptions. We used an information-theoretic model selection approach to determine whether 90-, 60-, 30-, 20-, 15-, 10-, 9-, 8-, 7-, 6-, 5-, 4-, 3-, 2-, or 1-day mean daily soil moisture conferred the best model fit for *β*. To do this, we constructed 16 linear mixed-effects models for each response variable where each soil moisture timestep was included as a single continuous fixed effect. Species were included as a random intercept term for all models. We used corrected Akaike Information Criterion (AICc) to select the soil moisture timescale that conferred the best model fit, indicated by the model with the lowest AICc score (Table S1; Fig. S2).

To explore environmental drivers of *χ*, we constructed two separate linear mixed effects models. In the first model, we included mean daily air temperature, vapor pressure deficit, *β*, and plant functional group as fixed effect coefficients. We also included two-way interaction terms between plant functional group and daily air temperature, vapor pressure deficit, or *β*. Species were designated as a random intercept term. Because *χ* is an input into the calculation of *β*, and is therefore autocorrelated with *β*, we constructed a second linear mixed effects model that included daily air temperature, vapor pressure deficit, soil moisture, soil nitrogen availability, and plant functional group as fixed effect coefficients. We also included all possible interactions between soil moisture, soil NO3-N availability, and plant functional group, and two-way interactions between plant functional group and daily air temperature or vapor pressure deficit as additional fixed effect coefficients. In both models, we used an information-theoretic model selection approach to determine whether 90-, 60-, 30-, 20-, 15-, 10-, 9-, 8-, 7-, 6-, 5-, 4-, 3-, 2-, or 1-day mean daily air temperature and vapor pressure deficit conferred the best model fit for *χ* using the same approach explained for the model investigating the relevant soil moisture timescale for *β*. In the second model, we used the same soil moisture timescale as was determined the best fit for *β*.

To explore environmental drivers of *N*area we constructed two separate linear mixed effects models. The first linear mixed effect model included soil nitrogen availability, *β*, *χ*, and plant functional group as fixed effect coefficients, with species again designated as a random intercept term. Two-way interactions between plant functional group and soil nitrogen availability, *β*, or *χ* were also included. The second linear mixed effect model substituted soil moisture for *β* to replicate the structure of the linear mixed effect model used to explain variance in *β* and avoid autocorrelation between *β* and *χ*. Two-way interactions between plant functional group and soil nitrogen availability, soil moisture, or *χ* were included, in addition to a two-way interaction between soil moisture and soil nitrogen availability and a three-way interaction between soil moisture, soil nitrogen availability, and plant functional group.

Finally, we conducted a path analysis using a structural equation model to examine direct and indirect pathways that drove variance in *N*area. The path analysis included plant functional group, soil nitrogen availability, *β*, and *χ* as direct predictors of *N*area. Vapor pressure deficit, air temperature, *β*, and plant functional group were included as direct predictors of *χ*. Soil moisture, soil nitrogen availability, and plant functional group were included as direct predictors of *β*. We also included soil moisture as a direct predictor of soil nitrogen availability, and air temperature as a covariate of vapor pressure deficit.

In all linear mixed-effects models, including those to select relevant aridity timescales, 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 '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). The structural equation model was build using the ‘sem’ function in the ‘lavaan’ R package (Rosseel, 2012). All analyses and plots were conducted in R version 4.1.1 (R Core Team, 2021).

**Results**

*Unit cost ratio (β)*

Model selection indicated that 3-day soil moisture was the timescale that conferred the best model fit for *β* (Table S1; Fig. S2). Variance in *β* across sites was driven by a strong two-way interaction between soil moisture and plant functional group (Table 2), which indicated a negative effect of increasing soil moisture on *β* in C4 non-legumes (Tukey: p=0.001) and no effect of soil moisture in C3 legumes (Tukey: p=0.641) and non-legumes (Tukey: p=0.415; Fig. 2A). We also found a strong negative effect of increasing soil nitrogen availability on *β* (Table 2; Fig. 2B), a pattern observed across all plant functional groups. Finally, C4 non-legumes generally had lower *β* values than both C3 legumes (Tukey: p=0.005) and C3 non-legumes (Tukey: p<0.001) when averaged across soil moisture and soil nitrogen availability values.

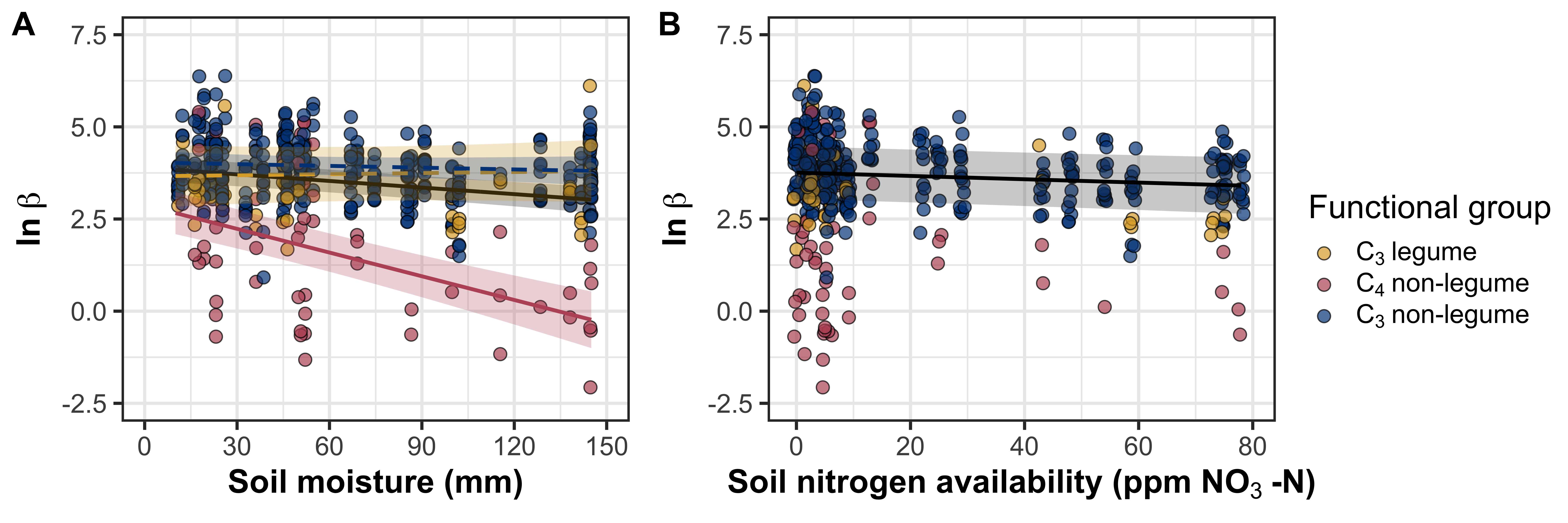
[SEM results]

**Table 2** Effects of soil moisture, soil nitrogen availability, and plant functional group on *β*\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | df | Coefficient | χ2 | *P*-value |
| Intercept |  | 3.786 |  |  |
| Soil moisture (SM) | 1 | 0.001 | 12.846 | **<0.001** |
| Soil NO3-N (N) | 1 | -0.021 | 9.670 | **0.002** |
| PFT | 2 | - | 65.020 | **<0.001** |
| SM \* N | 1 | <0.001 | 0.062 | 0.803 |
| SM \* PFT | 2 | - | 37.695 | **<0.001** |
| N \* PFT | 2 | - | 4.070 | 0.131 |
| SM \* N \* PFT | 2 | - | 0.037 | 0.982 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold. Model coefficients are on natural log scale, as beta was natural log transformed prior to fitting

**Figure 2**



**Fig. 2** Effects of soil moisture (panel A) and soil nitrogen availability (panel B) on *β*. Yellow shaded points and trendlines indicate C3 legumes, red shaded points and trendlines indicate C4 non-legumes, and blue shaded points and trendlines indicate C3 non-legumes. Black trendlines indicate the bivariate relationship between soil moisture or soil nitrogen availability and *β*. Solid trendlines indicate relationships where *P*<0.05 and dashed trendlines indicate relationships where *P*>0.05. Plant functional group-specific trendines in panel B are not included as there was no two-way interaction between soil nitrogen availability and plant functional group.

*Chi*

Model selection indicated that 4-day vapor pressure deficit and 4-day air temperature were the timescales that conferred the best model fit for *χ* (Table S1; Fig. S2). When the unit cost ratio was included as a direct predictor of *χ*, *χ* was driven by a series of two-way interactions between plant functional group and 4-day vapor pressure deficit, 4-day air temperature, and the unit cost ratio *β* (Table 3). Specifically, a two-way interaction between functional group and *β* revealed that, while increasing *β* increased *χ* across all functional groups, *χ* in C4 non-legumes was less sensitive to changes in *β* than C3 nonlegumes and C3 legumes (Tukey: p<0.001 in both cases). An additional two-way interaction between plant functional group and vapor pressure deficit indicated that increasing vapor pressure deficit increased χ in C4 nonlegumes (Tukey: p=0.002), decreased χ in C3 nonlegumes (Tukey: p<0.001), and did not change χ in C3 legumes (Tukey: p=0.271). A final two-way interaction between functional group and 4-day air temperature indicated that increasing air temperature increased χ in C4 nonlegumes (Tukey: p=0.002) and C3 legumes (Tukey: p=0.013) but did not influence χ in C3 nonlegumes (Tukey: p=0.509).

Similar patterns were observed when *β* was substituted for soil moisture and soil nitrogen availability (Table 3). Specifically, a two-way interaction between plant functional group and 3-day soil moisture indicated a negative effect of increasing soil moisture on χ in C4 nonlegumes (Tukey: p<0.001), with no apparent effect of soil moisture on χ in C3 legumes (Tukey: p=0.689) and C3 nonlegumes (Tukey: p=0.731). A weak two-way interaction between functional group and soil nitrogen availability indicated a marginal positive effect of increasing soil nitrogen availability on C4 nonlegumes (Tukey: p=0.082), with again no apparent effect on χ in C3 legumes (Tukey: p=0.401) and C3 nonlegumes (Tukey: p=0.849). A two-way interaction between functional group and 4-day vapor pressure deficit also indicated the same patterns as observed from the previous model, where increasing 4-day vapor pressure deficit increased χ in C4 nonlegumes (Tukey: p=0.002), decreased χ in C3 nonlegumes (Tukey: p<0.001), and did not change χ in C3 legumes (Tukey: p=0.262). Finally, a two-way interaction between functional group and 4-day air temperature indicated that increasing air temperature increased χ in C4 nonlegumes (Tukey: p<0.001) and C3 legumes (Tukey: p=0.027) but did not influence χ in C3 nonlegumes (Tukey: p=0.427).

**Table 3** Analysis of variance results exploring effects of vapor pressure deficit, temperature, the unit cost ratio, drivers of the unit cost ratio, and plant functional group on χ\*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | χ with β | | | χ without β | | |
| Treatment | df | Coefficient | χ2 | *P*-value | Coefficient | χ2 | *P*-value |
| Intercept | - | 0.457 | - | - | 0.445 | - | - |
| VPD | 1 | -0.008 | 34.332 | **<0.001** | -0.013 | 10.890 | **<0.001** |
| Temperature (T) | 1 | 0.020 | 0.603 | 0.437 | - | 3.430 | *0.064* |
| Unit cost ratio (*β*) | 1 | 0.001 | 327.005 | **<0.001** | - | - | - |
| Soil moisture | 1 | - | - | - | <0.001 | 17.503 | **<0.001** |
| Soil N | 1 | - | - | - | -0.001 | 1.209 | 0.272 |
| PFT | 2 | - | 122.582 | **<0.001** | - | 87.142 | **<0.001** |
| SM \* N | 1 | - | - | - | <0.001 | 0.441 | 0.507 |
| VPD \* PFT | 2 | - | 20.848 | **<0.001** | - | 24.786 | **<0.001** |
| T \* PFT | 2 | - | 16.407 | **<0.001** | - | 42.059 | **<0.001** |
| *β* \* PFT | 2 | - | 157.257 | **<0.001** | - | - | - |
| SM \* PFT | 2 | - | - | - | - | 56.680 | **<0.001** |
| N \* PFT | 2 | - | - | - | - | 9.128 | **0.010** |
| SM \* N \* PFT | 2 | - | - | - | - | 0.464 | 0.793 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold and 0.05<*P*<0.1 are italicized. The set of model coefficients, χ2 values, and *P* values on the left indicate results from a linear mixed-effect model where the unit cost ratio is included as a predictor of χ. The rightmost set of model coefficients, χ2 values, and *P* values indicate a linear mixed-effect model where the unit cost ratio is substituted for soil nitrogen availability and soil moisture, mimicking the structure of the model used to detect predictors of the unit cost ratio in Table 2. χ was not transformed prior to model fitting, so model coefficients are on the response scale.

*Leaf nitrogen content*

Variance in leaf nitrogen per leaf area was driven by a two-way interaction between the unit cost ratio *β* and plant functional group (Table 4). This interaction indicated a negative effect of increasing *β* on leaf nitrogen per leaf area in C3 legumes (Tukey: p=0.002) and a marginal negative effect in C3 nonlegumes (Tukey: p=0.083). There was no effect of *β* on leaf nitrogen per leaf area in C4 nonlegumes (Tukey: p=0.244). We also observed a negative effect of increasing *χ* on leaf nitrogen per leaf area, a pattern observed regardless of plant functional group (Table 4). Finally, increasing soil nitrogen availability had a marginal positive effect on leaf nitrogen per leaf area, a pattern observed regardless of plant functional group (Table 4).

**Table 4** Analysis of variance results exploring effects of soil nitrogen availability, the unit cost ratio, χ, and plant functional group on *N*area\*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | *N*area with *β* | | | *N*area without *β* | | |
| treatment | df | Coefficient | χ2 | P-value | Coefficient | χ2 | P-value |
| Intercept | - | 1.003 | - | - | 1.796 | - | - |
| Unit cost ratio (*β*) | 1 | -0.003 | 7.623 | **0.006** | - | - | - |
| *χ* | 1 | 0.327 | 1.164 | 0.281 | -1.059 | 7.158 | **0.007** |
| Soil N (N) | 1 | 0.003 | 2.991 | *0.084* | 0.016 | 3.977 | **0.046** |
| Soil moisture (SM) | 1 | - | - | - | 0.320 | <0.001 | 0.998 |
| PFT | 2 | - | 46.117 | **<0.001** | - | 62.024 | **<0.001** |
| SM \* N | 1 | - | - | - | -0.017 | 6.004 | 0.014 |
| *β* \* PFT | 2 | - | 6.993 | **0.030** | - | - | - |
| *χ* \* PFT | 2 | - | 3.656 | 0.161 | - | 14.34 | **<0.001** |
| N \* PFT | 2 | - | 1.789 | 0.409 | - | 1.990 | 0.370 |
| SM \* PFT | 2 | - | - | - | - | 0.594 | 0.743 |
| SM \* N \* PFT | 2 | - | - | - | - | 2.267 | 0.322 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold and 0.05<*P*<0.1 are italicized. Coefficients are expressed on natural-log scale, as *N*area was natural log transformed prior to model fitting

**Figure 4**

**Figure 5**

Diagram

Description automatically generated

**Figure 5** Structural equation model results exploring direct and indirect drivers of *β*, χ, and leaf nitrogen per unit leaf area. Blue solid arrows indicate bivariate relationships that are positively correlated and red solid arrows indicate bivariate relationships that are negatively correlated (P<0.05), while grey dashed lines indicate bivariate relationships where there is no correlation (P>0.05). Numbers indicate z-scores of each bivariate relationship and are noted in bold font when the correlation between the bivariate relationship occurs at P<0.05. Arrow thickness corresponds with the magnitude of the z-score.

**Discussion**

Widespread evidence exists documenting positive relationships between soil nutrient availability, leaf nutrient content, and leaf photosynthesis (cite). However, recent work indicates that leaf nutrient content and leaf photosynthesis are best predicted through interactions between aboveground climatic drivers and belowground edaphic factors.

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