***Ecology***

**“The cost of resource use for photosynthesis drives variation in leaf nitrogen content across a climate and resource availability gradient”**

Evan A. Perkowski1,\*, Helen G. Scott1, Nicholas G. Smith1

1Department of Biological Sciences, Texas Tech University, Lubbock, TX

**Appendix S1**

*SPLASH model overview and calculations for soil water holding capacity*

The SPLASH model, derived from the STASH model (Cramer & Prentice, 1988), spins up a bucket model using Priestley-Taylor equations (Priestley & Taylor, 1972) to calculate 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):

(S1)

In this study, models were spun up by equilibrating the previous day’s soil moisture using successive model iterations with daily mean air temperature, daily precipitation total, 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’ package in R (Thieurmel & Elmarhraoui, 2019). Water holding capacity (mm), or bucket size, was estimated as a function of soil texture using pedotransfer equations explained in (Saxton & Rawls, 2006), as done by (Stocker *et al.*, 2020) and (Bloomfield *et al.*, 2023). Specifically, water holding capacity (θWHC; mm) was calculated as a function of the volumetric soil water storage at field capacity, *W*PWP (m3 m-3), and the volumetric soil water storage at wilting point:

(S2)

where *W*FC (m3 m-3) is the volumetric soil water storage at field capacity, *W*PWP (m3 m-3) is the volumetric soil water storage at wilting point, *f*gravel (%) is the fraction of gravel content in soil, *z*bedrock (mm) is the distance to bedrock, and *z*max (mm) is the maximum allowable distance to bedrock, set to 2000mm. *W*FC is calculated as:

(S3)

where

(S4)

*W*PWP is calculated as:

(S5)

where

(S6)

In Equations (S4) and (S6), *f*sand (%) is the fraction of sand content in soil (%), *f*clay (%) is the fraction of clay content in soil (%), and *f*OM is the fraction of organic matter in soil (%). Organic matter in the soil was calculated in this study by converting soil organic carbon data extracted from SoilGrids 2.0 to soil organic matter using the van Bemmelen factor (1.724 conversion factor).

*Elevation correction equation*

Partial pressures were standardized using an elevation correction equation explained in Stocker et al. (2020):

(S7)

where *P*atm,z (Pa) is atmospheric pressure at elevation *z* (m.a.s.l.), *P*atm,0 is atmospheric pressure at 0 m.a.s.l. (101325 Pa), *L* is the mean adiabatic lapse rate (0.0065 K m-2), *T*K,0 is temperature in K at 25ºC (298.15 K), *g* is the gravitational acceleration rate (9.80665 m s-2), *M*a is the molar weight of dry air (0.028963 kg mol-1), and R is the universal gas constant (8.3145 J mol-1).

*Michaelis-Menten coefficients for Rubisco affinity to CO2 and O2*

*K* (Pa) is the Michaelis-Menten coefficient for Rubisco affinity to CO2 and O2, calculated as:

(S8)

where Kc (Pa) and *K*o (Pa) are the Michaelis-Menten coefficients for Rubisco affinity to CO2 and O2, respectively. Oi is the intercellular O2 concentration, set to 210 μmol mol-1 and converted to partial pressure (Pa) using the elevation correction explained in Eqn. 6. *Γ*\* (Pa) is the CO2 compensation point in the absence of dark respiration. *K*c, *K*o, and *Γ*\* were each calculated following Bernacchi et al. (2001), where:

(S9)

(S10)

(S11)

In all three equations, *T*k is leaf temperature (in Kelvin), estimated through mean daily air temperature of the seven days leading up to each site visit, while R is the universal gas constant (8.314 J mol-1 K-1). *K*c, *K*o, and *Γ*\* were each converted to partial pressure using the elevation correction equation explained in Appendix S1: Equation S7.

*Piecewise structural equation model loadings and fit parameters*

Seven linear mixed effect models were loaded into the structural equation model: (1) log-transformed *N*area regressed against log-transformed *N*mass and log-transformed *M*area, (2) log-transformed *M*area regressed against leaf *C*i:*C*a, nitrogen availability, and photosynthetic pathway, (3) log-transformed *N*mass regressed against leaf *C*i:*C*a, square-root transformed *β*, nitrogen availability, log-transformed *M*area, N-fixation ability, and photosynthetic pathway, (4) leaf *C*i:*C*a regressed against square-root transformed *β*, *VPD*, photosynthetic pathway, and soil moisture, (5) square-root transformed *β* regressed against nitrogen availability, soil moisture, N-fixation ability, and photosynthetic pathway, and *VPD*, (6) nitrogen availability regressed against soil moisture, and (7) soil moisture regressed against *VPD*. All models included the relevant timescale selected in the individual linear mixed-effect models explained above and included species as a random intercept term. Models were built using the ‘lme’ function in the ‘nlme’ R package (Pinheiro & Bates, 2022), and were loaded into the piecewise structural equation model using the ‘psem’ function in the ‘piecewiseSEM’ R package (Lefcheck, 2016).

Tests of directed separation indicated that the structural equation model was missing three correlations that contributed to poor overall model fit (Fisher’s *C*=162.814, *p*<0.001; df=42; AIC=246.814; BIC=418.649): a correlation between nitrogen availability and *VPD*90 (*p*<0.001), a correlation between *β* and *VPD* (*p*<0.05), and a correlation between soil moisture and *N*mass (*p*<0.05). These relationships were included in the model as correlated errors, as we did not have hypotheses to explain their direct relationships. The inclusion of these relationships improved model fit (Fisher’s *C*=23.899, *p*=0.939; df=36; AIC=107.899; BIC=279.734) and satisfied goodness-of-fit requirements (Lefcheck, 2016).

**Table S1** List of sampled species, including the NRCS symbol, photosynthetic pathway, growth duration, growth habit, N fixation capability, assigned plant functional group, and the number of collected individuals

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Symbol** | **Species** | **Photosynthetic pathway** | **Growth duration** | **Growth habit** | **N-fixer?** | **Plant functional group** | **Number sampled** |
| ACAN11 | *Acaciella angustissima* (Mill) Britton & Rose | c3 | perennial | forb | yes | c3\_legume | 3 |
| AMAR2 | *Ambrosia artemisiifolia* L. | c3 | annual | forb | no | c3\_nonlegume | 25 |
| AMPS | *Ambrosia psilostachya* DC. | c3 | perennial | forb | no | c3\_nonlegume | 32 |
| ARAL3 | *Argemone albiflora* Hornem. | c3 | annual | forb | no | c3\_nonlegume | 3 |
| ARPU9 | *Aristida purpurea* Nutt. | c4 | perennial | graminoid | no | c4\_nonlegume | 2 |
| ASAS | *Asclepias asperula* (Decne.) Woodson | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| ASLA4 | *Asclepias latifolia* (Torr.) Raf. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| ASSY | *Asclepias syriaca* L. | c3 | perennial | forb | no | c3\_nonlegume | 18 |
| BOIS | *Bothriochloa ischaemum* (L.) Keng | c4 | perennial | graminoid | no | c4\_nonlegume | 6 |
| BOSA | *Bothriochloa saccharoides* (Sw.) Rydb. | c4 | perennial | graminoid | no | c4\_nonlegume | 6 |
| CAPL3 | *Carex planostachys* Kunze | c4 | perennial | graminoid | no | c4\_nonlegume | 3 |
| CAREX | *Carex spp.* L. | c4 | perennial | graminoid | no | c4\_nonlegume | 16 |
| CHFE3 | *Chamaesyce fendleri* (Torr. & A. Gray) Small | c3 | perennial | forb | no | c3\_nonlegume | 2 |
| CHPI8 | *Chyrysopsis pilosa* Nutt. | c3 | annual | forb | no | c3\_nonlegume | 3 |
| COCO13 | *Conoclinium coelestinum* (L.) DC. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| COER | *Commelina erecta* L. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| CRGLL | *Croton glandulosus* L. | c3 | annual | forb | no | c3\_nonlegume | 22 |
| CYDA | *Cynodon dactylon* (L.) Pers. | c4 | perennial | graminoid | no | c4\_nonlegume | 15 |
| DIAN | *Dichanthium annulatum* (Forssk.) Stapf | c4 | perennial | graminoid | no | c4\_nonlegume | 8 |
| ENPE4 | *Engelmannia peristenia* (Raf.) Goodman & C.A. Lawson | c3 | perennial | forb | no | c3\_nonlegume | 6 |
| EUMA8 | *Euphorbia marginata* Pursh | c3 | annual | forb | no | c3\_nonlegume | 6 |
| GAPU | *Gaillardia pulchella* Foug. | c3 | annual | forb | no | c3\_nonlegume | 16 |
| GLGO | *Glandularia gooddingii* (Briq.) Solbrig | c3 | perennial | forb | no | c3\_nonlegume | 2 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Symbol** | **Species** | **Photosynthetic pathway** | **Growth duration** | **Growth habit** | **N-fixer?** | **Plant functional group** | **Number sampled** |
| HEAN3 | *Helianthus annuus* L. | c3 | annual | forb | no | c3\_nonlegume | 6 |
| HECA8 | *Heterotheca canescens* (DC.) Shinners | c3 | perennial | forb | no | c3\_nonlegume | 2 |
| HETE3 | *Heliotropium tenellum* (Nutt.) Torr | c3 | annual | forb | no | c3\_nonlegume | 3 |
| IVAX | *Iva axillaris* Pursh | c3 | perennial | forb | no | c3\_nonlegume | 4 |
| LIAT | *Lilaeopsis attenuata* auct. non (Hook. & Arn.) Fernald | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| LIPU | *Liatris punctata* Hook. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| LOPE | *Lolium perenne* L. | c3 | perennial | graminoid | no | c3\_nonlegume | 9 |
| MIQU2 | *Mimosa quadrivalvis* L. | c3 | perennial | forb | yes | c3\_legume | 15 |
| NALE3 | *Nassella leucotricha* (Trin. & Rupr.) Pohl | c3 | perennial | graminoid | no | c3\_nonlegume | 19 |
| OECU2 | *Oenothera curtiflora* W.L. Wagner & Hoch | c3 | annual | forb | no | c3\_nonlegume | 3 |
| OENOT | *Oenothera spp.* L. | c3 | annual | forb | no | c3\_nonlegume | 1 |
| PAVI2 | *Panicum virgatum* L. | c4 | perennial | graminoid | no | c4\_nonlegume | 12 |
| RACO3 | *Ratibida columnifera* (Nutt) Wooton & Standl. | c3 | perennial | forb | no | c3\_nonlegume | 40 |
| RHSET | *Rhynchosia senna* Gillies ex Hook. var. *texana* (Torr. & A. Gray) M.C. Johnst. | c3 | perennial | forb | yes | c3\_legume | 1 |
| RUHI2 | *Rudbeckia hirta* L. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| RUNU | *Ruellia nudiflora* (Engelm. & A. Gray) Urb. | c3 | perennial | forb | no | c3\_nonlegume | 15 |
| RUTR | *Rubus trivialis* Michx. | c3 | perennial | vine | no | c3\_nonlegume | 3 |
| SAFA2 | *Salvia farinacea* Benth. | c3 | perennial | forb | no | c3\_nonlegume | 7 |
| SCHIZ4 | *Schizachyrium spp.* Nees | c4 | perennial | graminoid | no | c4\_nonlegume | 8 |
| SCSC | *Schizachyrium scoparium* (Michx.) Nash | c4 | perennial | graminoid | no | c4\_nonlegume | 3 |
| SODI | *Solanum dimidiatum* Raf. | c3 | perennial | forb | no | c3\_nonlegume | 1 |
| SOEL | *Solanum elaeagnifolium* Cav. | c3 | perennial | forb | no | c3\_nonlegume | 53 |
| SOHA | *Sorghum halapense* (L.) Pers. | c4 | perennial | graminoid | no | c4\_nonlegume | 38 |
| STTE3 | *Stillingia texana* I.M. Johnst. | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| VEOC | *Verbesina occidentalis* (L.) Walter | c3 | perennial | forb | no | c3\_nonlegume | 3 |
| VEST | *Verbena stricta* Vent. | c3 | perennial | forb | no | c3\_nonlegume | 3 |

**Table S2** Model selection results for soil moisture, air temperature, and vapor pressure deficit. Soil moisture was used in a bivariate regression against *β*, while vapor pressure deficit was used in bivariate regressions against leaf *C*i:*C*a\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Soil moisture** | | **Vapor pressure deficit** | |
| Day | AICc | RMSE | AICc | RMSE |
| 1 | 2966.46 | 4.7692 | -857.3 | 0.0741 |
| 2 | 2966.27 | 4.7680 | -856.94 | 0.0741 |
| 3 | 2966.20 | 4.7676 | -856.84 | 0.0741 |
| 4 | 2966.27 | 4.7681 | -856.34 | 0.0741 |
| 5 | 2966.39 | 4.7689 | -856.29 | 0.0741 |
| 6 | 2966.44 | 4.7692 | -856.22 | 0.0741 |
| 7 | 2966.50 | 4.7696 | -856.18 | 0.0741 |
| 8 | 2966.54 | 4.7700 | -856.08 | 0.0741 |
| 9 | 2966.58 | 4.7704 | -856.17 | 0.0741 |
| 10 | 2966.67 | 4.7710 | -856.95 | 0.0740 |
| 15 | 2966.65 | 4.7722 | -856.52 | 0.0741 |
| 20 | 2966.47 | 4.7722 | -856.66 | 0.0742 |
| 30 | 2966.30 | 4.7715 | -857.53 | 0.0741 |
| 60 | 2963.35 | 4.7583 | -857.51 | 0.0742 |
| **90** | **2958.59** | **4.7352** | **-873.11** | **0.0730** |

\*Timescale that conferred lowest AICc value is indicated in bold.

**Figure S1**

A graph of a normal and a normal curve

Description automatically generated

**Fig. S1** Model selection results exploring relevant timescales for soil moisture (left panel) and vapor pressure deficit (right panel). The x-axis indicates the number of days before each site visit and the y-axis notes the corrected Akaike Information Criterion value. The timescale with the lowest AICc value, and therefore most relevant timescale to include in statistical models, is noted as a red point.

**Figure S2**

**A graph with a number of numbers and a number of objects

Description automatically generated**

**Fig. S2** Density plot demonstrating the observed variance in *β* across the environmental gradient. Square root transformed *β* is included on the x-axis. Blue shading indicates the distribution of *β* values for C3 species, while red shading indicates the distribution of *β* values for C4 species.

**References**

**Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP**. **2001**. Improved temperature response functions for models of Rubisco-limited photosynthesis. *Plant, Cell and Environment* **24**: 253–259.

**Bloomfield KJ, Stocker BD, Keenan TF, Prentice IC**. **2023**. Environmental controls on the light use efficiency of terrestrial gross primary production. *Global Change Biology* **29**: 1037–1053.

**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.

**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.

**Lefcheck JS**. **2016**. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution* **7**: 573–579.

**Pinheiro J, Bates D**. **2022**. nlme: linear and nonlinear mixed effects models.

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

**Saxton KE, Rawls WJ**. **2006**. Soil water characteristic estimates by texture and organic matter for hydrologic solutions. *Soil Science Society of America Journal* **70**: 1569–1578.

**Stocker BD, Wang H, Smith NG, Harrison SP, Keenan TF, Sandoval D, Davis T, Prentice IC**. **2020**. P-model v1.0: An optimality-based light use efficiency model for simulating ecosystem gross primary production. *Geoscientific Model Development* **13**: 1545–1581.

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