***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* spp. | 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.

**Table S3** Effects of soil moisture, nitrogen availability, and plant functional group on the photosynthetic cost to acquire nitrogen relative to water (*β*)\*

|  |  |  |  |
| --- | --- | --- | --- |
|  | df | χ2 | *p* |
| Soil moisture (*SM*90) | 1 | 7.982 | **0.004** |
| N availability (*N*) | 1 | 10.461 | **0.001** |
| PFT | 2 | 98.254 | **<0.001** |
| *SM90* \* *N* | 1 | 0.741 | 0.389 |
| *SM90* \* PFT | 2 | 13.922 | **0.001** |
| *N* \* PFT | 2 | 1.588 | 0.452 |
| *SM90* \* *N* \* PFT | 2 | 1.061 | 0.588 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold. Key: df=degrees of freedom, χ2=Wald Type II chi-square test statistic

**Table S4** Effects of *VPD*, drivers of the photosynthetic cost to acquire nitrogen relative to water, and functional group on leaf *C*i:*C*a\*

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | df | χ2 | *p* |
| Vapor pressure deficit (*VPD*90) | 1 | 20.775 | **<0.001** |
| Soil moisture (*SM*90) | 1 | 7.711 | **0.005** |
| N availability (N) | 1 | 6.359 | **0.012** |
| PFT | 2 | 721.308 | **<0.001** |
| *SM*90 \* N | 1 | 0.091 | 0.763 |
| *VPD*90 \* PFT | 2 | 3.675 | 0.159 |
| *SM*90 \* PFT | 2 | 17.509 | **<0.001** |
| N \* PFT | 2 | 22.486 | **<0.001** |
| *SM*90 \* N \* PFT | 2 | 1.826 | 0.401 |

\*Significance determined using Type II Wald χ2 tests (α=0.05). *P*-values less than 0.05 are in bold. Leaf *C*i:*C*a was not transformed prior to model fitting, so model coefficients are reported on the response scale. Key: df=degrees of freedom, χ2=Wald Type II chi-square test statistic

**Table S5** Effects of leaf *C*i:*C*a, drivers of the cost to acquire nitrogen relative to water, and functional group on area-based leaf nitrogen content, mass-based leaf nitrogen content, and leaf mass per area\*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | *N*area | | *N*mass | | *M*area | |
|  | df | χ2 | *p* | χ2 | *p* | χ2 | *p* |
| Leaf *C*i:*C*a | 1 | 5.579 | **0.018** | 0.127 | 0.722 | 6.560 | **0.010** |
| N availability (N) | 1 | 5.807 | **0.016** | 82.829 | **<0.001** | 43.217 | **<0.001** |
| Soil moisture (*SM*90) | 1 | 9.107 | **0.003** | 5.094 | **0.024** | 0.538 | 0.463 |
| PFT | 2 | 40.208 | **<0.001** | 13.582 | **0.001** | 7.471 | **0.024** |
| *SM*90 \* N | 1 | 1.796 | 0.180 | 3.868 | **0.049** | 0.091 | 0.763 |
| Leaf *C*i:*C*a \* PFT | 2 | 23.869 | **<0.001** | 3.546 | 0.170 | 26.294 | **<0.001** |
| N \* PFT | 2 | 6.138 | **0.046** | 1.702 | 0.427 | 16.622 | **<0.001** |
| *SM*90 \* PFT | 2 | 1.354 | 0.508 | 1.721 | 0.423 | 0.454 | 0.797 |
| *SM*90 \* N \* PFT | 2 | 4.870 | *0.088* | 0.055 | 0.973 | 7.996 | **0.018** |

\*Significance determined using Type II Wald *χ*2 tests (α=0.05). *P*-values less than 0.05 are in bold and *p*-values where 0.05<*p*<0.1 are italicized. Key: df=degrees of freedom, χ2=Wald Type II chi-square test statistic; *N*area=area-based leaf nitrogen content (gN m-2); *N*mass=mass-based leaf nitrogen content (gN g-1); leaf mass per area (*M*area; g

m-2)

**Table S6** Structural equation model results investigating drivers of variance in area-based leaf nitrogen content\*

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Predictor** | **Coefficient** | ***p*** |
| ***N*area** | | |  |
|  | *N*mass | 0.899 | **<0.001** |
| *M*area | 0.822 | **<0.001** |
| ***N*mass** | | |  |
|  | *Photo. pathway* | -0.378 | **0.004** |
| *M*area | -0.373 | **<0.001** |
| *N-fixing ability* | 0.288 | **<0.001** |
| Leaf *C*i:*C*a | 0.185 | 0.154 |
| *β* | -0.171 | **0.012** |
| *Soil N* | 0.166 | **<0.001** |
| ***M*area** | | |  |
|  | *Photo. pathway* | -0.461 | **0.010** |
|  | *Leaf Ci:Ca* | -0.309 | **0.006** |
|  | *Soil N* | -0.255 | **<0.001** |
| **Leaf *C*i:*C*a** | | |  |
|  | *Photo. pathway* | -0.738 | **<0.001** |
| *β* | 0.316 | **<0.001** |
| *VPD90* | -0.096 | **<0.001** |
| *SM90* | -0.070 | **<0.001** |
| ***β*** | | |  |
|  | *Photo. pathway* | -0.816 | **<0.001** |
| *N-fixing ability* | -0.114 | 0.189 |
| *SM90* | 0.091 | **0.005** |
| *Soil N* | -0.086 | **0.002** |
| ***Soil N*** | | |  |
|  | *SM90* | -0.198 | **<0.001** |
| ***Soil moisture*** | | | |
|  | *VPD90* | -0.675 | **<0.001** |
| ***Correlated Errors*** | |  |  |
|  | *Soil N* ~ *VPD90* | -0.267 | **<0.001** |
|  | *N*mass ~ *SM*90 | -0.049 | 0.135 |
|  | *β* ~ *VPD90* | -0.062 | 0.080 |

\*Coefficients, listed in order of magnitude for each predictor, are standardized across the structural equation model. *P*-values less than 0.05 are in bold. Negative coefficients for photosynthetic pathway indicate reduced values in C4 species, while positive coefficients for N-fixing ability indicate greater values in N-fixing species.

**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.

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