***Ecology***

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

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**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).

**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.

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