***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**

**Text 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*

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.

**Text S2**

*Piecewise structural equation model loadings and fit parameters*

Five linear mixed effect models were loaded into the C3 and C4 structural equation models: (1) *N*area regressed against *N*mass and *M*area, (2) *M*area regressed against leaf *C*i:*C*a, nitrogen availability, and soil moisture, (3) *N*mass regressed against leaf *C*i:*C*a, *M*area, nitrogen availability, and soil moisture, (4) leaf *C*i:*C*a regressed against *β*, *VPD*, soil moisture, and soil nitrogen availability, (5) *β* regressed against nitrogen availability and soil moisture. All models included the relevant timescale selected for soil moisture and *VPD* and included species as a random intercept term. Models were built using the ‘lmer’ function in the ‘lme4’ R package (Bates *et al.*, 2015) 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 C3 and C4 structural equation models were missing three correlations that contributed to poor overall model fit: a correlation between soil nitrogen availability and *VPD*, a correlation between *β* and *VPD*, and a correlation between soil moisture and *N*mass (*p*<0.05 in all cases). 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 correlated errors improved the overall model fit, but did not modify the outcomes of the original terms included in the structural equation model. Model fits for both photosynthetic pathways satisfied goodness-of-fit recommendations for piecewise structural equation modeling (C3: Fisher’s *C*=17.740, *p*=0.339, df=16, AIC=77.740, BIC=188.247; C4: Fisher’s *C*=9.357, *p*=0.898, df=16, AIC=69.357, BIC=146.598).

**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?** | **Number sampled** |
| ACAN11 | *Acaciella angustissima* (Mill) Britton & Rose | C3 | perennial | forb | yes | 3 |
| AMAR2 | *Ambrosia artemisiifolia* L. | C3 | annual | forb | no | 22 |
| AMPS | *Ambrosia psilostachya* DC. | C3 | perennial | forb | no | 32 |
| ARAL3 | *Argemone albiflora* Hornem. | C3 | annual | forb | no | 3 |
| ARPU9 | *Aristida purpurea* Nutt. | C4 | perennial | graminoid | no | 2 |
| ASAS | *Asclepias asperula* (Decne.) Woodson | C3 | perennial | forb | no | 3 |
| ASLA4 | *Asclepias latifolia* (Torr.) Raf. | C3 | perennial | forb | no | 3 |
| ASSY | *Asclepias syriaca* L. | C3 | perennial | forb | no | 18 |
| BOIS | *Bothriochloa ischaemum* (L.) Keng | C4 | perennial | graminoid | no | 6 |
| BOSA | *Bothriochloa saccharoides* (Sw.) Rydb. | C4 | perennial | graminoid | no | 6 |
| CAPL3 | *Carex planostachys* Kunze | C4 | perennial | graminoid | no | 3 |
| CAREX | *Carex spp.* L. | C4 | perennial | graminoid | no | 16 |
| CHFE3 | *Chamaesyce fendleri* (Torr. & A. Gray) Small | C3 | perennial | forb | no | 2 |
| CHPI8 | *Chyrysopsis pilosa* Nutt. | C3 | annual | forb | no | 3 |
| COCO13 | *Conoclinium coelestinum* (L.) DC. | C3 | perennial | forb | no | 3 |
| COER | *Commelina erecta* L. | C3 | perennial | forb | no | 3 |
| CRGLL | *Croton glandulosus* L. | C3 | annual | forb | no | 22 |
| CYDA | *Cynodon dactylon* (L.) Pers. | C4 | perennial | graminoid | no | 15 |
| DIAN | *Dichanthium annulatum* (Forssk.) Stapf | C4 | perennial | graminoid | no | 8 |
| ENPE4 | *Engelmannia peristenia* (Raf.) Goodman & C.A. Lawson | C3 | perennial | forb | no | 6 |
| EUMA8 | *Euphorbia marginata* Pursh | C3 | annual | forb | no | 6 |
| GAPU | *Gaillardia pulchella* Foug. | C3 | annual | forb | no | 16 |
| GLGO | *Glandularia gooddingii* (Briq.) Solbrig | C3 | perennial | forb | no | 2 |
| HEAN3 | *Helianthus annuus* L. | C3 | annual | forb | no | 6 |
| HECA8 | *Heterotheca canescens* (DC.) Shinners | C3 | perennial | forb | no | 2 |
| HETE3 | *Heliotropium tenellum* (Nutt.) Torr | C3 | annual | forb | no | 3 |
| IVAX | *Iva axillaris* Pursh | C3 | perennial | forb | no | 4 |
| LIAT | *Lilaeopsis attenuata* auct. non (Hook. & Arn.) Fernald | C3 | perennial | forb | no | 3 |
| LIPU | *Liatris punctata* Hook. | C3 | perennial | forb | no | 3 |
| LOPE | *Lolium perenne* L. | C3 | perennial | graminoid | no | 9 |
| MIQU2 | *Mimosa* spp. | C3 | perennial | forb | yes | 16 |
| NALE3 | *Nassella leucotricha* (Trin. & Rupr.) Pohl | C3 | perennial | graminoid | no | 18 |
| OECU2 | *Oenothera curtiflora* W.L. Wagner & Hoch | C3 | annual | forb | no | 3 |

**(Table S1, continued)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Symbol** | **Species** | **Photosynthetic pathway** | **Growth duration** | **Growth habit** | **N-fixer?** | **Number sampled** |
| OENOT | *Oenothera spp.* L. | C3 | annual | forb | no | 1 |
| PAVI2 | *Panicum virgatum* L. | C4 | perennial | graminoid | no | 12 |
| RACO3 | *Ratibida columnifera* (Nutt) Wooton & Standl. | C3 | perennial | forb | no | 40 |
| RHSET | *Rhynchosia senna* Gillies ex Hook. var. *texana* (Torr. & A. Gray) M.C. Johnst. | C3 | perennial | forb | yes | 1 |
| RUHI2 | *Rudbeckia hirta* L. | C3 | perennial | forb | no | 3 |
| RUNU | *Ruellia nudiflora* (Engelm. & A. Gray) Urb. | C3 | perennial | forb | no | 15 |
| SAFA2 | *Salvia farinacea* Benth. | C3 | perennial | forb | no | 7 |
| SCHIZ4 | *Schizachyrium spp.* Nees | C4 | perennial | graminoid | no | 8 |
| SCSC | *Schizachyrium scoparium* (Michx.) Nash | C4 | perennial | graminoid | no | 3 |
| SODI | *Solanum dimidiatum* Raf. | C3 | perennial | forb | no | 1 |
| SOEL | *Solanum elaeagnifolium* Cav. | C3 | perennial | forb | no | 53 |
| SOHA | *Sorghum halapense* (L.) Pers. | C4 | perennial | graminoid | no | 38 |
| STTE3 | *Stillingia texana* I.M. Johnst. | C3 | perennial | forb | no | 3 |
| VEOC | *Verbesina occidentalis* (L.) Walter | C3 | perennial | forb | no | 3 |
| VEST | *Verbena stricta* Vent. | C3 | perennial | forb | no | 3 |

**Table S2** Site locality information, sampling year(s), 2006-2020 mean annual precipitation, 2006-2020 mean annual temperature, and water holding capacity\*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Latitude** | **Longitude** | **Year** | **MAP** | **MAT** | **WHC** |
| Edwards\_2019\_17 | 29.95 | -100.36 | 2020 | 563.5 | 19.0 | 224.7 |
| Uvalde\_2020\_02 | 29.59 | -100.09 | 2020, 2021 | 648.5 | 19.5 | 235.2 |
| Menard\_2020\_01 | 30.91 | -99.57 | 2020 | 641.9 | 18.3 | 220.2 |
| Kerr\_2020\_03 | 30.06 | -99.34 | 2021 | 672.4 | 18.3 | 237.5 |
| Bandera\_2020\_03 | 29.85 | -99.30 | 2021 | 789.4 | 18.8 | 235.1 |
| Sansaba\_2020\_01 | 31.29 | -98.62 | 2020 | 733.0 | 18.8 | 234.3 |
| Comal\_2020\_21 | 29.79 | -98.43 | 2020 | 878.5 | 19.9 | 220.7 |
| Blanco\_2019\_16 | 29.99 | -98.43 | 2020 | 833.0 | 19.2 | 222.2 |
| Bexar\_2019\_13 | 29.24 | -98.43 | 2020 | 759.3 | 21.5 | 206.0 |
| Burnet\_2020\_14 | 30.84 | -98.34 | 2021 | 763.3 | 19.5 | 217.8 |
| Comal\_2020\_19 | 30.01 | -98.32 | 2021 | 845.0 | 19.3 | 220.4 |
| Hays\_2021\_54 | 29.96 | -98.17 | 2021 | 861.3 | 20.0 | 225.6 |
| Burnet\_2020\_12 | 30.82 | -98.06 | 2021 | 815.1 | 19.4 | 245.3 |
| Williamson\_2019\_09 | 30.71 | -97.86 | 2020 | 867.7 | 19.7 | 270.2 |
| Williamson\_2019\_10 | 30.54 | -97.77 | 2020 | 819.5 | 19.9 | 239.8 |
| Bell\_2021\_08 | 31.06 | -97.55 | 2021 | 937.3 | 19.6 | 232.3 |
| Fayette\_2021\_12 | 29.86 | -97.21 | 2021 | 985.7 | 20.4 | 165.6 |
| Fayette\_2020\_09 | 29.86 | -96.71 | 2021 | 1002.7 | 20.8 | 187.6 |
| Washington\_2020\_08 | 30.28 | -96.41 | 2021 | 1077.4 | 20.4 | 203.9 |
| Austin\_2020\_03 | 29.78 | -96.24 | 2021 | 1108.7 | 20.6 | 253.0 |
| Brazos\_2020\_16 | 30.93 | -96.23 | 2021 | 1078.0 | 20.1 | 202.2 |
| Brazos\_2020\_18 | 30.52 | -96.21 | 2020, 2021 | 1099.4 | 20.4 | 233.5 |
| Harris\_2020\_03 | 29.88 | -95.31 | 2020, 2021 | 1492.0 | 21.6 | 265.6 |

**\***Rows are arranged by longitude to visualize precipitation variability. Climate data were calculated using monthly PRISM data between 2006 and 2020 from the 4-km grid cell that contained each site. Key: MAP=2006-2020 mean annual precipitation (mm yr-1); MAT=2006-2020 mean annual temperature (°C); WHC=water holding capacity (mm)

**Table S3** Model results explaining the effects of ability to form associations with symbiotic nitrogen-fixing bacteria on components of leaf nitrogen content in C3 species\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | ***N*area** | | ***N*mass** | | ***M*area** | |
|  |  | df | *χ*2 | *p* | *χ*2 | *p* | *χ*2 | *p* |
|  | Leaf *C*i:*C*a | 1 | 27.931 | **<0.001** | 1.239 | 0.270 | 22.334 | **<0.001** |
|  | Soil moisture (*SM*90) | 1 | 8.196 | **0.004** | 2.613 | 0.106 | 1.137 | 0.286 |
|  | N availability (*N*) | 1 | 3.598 | *0.058* | 59.213 | **<0.001** | 35.626 | **<0.001** |
|  | N-fixation ability | 1 | 12.007 | **<0.001** | 3.692 | *0.055* | 1.887 | 0.170 |
|  | *SM90* \* *N* | 1 | 3.422 | *0.064* | 3.652 | *0.056* | 0.644 | 0.422 |

\*Significance determined using Type II Wald *χ*2 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; *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 S4** Model selection results for soil moisture, air temperature, and vapor pressure deficit for C3 species. 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 | 5729.16 | 226.837 | -954.38 | 0.045 |
| 2 | 5729.10 | 226.829 | -954.30 | 0.045 |
| 3 | 5729.08 | 226.824 | -950.18 | 0.045 |
| 4 | 5729.09 | 226.831 | -954.23 | 0.045 |
| 5 | 5729.11 | 226.837 | -955.03 | 0.044 |
| 6 | 5729.12 | 226.840 | -954.87 | 0.045 |
| 7 | 5729.13 | 226.841 | -954.32 | 0.045 |
| 8 | 5729.12 | 226.840 | -954.10 | 0.045 |
| 9 | 5729.09 | 226.837 | -953.06 | 0.045 |
| 10 | 5729.06 | 226.830 | -950.69 | 0.045 |
| 15 | 5728.62 | 226.758 | -955.40 | 0.044 |
| 20 | 5728.40 | 226.749 | -959.87 | 0.044 |
| 30 | 5728.02 | 226.665 | -972.00 | 0.043 |
| 60 | 5725.41 | 226.112 | -970.22 | 0.043 |
| 90 | **5719.41** | **224.761** | **-1010.68** | **0.041** |

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

**Table S5** Model selection results for soil moisture, air temperature, and vapor pressure deficit for C4 species. 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 | 1042.82 | 48.832 | -94.19 | 0.1220 |
| 2 | 1042.93 | 48.826 | -95.14 | 0.121 |
| 3 | 1042.96 | 48.824 | -95.02 | 0.121 |
| 4 | 1042.96 | 48.825 | -95.16 | 0.121 |
| 5 | 1042.93 | 48.824 | -96.85 | 0.120 |
| 6 | 1042.92 | 48.823 | -96.03 | 0.120 |
| 7 | 1042.96 | 48.827 | -97.08 | 0.119 |
| 8 | 1042.76 | 48.820 | -97.05 | 0.119 |
| 9 | 1042.56 | 48.812 | -97.28 | 0.119 |
| 10 | 1042.40 | 48.808 | -96.65 | 0.119 |
| 15 | 1041.42 | 48.763 | -98.22 | 0.119 |
| 20 | 1039.71 | 48.633 | -97.95 | 0.120 |
| 30 | 1039.12 | 48.683 | -95.43 | 0.122 |
| 60 | 1041.04 | 49.150 | **-102.01** | **0.119** |
| 90 | **1038.55** | **49.088** | -83.81 | 0.131 |

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

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | ***C3 model*** | | ***C4 model*** | |
|  | **Predictor** | **Coefficient** | ***p*** | **Coefficient** | ***p*** |
| ***N*area** | |  |  |  |  |
|  | *M*area | 0.928 | **<0.001** | 0.762 | **<0.001** |
| *N*mass | 0.872 | **<0.001** | 0.784 | **<0.001** |
| ***N*mass** | |  |  |  |  |
|  | *M*area | -0.355 | **<0.001** | -0.437 | **<0.001** |
|  | *Soil N* | 0.274 | **<0.001** | 0.279 | **0.006** |
| Leaf *C*i:*C*a | -0.155 | **0.011** | -0.029 | 0.785 |
| *Soil moisture* | 0.112 | **0.039** | -0.006 | 0.957 |
| ***M*area** | |  |  |  |  |
|  | *Soil N* | -0.281 | **<0.001** | -0.183 | *0.090* |
|  | *Leaf Ci:Ca* | -0.218 | **<0.001** | -0.107 | 0.343 |
|  | *Soil moisture* | 0.075 | 0.137 | 0.044 | 0.723 |
| **Leaf *C*i:*C*a** | |  |  |  |  |
|  | *β* | 0.606 | **<0.001** | 0.553 | **<0.001** |
| *VPD* | -0.289 | **<0.001** | 0.177 | 0.113 |
| *Soil N* | 0.117 | **<0.001** | -0.087 | 0.335 |
| *Soil moisture* | -0.009 | 0.851 | -0.150 | 0.190 |
| ***β*** | |  |  |  |  |
|  | *Soil moisture* | 0.167 | **0.004** | -0.310 | **0.015** |
|  | *Soil N* | -0.146 | **0.007** | -0.130 | 0.201 |
| ***Correlated Errors*** | |  |  |  |  |
|  | *N*mass *~ VPD* | -0.170 | **0.001** | -0.135 | *0.080* |
|  | *β* ~ *VPD* | -0.06 | 0.144 | 0.129 | *0.090* |
|  | *Soil N* ~ *VPD* | -0.301 | **<0.001** | -0.476 | **<0.001** |

\*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.

**Table S7** Effects of leaf *C*i:*C*a and soil resource availability area-based leaf nitrogen content, mass-based leaf nitrogen content, and leaf mass per area in C3 and C4 species\*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | ***N*area (gN m-2)** | | ***N*mass (gN g-1)** | | ***M*area (g m-2)** | |
|  |  | df | χ2 | *p* | χ2 | *p* | χ2 | *p* |
| **C3 species** | |  |  |  |  |  |  |  |
|  | Leaf *C*i:*C*a | 1 | 19.672 | **<0.001** | 1.248 | 0.264 | 13.340 | **<0.001** |
|  | Soil moisture (*SM*90) | 1 | 5.125 | **0.024** | 1.311 | 0.252 | 0.145 | 0.703 |
|  | N availability (*N*) | 1 | 1.151 | 0.283 | 57.552 | **<0.001** | 53.926 | **<0.001** |
|  | *SM90* \* *N* | 1 | 3.306 | *0.069* | 2.795 | *0.095* | 0.452 | 0.502 |
| **C4 species** | |  |  |  |  |  |  |  |
|  | Leaf *C*i:*C*a | 1 | 0.789 | 0.374 | 1.255 | 0.263 | 0.006 | 0.938 |
|  | Soil moisture (*SM*90) | 1 | 0.619 | 0.432 | 0.558 | 0.455 | 0.099 | 0.753 |
|  | N availability (*N*) | 1 | 2.043 | 0.153 | 12.904 | **<0.001** | 4.672 | **0.031** |
|  | *SM90* \* *N* | 1 | 0.018 | 0.894 | 0.459 | 0.498 | 0.301 | 0.583 |

\*Significance determined using Type II Wald *χ*2 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; *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 S8** Effects of vapor pressure deficit and soil resource availability on leaf *C*i:*C*a in C3 and C4 species\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | df | χ2 | *p* |
| **C3 species** | |  |  |  |
|  | Vapor pressure deficit (*VPD*90) | 1 | 29.830 | **<0.001** |
|  | Soil moisture (*SM*90) | 1 | 0.022 | 0.882 |
|  | N availability (*N*) | 1 | 0.142 | 0.707 |
|  | *SM90* \* *N* | 1 | 0.514 | 0.474 |
| **C4 species** | |  |  |  |
|  | Vapor pressure deficit (*VPD*60) | 1 | 9.608 | **0.002** |
|  | Soil moisture (*SM*90) | 1 | 0.130 | 0.718 |
|  | N availability (*N*) | 1 | 0.002 | 0.961 |
|  | *SM90* \* *N* | 1 | 0.025 | 0.874 |

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

**Table S9** Effects of soil moisture and nitrogen availability on the photosynthetic cost to acquire nitrogen relative to water (*β*) in C3 and C4 species\*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | df | χ2 | *p* |
| **C3 species** | |  |  |  |
|  | Soil moisture (*SM*90) | 1 | 10.418 | **0.001** |
|  | N availability (*N*) | 1 | 7.624 | **0.006** |
|  | *SM90* \* *N* | 1 | 1.048 | 0.306 |
| **C4 species** | |  |  |  |
|  | Soil moisture (*SM*90) | 1 | 17.642 | **<0.001** |
|  | N availability (*N*) | 1 | 11.671 | **<0.001** |
|  | *SM90* \* *N* | 1 | 0.038 | 0.845 |

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

**Figure S1**

A graph of different types of soil moisture

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**Fig. S1** Model selection results exploring relevant timescales for soil moisture (top row of panels) and vapor pressure deficit (bottom row of panels) for C3 (left column of panels) and C4 (right column of panels) species. 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 is noted as a red point.

**Figure S2**

**A diagram of photosynthetic pathway

Description automatically generated**

**Fig. S2** Paired violin plot and boxplot demonstrating the observed variance in *β* across the environmental gradient. Photosynthetic pathway is on the x-axis and square root transformed *β* is on the y-axis.

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