**Trait-mediated growth and survival of tropical wet forest tree seedlings across an experimental soil moisture gradient in Puerto Rico**

David Matlaga, Roel Lammerant, J. Aaron Hogan, María Uriarte, Celimar Rodriguez Valle, Jess K. Zimmerman, Robert Muscarella

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**Figure S1**. The experimental seedling plot design. Seedlings were planted in 80x80 cm plots using a 4x4 grid arrangement with 10 cm spacing and 40 cm exterior buffer area (left, drawing not to scale). Sixteen seedlings (two seedlings each of eight species) were assigned to locations within the grid randomly. Rainout shelters were constructed above each seedling plot (right). The rainout shelters were approximately two feet off the ground (exact height varied depending on topography) and constructed from plastic materials (see methods).

**Figure S2**. (A) Percent volumetric soil moisture (average of 4 measurements – one in each plot corner) for each seedling plot over time, recorded every two weeks (May 2019 - January 2020). Shelters where precipitation was excluded are colored red (i.e., the drought treatment, n = 30). Shelters where precipitation was collected and hand-watered back onto plots are colored blue (i.e., the control treatment, n =30). Panels (B) and (C) show the mean and minimum soil moisture recorded in each plot during the experiment by treatment.

**Figure S3**. Relationships between intrinsic water-use efficiency (iWUE) of seedlings (N=120) and soil moisture for eight species. Solid lines show statistically-significance linear relationships for species (*Guarea guidonia*, *Inga laurina*, *Manilkara bidentata*, and *Schefflera morototoni*), whereas dotted lines show non-significant relationships (*Prestoea acuminata* var. *montana*, and *Tetragastris balsamifera*).

**Figure S4**. Survival for the eight species over the duration of the experiment.

**Figure S5.** Total leaf area growth trajectories for all planted seedlings during the experiment. Lines are colored on a continuous scale indicating plot average soil moisture, with blue shades showing wetter plots and red shades showing drier plots

**Table S1 (Supplementary materials)**. Hypotheses relating to tropical tree seedling’s response to drought.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait** | **Units** | **Predicted response to low soil moisture (i.e., more conservative traits)** | **Rationale** | **References** |
| Leaf mass per area (LMA) | g/m2 | **+** | High LMA values relate to high leaf tissue density which tends to increase tolerance towards water limitations. | Niinemets 2001, La riva et al. 2016 |
| Average leaf area | cm2 | **–** | The leaf boundary layer is thinner for smaller leaves, which lowers the risk of heat damage under high air temperatures and low soil water potential. | Wright et al. 2017, Marechaux et al. 2019 |
| Leaf thickness | mm | **?** | Leaf thickness is positively correlated with irradiance and shows a mixed response to drought. | Niinemets 2001, La riva et al. 2016, Tng et al. 2018 |
| Stem wood density (WD) | g/cm3 | **+** | Dense wood tends to be correlated with a lower risk of drought induced cavitation. | Castro-Diez et al. 1998, Hacke et al. 2001, Markesteijn et al. 2011 |
| Leaf dry matter content (LDMC) | % | **+** | High LDMC values imply a low amount of water in leaf fresh mass and tend to relate with thicker and more rigid cell walls. | Markesteijn et al. 2011 |
| Stem dry matter content (SDMC) | % | **+** | High SDMC values imply a low amount of water in stem fresh mass and tend to be correlated with a lower risk of drought induced cavitation. | Castro-Diez et al. 1998, Hacke et al. 2001 |
| Root dry mass fraction | % | **+** | Under increasing drought stress, plants tend to allocate proportionately more biomass towards their root system. The increase in root mass fraction positively influences the ability to retrieve water from the soil. | Poorter et al. 2012, Larson et al. 2016, Eziz et al. 2017 |
| Specific root length (SRL) | cm/g | **–** | Low SRL values imply a higher investment in root tissue per length which tend to relate with a longer root lifespan and a possible lower risk of drought induced cavitation. | Eissenstat 1997, Hernandez et al. 2010, McCormack et al. 2012, Pérez-Harguindeguy et al. 2013, Larson et al. 2016, |
| Root tissue density | g/cm3 | **+** | Dense root tissue implies narrow vessels with thick cell walls and a lower risk of drought induced cavitation. | Köcher et al. 2012, Eldhuset et al. 2013 |
| Average root diameter | mm | **+** | High root diameter values imply a higher investment in root tissue per length which tend to relate with a longer root lifespan. | McCormack et al. 2012, Wang et al. 2016 |
| Root length | Cm | **–** | Reduced transpiration and respiration rates under increasing soil moisture stress tend to associate with a reduction in carbon transportation from the leaves to the root system, which implies a decrease in root length. | Ruehr et al. 2009, Eldhuset et al. 2013, Brunner et al. 2015, Blessing et al. 2016 |
| Number of root tips | # | **–** | Reduced transpiration and respiration rates under increasing soil moisture stress tend to associate with a reduction in carbon transportation from the leaves to the root system, which implies a decrease in number of root tips. | Ruehr et al. 2009, Eldhuset et al. 2013, Brunner et al. 2015, Blessing et al. 2016 |
| Root depth | cm | **–** | Reduced transpiration and respiration rates under increasing soil moisture stress tend to associate with a reduction in carbon transportation from the leaves to the root system, which implies a decrease in root depth. | Ruehr et al. 2009, Eldhuset et al. 2013, Blessing et al. 2016 |

**Table S2 (Supplementary materials)**. Successional status, peak timing of their germination (Uriarte et al. 2005, Comita et al. 2010, Taylor 1993) and authority for the eight study species.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species**  **(Family)** | **Successional status** | **Peak germination** | **Authority** |
| *Cecropia schreberiana* (Urticaceae) | Pioneer | Late March | Miq. |
| *Schefflera morototoni* (Araliaceae) | Pioneer | Late February/early March | (Aubl.) Maguire, Steyerm. & Frondin |
| *Urera baccifera* (Urticaceae) | Pioneer | Late March | (L.) Guadich. Ex Wedd. |
| *Manilkara bidentata* (Sapotaceae) | Late | Mid March | (A.DC.) A.Chev. |
| *Guarea guidonia* (Meliaceae) | Late | Late February | (L.) Sleumer |
| *Tetragastris balsamifera* (Burseraceae) | Late | Late February | Sw. Oken |
| *Inga laurina* (Leguminosae) | Secondary | Early January | Sw. Wild |
| *Prestoea acuminata* var*. montana* (Arecaceae) | Secondary | Late February/early March | (Graham) A.J.Hend. & Galeano |

**Table S3 (Supplementary materials).** Relationship between leaf length and leaf area for shadehouse grown seedlings.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Relationship | R2 | Leaves (n) | Seedlings (n) |
| *Prestoea acuminata* var*. montana* | *y = 1.3478x - 4.3908*  (*F1,19 = 37.3, p<0.0001)* | 0.66 | 20 | 20 |
| *Inga Laurina* | y= 3.42x -10.296  (*F1,236 = 981.3, p<0.0001)* | 0.81 | 238 | 30 |
| *Tetragastris balsamifera* | y= 4.34x-17.536  (*F1,82 = 336.2, p<0.0001)* | 0.81 | 84 | 30 |
| *Guarea guidonia* | y= 2.821x-4.305  (*F1,110 = 703.7, p<0.0001)* | 0.87 | 112 | 30 |
| *Manilkara bidentata* | y= 3.4113x-6.06  (*F1,66 = 107.6, p<0.0001)* | 0.62 | 67 | 29 |
| *Urera baccifera* | y= 2.305x-3.05  (*F1,79 = 1181.9, p<0.0001)* | 0.98 | 80 | 16 |
| *Schefflera morototoni* | y= 2.377x-2.765  (*F1,102 = 1081.3, p<0.0001)* | 0.92 | 103 | 30 |
| *Cecropia schreberiana* | y= 2.393x-3.0614  (*F1,83 = 495.5, p<0.0001)* | 0.86 | 84 | 27 |

**Table S4 (Supplementary materials).** Correlation between intrinsic water-use efficiency (*iWUE*) and soil moisture for six of the eight study species. Due to low sample size at the conclusion of the experiment, resulting from high mortality, *Urera baccifera* and *Cecropia schreberiana* were not included in water-use efficiency measurements.

|  |  |  |
| --- | --- | --- |
| **Species** | **Pearson´s *r*** | **p-value** |
| *Inga laurina* | **-0.78** | **< 0.001** |
| *Tetragastris balsamifera* | **0.05** | **0.85** |
| *Manilkara bidentata* | **-0.61** | **< 0.05** |
| *Guarea guidonia* | **-0.60** | **< 0.05** |
| *Prestoea acuminata* var*. montana* | **-0.22** | **0.35** |
| *Schefflera morototoni* | **-0.71** | **< 0.001** |

**Table S5 (Supplementary materials).** Pairwise Pearson correlation of the 11 traits included in the principal component analysis (PCA) (leaf mass per area (LMA), leaf dry matter content (LDMC), specific root length (SRL), root tissue density (RTD). Correlations with a p < 0.05 indicated with bold and \*.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Average leaf area** | **LMA** | **LDMC** | **Mean leaf thickness** | **Root dry mass fraction** | **Root length** | **Root Depth** | **Average root diam.** | **Root tips** | **SRL** | **RTD** |
| Average leaf area | 1 | **0.20\*** | **0.35\*** | 0,04 | -0.03 | **0.23\*** | **0.17\*** | 0.01 | **0.20\*** | **-0.27\*** | **0.32\*** |
| LMA | **0.20\*** | 1 | **0.67\*** | **0.44\*** | **-0.12\*** | **0,38\*** | **0.30\*** | **-0.15\*** | **0.38\*** | **-0.39\*** | **0.47\*** |
| LDMC | **0.35\*** | **0.67\*** | 1 | **0,18\*** | -0.07 | **0.43\*** | **0.41\*** | **-0.13\*** | **0.41\*** | **-0.47\*** | **0.63\*** |
| Mean leaf thickness | 0.04 | **0.44\*** | **0.18\*** | 1 | **-0.19\*** | -0.01 | -0.01 | -0.01 | 0.01 | **-0.19\*** | **0.09\*** |
| Root dry massvfraction | -0.03 | **-0.12\*** | -0.07 | -**0.19\*** | 1 | **0.27\*** | **0.30\*** | **0.12\*** | **0.25\*** | **-0.17\*** | **0.14\*** |
| Root length | **0.23\*** | **0.38\*** | **0.43\*** | -0.01 | **0.27\*** | 1 | **0.70\*** | **-0.36\*** | **0.93\*** | **0.17\*** | **0.26\*** |
| Root Depth | **0.17\*** | **0.30\*** | **0.41\*** | -0.01 | **0.30\*** | **0,70\*** | 1 | **-0.16\*** | **0.67\*** | **-0.27\*** | **0.31\*** |
| Average root diam. | 0.01 | **-0.15\*** | **-0.13\*** | -0.01 | **0.12\*** | **-0.36\*** | **-0.16\*** | 1 | **-0.38\*** | **-0.31\*** | **-0.18\*** |
| Root tips | **0.20**\* | **0.38\*** | **0.41\*** | 0.01 | **0.25\*** | **0.93\*** | **0.67\*** | **-0.38\*** | 1 | **-0.13\*** | **0.25\*** |
| SRL | **-0.27\*** | **-0.39\*** | **-0.47\*** | **-0.19\*** | **-0.17\*** | **0.17\*** | **-0.27\*** | **-0.31\*** | **-0.13\*** | 1 | **-0.47\*** |
| RTD | **0.32\*** | **0.47\*** | **0.63\*** | **0.09\*** | **0.14\*** | **0.26\*** | **0.31\*** | **-0.18\*** | **0.25\*** | **-0.47\*** | 1 |

**Table S6 (Supplementary materials).** Correlation of traits of eight seedling species in soil manipulation experiment to varimax rotated PCA dimensions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait (units)** |  | **Abbreviation** | **RC1** | **RC2** |
| Average leaf area (cm2) |  | AvgLeafArea | 0.47 | 0.09 |
| Leaf mass per area (g/m2) |  | LMA | 0.80 | 0.15 |
| Leaf dry matter content (%) |  | LDMC | 0.82 | 0.25 |
| Leaf thickness (mm) |  | LeafThicknessMean | 0.48 | -0.24 |
| Root dry mass fraction (%) |  | RootDryMassFraction | -0.12 | 0.42 |
| Root length (cm) |  | RootLength | 0.28 | 0.90 |
| Root depth (cm) |  | RootDepth | 0.32 | 0.74 |
| Average root diameter (mm) |  | RootAvgDiam | 0.05 | -0.50 |
| Total amount of root tips |  | RootTips | 0.27 | 0.89 |
| Specific root length (cm/g) |  | SRL | -0.72 | 0.05 |
| Root tissue density (g/cm3) |  | RTD | 0.71 | 0.19 |

**Table S7 (Supplementary materials).** Summary of Cox proportional hazard models for survival. Model structure and description in methods. Values given for the overall model determined by ‘penalized’ loglikelihood. HR is the hazard ratio.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Model component | Variable | X2 | p | AIC | Coef | HR | p | Std Dev |
| *Cecropia schreberiana* | Overall model |  | 51.95 | <0.0001 | 21.31 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.01 | 0.99 | 0.590 |  |
|  |  | Densiometer |  |  |  | -0.02 | 0.97 | 0.520 |  |
|  |  | Starting LA |  |  |  | -0.04 | 0.96 | 0.470 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 0.413 |
| *Guarea guidonia* | Overall model |  | 82.68 | <0.0001 | 38.05 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.16 | 0.84 | <0.0001 |  |
|  |  | Densiometer |  |  |  | 0.05 | 1.05 | <0.0001 |  |
|  |  | Starting LA |  |  |  | 0.00 | 1.00 | <0.0001 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 1.32 |
| *Inga laurina* | Overall model |  | 18.69 | <0.0001 | 12.69 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.36 | 0.69 | 0.0170 |  |
|  |  | Densiometer |  |  |  | 0.41 | 1.52 | 0.0320 |  |
|  |  | Starting LA |  |  |  | -0.05 | 0.94 | 0.1800 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 0.01 |
| *Manilkara bidentata* | Overall model |  | 27.62 | <0.0001 | 12.35 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.21 | 0.81 | 0.0057 |  |
|  |  | Densiometer |  |  |  | 0.02 | 1.03 | 0.8400 |  |
|  |  | Starting LA |  |  |  | -0.22 | 0.79 | 0.0780 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 0.96 |
| *Prestoea acuminata* var*. montana* | Overall model |  | 88.78 | <0.0001 | 44.70 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.14 | 0.86 | <0.0001 |  |
|  |  | Densiometer |  |  |  | 0.00 | 1.00 | <0.0001 |  |
|  |  | Starting LA |  |  |  | -0.02 | 0.97 | <0.0001 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 1.06 |
| *Tetragastris balsamifera* | Overall model |  | 31.67 | 0.004 | 3.49 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.04 | 0.96 | 0.130 |  |
|  |  | Densiometer |  |  |  | 0.04 | 1.04 | 0.470 |  |
|  |  | Starting LA |  |  |  | -0.01 | 0.98 | 0.230 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 0.75 |
| *Urera baccifera* | Overall model |  | 51.95 | <0.0001 | 21.31 |  |  |  |  |
|  | Fixed effects | Soil moisture |  |  |  | -0.03 | 0.97 | 0.0570 |  |
|  |  | Densiometer |  |  |  | 0.09 | 1.10 | 0.0020 |  |
|  |  | Starting LA |  |  |  | 0.17 | 0.83 | 0.0048 |  |
|  | Random effects | Plot (intercept) |  |  |  |  |  |  | 0.41 |

**Table S8 (Supplementary materials).** Summary of lme growth model. R2C is the conditional R2 and is interpreted as a variance explained by the entire model, including both fixed and random effects.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Model component | Variable | R2C | AIC | Estimate | t-value | Variance |
| *Cecropia schreberiana* | Overall model |  | 0.385 | -103.133 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.160 | 2.195 |  |
|  |  | Soil moisture |  |  | 0.000 | 0.110 |  |
|  |  | Densiometer |  |  | -0.003 | -0.887 |  |
|  |  | Starting LA |  |  | -0.019 | -7.302 |  |
|  | Random effects | Individual |  |  |  |  | 0.000 |
|  |  | Plot |  |  |  |  | 0.004 |
|  |  | Residual |  |  |  |  | 0.022 |
| *Guarea guidonia* | Overall model |  | 0.539 | 756.164 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.353 | 2.626 |  |
|  |  | Soil moisture |  |  | 0.014 | 3.939 |  |
|  |  | Densiometer |  |  | 0.002 | 0.254 |  |
|  |  | Starting LA |  |  | -0.016 | -17.673 |  |
|  | Random effects | Individual |  |  |  |  | 0.037 |
|  |  | Plot |  |  |  |  | 0.019 |
|  |  | Residual |  |  |  |  | 0.109 |
| *Inga laurina* | Overall model |  | 0.547 | 756.164 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.335 | 1.068 |  |
|  |  | Soil moisture |  |  | 0.042 | 5.106 |  |
|  |  | Densiometer |  |  | 0.050 | 2.627 |  |
|  |  | Starting LA |  |  | -0.012 | -18.494 |  |
|  | Random effects | Individual |  |  |  |  | 0.131 |
|  |  | Plot |  |  |  |  | 0.159 |
|  |  | Residual |  |  |  |  | 0.548 |
| *Manilkara bidentata* | Overall model |  | 0.518 | -647.259 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.230 | 4.005 |  |
|  |  | Soil moisture |  |  | 0.005 | 3.681 |  |
|  |  | Densiometer |  |  | 0.006 | 1.759 |  |
|  |  | Starting LA |  |  | -0.012 | -19.352 |  |
|  | Random effects | Individual |  |  |  |  | 0.006 |
|  |  | Plot |  |  |  |  | 0.003 |
|  |  | Residual |  |  |  |  | 0.022 |
| *Prestoea**montana* | Overall model |  | 0.436 | -644.417 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.071 | 1.331 |  |
|  |  | Soil moisture |  |  | 0.003 | 2.309 |  |
|  |  | Densiometer |  |  | 0.006 | 2.064 |  |
|  |  | Starting LA |  |  | -0.013 | -13.307 |  |
|  | Random effects | Individual |  |  |  |  | 0.003 |
|  |  | Plot |  |  |  |  | 0.003 |
|  |  | Residual |  |  |  |  | 0.019 |
| *Tetragastris balsamifera* | Overall model |  | 0.247 | 1308.641 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.377 | 2.484 |  |
|  |  | Soil moisture |  |  | 0.007 | 1.991 |  |
|  |  | Densiometer |  |  | 0.007 | 0.816 |  |
|  |  | Starting LA |  |  | -0.006 | -8.591 |  |
|  | Random effects | Individual |  |  |  |  | 0.0269 |
|  |  | Plot |  |  |  |  | 0.0168 |
|  |  | Residual |  |  |  |  | 0.260 |
| *Urera baccifera* | Overall model |  | 0.154 | -93.727 |  |  |  |
|  | Fixed effects | Intercept |  |  | 0.377 | 2.484 |  |
|  |  | Soil moisture |  |  | 0.007 | 1.991 |  |
|  |  | Densiometer |  |  | 0.007 | 0.816 |  |
|  |  | Starting LA |  |  | -0.007 | -8.591 |  |
|  | Random effects | Individual |  |  |  |  | 0.027 |
|  |  | Plot |  |  |  |  | 0.016 |
|  |  | Residual |  |  |  |  | 0.260 |

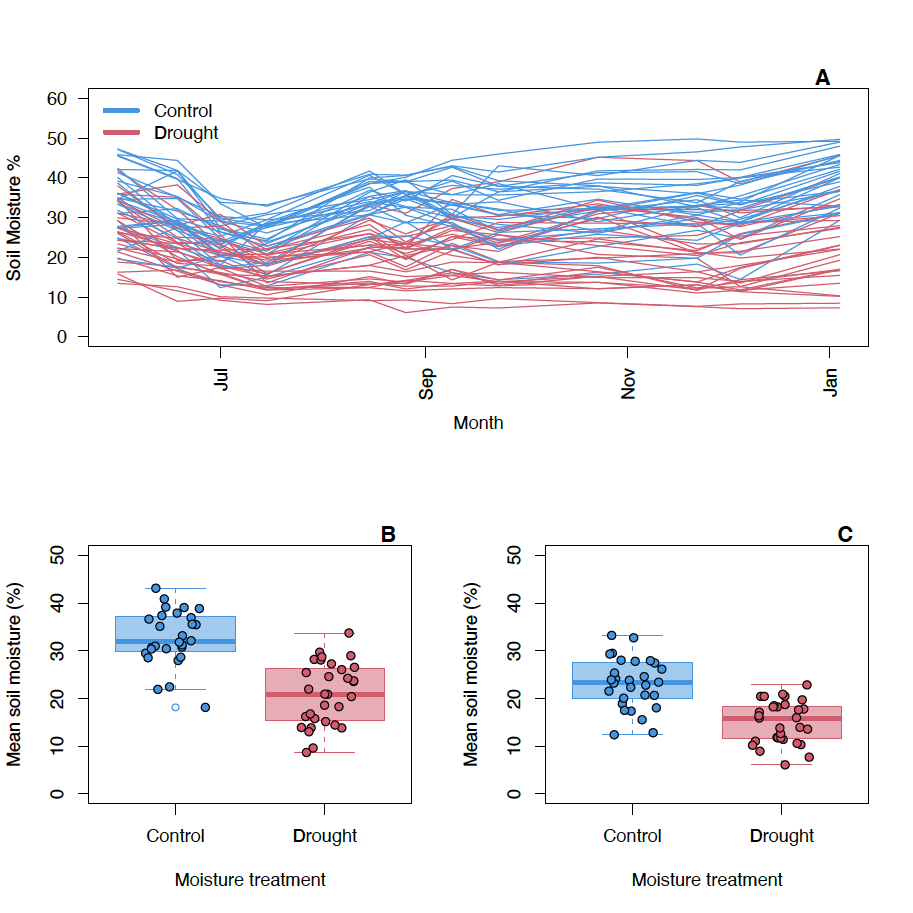
**Table S9 (Supplementary materials).** Pearson correlation (and p-value) for trait associations with species-level demographic rates.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Trait** | **Average Growth** | **Average Survival** | **Growth**  **Sensitivity** | **Growth**  **Tolerance** | **Survival**  **Sensitivity** | **Survival**  **Tolerance** |
| Log leaf area | 0.64 (0.09) | 0.69 (0.06) | 0.66 (0.08) | -0.67 (0.07) | -0.02 (0.96) | **0.72 (0.04)** |
| Log LMA | 0.57 (0.14) | **0.85 (0.01)** | 0.52 (0.19) | -0.46 (0.28) | 0.16 (0.71) | **0.79 (0.02)** |
| Log Leaf thickness | 0.33 (0.43) | **0.78 (0.02)** | 0.24 (0.57) | -0.15 (0.77) | 0.28 (0.50) | 0.64 (0.09) |
| LDMC | 0.67 (0.07) | **0.74 (0.04)** | 0.64 (0.08) | -0.59 (0.14) | -0.21 (0.62) | **0.86 (0.01)** |
| Log R:S ratio | 0.34 (0.41) | 0.53 (0.17) | 0.52 (0.19) | -0.69 (0.06) | 0.62 (0.10) | 0.22 (0.60) |
| Log SRL | -0.46 (0.26) | -0.69 (0.06) | -0.48 (0.23) | 0.51 (0.20) | 0.05 (0.91) | **-0.73 (0.04)** |
| Log RTD | 0.64 (0.09) | **0.72 (0.04)** | 0.60 (0.11) | -0.55 (0.16) | -0.19 (0.66) | **0.84 (0.01)** |
| Log Root length | **0.74 (0.03)** | **0.75 (0.03)** | **0.83 (0.01)** | **-0.86 (0.01)** | 0.25 (0.56) | 0.64 (0.08) |
| Log Root Avg Diam | -0.21 (0.61) | 0.22 (0.6) | -0.08 (0.85) | -0.11 (0.79) | 0.28 (0.50) | 0.06 (0.89) |
| Log Root Depth | **0.77 (0.02)** | 0.71 (0.05) | **0.86 (0.01)** | **-0.90 (0.001)** | 0.07 (0.86) | 0.69 (0.06) |
| Log Root Tips | **0.78 (0.02)** | 0.68 (0.06) | **0.82 (0.01)** | **-0.81 (0.02)** | 0.21 (0.61) | 0.60 (0.12) |
| RC axis 1 | 0.53 (0.18) | **0.79 (0.02)** | 0.49 (0.22) | -0.45 (0.27) | -0.07 (0.87) | **0.85 (0.01)** |
| RC axis 2 | **0.76 (0.03)** | 0.58 (0.13) | **0.84 (0.01)** | **-0.87 (0.01)** | 0.22 (0.60) | 0.49 (0.22) |

**A picture containing diagram

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**Figure S1.**

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**Figure S2.**

Chart, scatter chart

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**Figure S3.**

Chart

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**Figure S4.**

Map

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**Figure S5**

**Methods supplement (iWUE)**

To directly assess the effect of the experimental treatments on seedling physiological performance, we measured leaf intrinsic water-use efficiency (iWUE) using carbon (C) isotopes.

Isotopically-derived iWUE is an indicator of plant carbon assimilation and *gsw*(stomatal conductance to water vapor slope) at intermediate time scales (weeks to months in the context of this experiment). It integrates drought stress through isotopically-derived estimates of intracellular CO2 (*ci*), which decreases as *gsw* decreases with increasing drought stress (Cernusak et al 2013 Farquhar et al. 1982). Leaf tissue elemental analyses of carbon (C), nitrogen (N) and their isotopes (13C and 15N) were completed on selected seedlings from the wettest and driest plots of the experimental soil moisture gradient. *U. baccifera* and *C. shreberiana* were not included in leaf tissue elemental analyses because of low overall survival. Ten individuals of each of the remaining six species were harvested from relatively wet and relatively dry plots (20, in total) and used for leaf elemental analysis. Dried leaf tissues were homogenized in individual 15 mL sterile plastic vials using sterile stainless-steel beads and a Mini-G 1600 tissue homogenizer (SPEX Sample Prep, Metuchen, NJ). Samples were analyzed using a Carlo Erba NA 1500 Elemental Analyzer (Thermo Scientific, Waltham, MA USA), fitted with a Costech zero-blank autosampler at the Duke Environmental Stable Isotope Laboratory (Durham, North Carolina).

Because the photosynthetic enzyme, Rubisco, fractionates ambient CO2 during carboxation at 4.4 ‰ (Farquhar et al. 1982), calculations, leaf δ13C can be used to calculate intrinsic *ci*. Lower intrinsic *ci* values typically indicate lower gsw, assuming a constant leaf mesophyll conductance and linear relationship of *ci* to *cc* (mole fraction of CO2 in the chloroplast). Carbon isotope values were converted to intrinsic (i.e., long-term) concentration of intracellular CO2 (*ci*) estimates using the following equation for C3 plants and a *ca* value of 408 μmols mol-1 CO2.: Δ= (4.4+2.26*c*i/*ca*) (Farquhar et al. 1982, Lambers Chapin and Pons 2008). Intrinsic water use efficiency (iWUE) was determined from calculated intrinsic ci values using the following equation, from Lambers, Chapin and Pons (2008): iWUE = (c*a* \*(1-*c*i/*ca*))/1.6, We used linear regressions to evaluate how seedling *iWUE* varied with soil moisture, fitting separate regressions for each of the six species for which were measured *iWUE*.