

## THESIS

IS IT PLASTIC OR JUST FANTASTIC? UNDERSTANDING THE ROLE OF  
PLASTICITY AND LOCAL ADAPTATION IN THE DROUGHT TOLERANCE OF  
*BOUTELOUA GRACILIS*

Submitted by

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## ABSTRACT

### IS IT PLASTIC OR JUST FANTASTIC? UNDERSTANDING THE ROLE OF PLASTICITY AND LOCAL ADAPTATION IN THE DROUGHT TOLERANCE OF *BOUTELOUA GRACILIS*

The ability of an organism to tolerate or acclimate to drought may become a major driver of changes in community composition, carbon and water cycles and ecosystem services as we encounter increasing severity and frequency of droughts in the face of global change. Drought tolerance traits allow us to quantify functional attributes of individual species, but the expected variability of drought tolerance traits within a species is uncertain. It is unknown whether the local adaptation of populations can explain the expected variability. The objective of this study is the quantification of plasticity of drought tolerance responses across populations of *Bouteloua gracilis* to different soil moisture levels. *B. gracilis* is a C<sub>4</sub> perennial grass that dominates grasslands across a range of climates and is a major contributor of ecosystem function and services within these systems. Populations from less arid sites showed greater osmotic adjustment and higher midday water potentials when grown under limited soil moisture conditions. Populations from arid sites did not adjust osmotic potential but showed more negative midday water potentials while maintaining higher growth rates. This variation in response to lowered soil moisture indicates a potential shift in water use strategy across an aridity gradient that has implications for land managers seeking to restore *B. gracilis* dominated ecosystems with drought tolerant material.

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# THE LEGACY OF *BOUTELOUA GRACILIS* PHYSIOLOGY RESEARCH ON THE NORTH AMERICAN PRAIRIE

## HISTORICAL OVERVIEW

Interest in the performance and physiology of Blue Grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) has roots in the severe droughts of the 1930's and has become increasingly relevant again as models of global change predict greater frequency and duration of droughts in the next century (IPCC 2013). As early as 1928, the properties of *B. gracilis* were studied as an important forage species (McGinnies 1928), but it was not until the 1930's that this species' importance in stabilizing the prairie during drought was acknowledged.

In 1940, after almost seven years of drought and blowing dust in prairie ecosystems as far east as Kansas, J. E. Weaver writes that "almost complete destruction of the vegetation" had occurred (Weaver and Albertson 1940). In 1939, the final wave of intense drought reduced percent cover from 85% to just 16% leaving these systems at risk of increased erosion and nearly complete loss of productivity for grazing (Weaver and Albertson 1940). Much of this destruction was caused by a layer of dust 0.5 to 2.5 inches thick that compacted with the rains and intensified the drought by forming a barrier to precipitation (Albertson and Weaver 1946). In the most affected sites of western Kansas, up to three fourths of the landscape was covered by *B. gracilis* with only one to two other species remaining- both of the *Bouteloua* genus (*B. dactyloides* and *B. curtipendula*). Losses were heavy for all grass species but *B. dactyloides* and *B. gracilis* were frequently the only grasses that survived. Work during this era indicated that *B. gracilis* is the most drought tolerant of the North American

prairie species studied (Mueller and Weaver 1942) and that this species increased in territory over the seven years of sporadic drought in the 1930's (Weaver and Albertson 1939). The advancement of *B. gracilis* into territory formerly occupied by little bluestem (*Schizachyrium scoparium*) was most pronounced under conditions of heavy grazing and provided some forage to areas that were otherwise almost bare or dominated by unpalatable forbs (Albertson 1938) reflecting the ability of *B. gracilis* to preserve some ecosystem functions under heavy use and adverse conditions.

After the deterioration of the North American prairies, interest increased in restoration of the prairie ecosystem services of erosion control and forage for grazing animals. Andrew Riegel hints at the importance of *B. gracilis* as a range species; in addition to being extraordinarily drought tolerant, it was favored in restoration because it was eaten "with relish" by all grazing animals (Riegel 1941). Riegel states that there is so much variation in *B. gracilis* throughout its range from Alberta to Mexico that many early managers believed there to be multiple species of *B. gracilis*. In 1944, Riegel examined the question of plasticity and genetic adaptation of *B. gracilis* by growing seeds from Montana to Arizona in a common garden in Kansas. He found differences in phenology and growth patterns that suggest early maturity and smaller plants in the northern populations, with taller plants and greater rooting depth in the southern populations. He also speculates at a possible loss of freezing tolerance in populations from Arizona (Riegel 1944).

In 1959 Calvin McMillan conducted a study of ecotypic variation among different regions of the shortgrass steppe by comparing *B. gracilis* clones from southeastern Canada to Texas (McMillan 1959). His results support Riegel's in that he observed

earlier flowering dates from northern and western populations and later flowering dates from southern populations- with a distinct pattern of those in the west flowering earlier than those in the east across Colorado, Nebraska and Kansas. Riegel hypothesized that these differences may be closely linked to day length, but acknowledged that variability in biotic conditions such as temperature and soil moisture affect the phenological attributes of *B. gracilis* and that this indicates great plasticity in the behavior of this species.

Presumably, as the prairie recovered following the drought of the 1930's, research interests returned to the effect of grazing and management techniques on shortgrass prairie dominated by *B. gracilis*. Research focused primarily on the effects of grazing frequency (Holscher 1945, Dodd 1958), burning (Hopkins 1948) and fertilization (Kipple 1959, Rogler 1957) on forage production. Researchers began to quantify the interaction between productivity and climatic factors, and forage production was modeled as a linear function of spring precipitation (Smoliak 1956). Also emerging at this time was the examination of variation of the ploidy-level of *B. gracilis* with geographical location. Snyder and Harlan (1953) indicated that the polyploid forms of *B. gracilis* were found in a wider geographical range, but that diploid forms occupied more severe arid habitats in Texas and New Mexico. These correlations were only with geographic location and not with the morphology of *B. gracilis*.

Advances in technology and funding from the International Biome Program allowed for the study of *B. gracilis* physiology at a much finer scale. In 1973, Williams and Markley demonstrated that *B. gracilis* exhibits a C<sub>4</sub> photosynthetic pathway and paved the way for a long-standing comparison of *B. gracilis* as a model C<sub>4</sub> grass and



*Pascopyrum smithii* as a model C<sub>3</sub> grass in these ecosystems (Williams and Markley 1974, Williams 1974). Technological advances allowed for the quantification of the movement of carbon, water and nutrients as flows within a larger ecosystem (Nyhan 1975, Detling et al. 1979) and the modeling of *B. gracilis* biomass dynamics (Ares 1975, Uresk et al. 1975).

With the development of ecosystem ecology in the early 1970's, study of *B. gracilis* evolved to examine interactions of this species with the prairie at large. Studies examining the effect of primary consumers other than cattle appeared (Peden 1974, Dyer 1976) along with studies on the interactions between plant species (Jameson 1970, Bement 1968). With the establishment of the Long Term Ecological Research (LTER) program in 1980, long term projects in the grasslands of northeastern Colorado (SGS), Kansas (KNZ) and New Mexico (SEV) gained funding to study topics such as the effect of diversity or climate forcings on productivity, the legacy of land use, disturbance responses and the modeling of ecosystem properties in space and time (Hobbie 2003). Studies appeared that examine the ecosystem as a much more inclusive scale. Lauenroth, Milchunas and Burke (1998) examined the functioning of the shortgrass steppe in such comprehensive terms as soil carbon and nitrogen pools, microbial biomass, vegetative structure and soil and macrofauna. They found that this ecosystem is extremely resilient to moderate grazing and that it is likely that bison and now cattle formed an essential component in the maintenance of this ecosystem.

Large scale studies focused more on ecosystem and vegetation structure and synthesis across ecosystems became a critical component of the research in this area. Generalized models of grazing (Milchunas et al. 1987, Milchunas and Lauenroth 1993)

and water availability (Huxman and Smith et al. 2004, Knapp et al. 2008) have been increasingly prevalent at the regional or global scale. Community dynamics and intraspecific competition, particularly between C<sub>3</sub> and C<sub>4</sub> functional groups, are important in the inference of mechanisms behind shifts in community or structure in *B. gracilis* dominated grasslands. These groups are often simplified to comparisons between two dominant species representing each functional group to maximize understanding of competitive dynamics within the community.

#### PHYSIOLOGICAL COMPARISONS OF *B. GRACILIS* AND *P. SMITHII*

Some of the most detailed *B. gracilis* physiology work from 1980 until the present emerged as a comparison of physiology in *B. gracilis* and *Pascopyrum smithii* (formerly *Agropyron smithii*). These two species have been contrasted under a variety of conditions to examine possible niche partitioning for C<sub>3</sub> and C<sub>4</sub> grasses. These studies (with other relevant studies) are the focal point of this section because they cover several relevant areas of study for *B. gracilis* physiology while offering insight into how these two plants may compete with or complement each other under novel conditions.

#### Temperature and Water Relations

In one of the fundamental papers exploring the niche differences in *P. smithii* and *B. gracilis*, Kemp and Williams find that each species responds to temperature as is to be expected for their respective photosynthetic pathways. *P. smithii*, the C<sub>3</sub> grass, showed highest rates of net photosynthesis and root respiration at approximately 20°C while *B. gracilis* maximized performance at approximately 35°C (Kemp and Williams

1980). They also demonstrated that leaf gas exchange declined with changes in leaf water potential and that this relationship was not different between the two species or different temperatures. Kemp and Williams concluded that the adaptation to different temperatures meant that the primary niche separation in these species was temporal- i.e. cool-season grasses growing primarily in the spring and warm-season grasses in the summer.

Although the relationship with leaf water potential and photosynthesis did not change between these species, diurnal patterns of leaf water potential and conductance is markedly different between these species. Sala et al. (1982) examined water relations to differentiate the physiology and ecology of these two species. *P. smithii* exhibited characteristics common in other species; leaf level conductance increased throughout the morning and peaked midmorning. *B. gracilis* exhibited a unique stomatal behavior in that it increased leaf level conductance prior to dawn- the time of least evaporative water stress. This behavior is highly responsive to rainfall events as small as 5mm, with the time of maximum photosynthesis shifting to later in the morning following rain events (Sala and Lauenroth 1982). Limitation in water availability has, however, been shown to decrease leaf water potentials and photosynthesis in *B. gracilis* in the later growing season so much that the temporal niche between *P. smithii* and *B. gracilis* is all but eliminated (Monson et al. 1986). This same study revealed that *B. gracilis* did increase water use efficiency later in the season but that most of the growth occurred earlier in the season. Thus the exact niche separation between *P. smithii* and *B. gracilis* remains unclear. It is likely that *B. gracilis* is adapted to increase

conductance quickly in response to smaller size rain events (Lauenroth et al. 1987) that may lend an advantage to this species.

### Global Change Conditions

The ability of *B. gracilis* to increase carbon uptake before dawn in a water limited environment lends it a unique competitive advantage in this ecosystem that may not be quite as clear in an environment rich with CO<sub>2</sub>. Studies comparing these species aim to answer the question of whether increased CO<sub>2</sub> concentrations will benefit C<sub>3</sub> plants enough to allow them to compete with C<sub>4</sub> plants under higher temperatures. Morgan et al. (1994) found that short term exposure to elevated CO<sub>2</sub> increased photosynthetic rates in both species (with greater increases seen in *P. smithii*), but plants grown long term under elevated CO<sub>2</sub> conditions exhibited lower rates of photosynthesis due to acclimation. The authors hypothesized that lower rates of CO<sub>2</sub> assimilation was related to lower nitrogen concentration in leaves and higher nitrogen use efficiency. This study also indicated that while increasing in CO<sub>2</sub> dramatically increases photosynthesis in C<sub>3</sub> species, *B. gracilis* (C<sub>4</sub>) also has moderate potential to increase photosynthesis under elevated CO<sub>2</sub>. Read et al. (1997) found somewhat variable responses acclimation to elevated temperature and atmospheric CO<sub>2</sub> concentration. Similarly, additional studies found that responses in growth, biomass partitioning and nitrogen concentrations may vary depending on whether nutrients are supplied (Read and Morgan 1996).

There is indication that *P. smithii* may increase carbon storage in belowground biomass under elevated carbon conditions but that *B. gracilis* does not exhibit any changes in partitioning. This may, however, be related to the conditions of growth in a

greenhouse: summer-like conditions may prompt preparation for dormancy in *P. smithii* that would not be seen until later in the season for *B. gracilis* (Morgan et al. 1998). There is evidence that rather than increase photosynthetic rates and growth under elevated CO<sub>2</sub> conditions, both species, but *P. smithii* in particular, will reduce photosynthetic rates, preserve leaf water potentials and improve levels of soil water content in the field (LeCain et al. 2003). Conservation of soil moisture may minimize the competitive advantage of *B. gracilis*, but as Riegel (1944) and later McMillian (1959) found, *B. gracilis* may be highly plastic in response to a variety of environmental cues such as day length and soil moisture making the response of this species to future conditions uncertain.

#### Mycorrhizal Associations

Association with the vesicular-arbuscular (VA) mycorrhizal symbiont *Glomus fasciculatum* has been shown to increase both transpiration and photosynthetic rates in *B. gracilis* that does not result in increased biomass (Allen et al. 1981) by increasing the availability of limiting nutrients. Plants infected with *G. fasciculatum* exhibit smaller shoots and slower growth rates than those not infected but at similar nutrient levels due to loss of photosynthate (Hays 1982). Hays et al. (1982) found that infection of *B. gracilis* did not occur under high nitrogen conditions, suggesting that this symbiotic relationship occurs in response to nitrogen limitation. Research also suggests that the increased surface area of hyphae in contact with the soil reduces plant level resistance to water movement and increases water transport in this species without changing leaf properties or water status. *G. fasciculatum* forms associations with both

*B. gracilis* and *P. smithii* and questions of niche partitioning can arise in these interactions. Allen et al. (1984) found that association with *G. fasciculatum* increased the rate of photosynthesis in both species but only significantly increased leaf biomass in *P. smithii*. The association with *G. fasciculatum* did not change the nature of the niche partitioning with *P. smithii* and *B. gracilis* as the mycorrhizae mirrored the temporal behavior of each species. Mycorrhizal infection can facilitate the transport of water and nitrogen into the roots of these plants but because the relationship seems to be plant-driven, the effects likely mirror or enhance the niche separation between these two species instead of fundamentally altering them.

## SUMMARY

The performance of *B. gracilis* has been strongly linked to the ecosystem function of the shortgrass steppe under conditions of drought and heavy grazing. Early studies showed significant ecotypic variation in the performance of *B. gracilis* (Riegel 1944, McMillan 1959). Many aspects of the physiology and response of this species have been examined in the fifty year period since Riegel and McMillan. What is yet to be examined is the role of genetic variability and local adaptation in the observed responses of *B. gracilis*. The response of novel genetic sources of *B. gracilis* may be very different from those exhibited by *B. gracilis* examined in the heart of its range.

Land managers and scientists may share an interest in understanding whether *B. gracilis* in New Mexico and Canada will share the same responses as *B. gracilis* from the shortgrass steppe. Because temperature plays such an important role in the photosynthetic rate of this species, it is critical to evaluate whether *B. gracilis* from cold

climates performs the same way as *B. gracilis* from warm climates. Does *B. gracilis* from Canada still exhibit optimal rates of photosynthesis at 35°C? If *B. gracilis* from cold climates operates in a temperature closer to that of *P. smithii*, will competition increase as the gap between niches closes? *B. gracilis* has demonstrated changes in physiology and phenology in relation to water availability. One question of relevance for land managers is whether all ecotypes of *B. gracilis* exhibit similar sensitivity to soil moisture—do they adjust flowering time and stomatal opening with the same sensitivity? And perhaps most importantly, how much plasticity exists in these responses to soil moisture and how much can existing populations of *B. gracilis* acclimate to changing precipitation regimes? If ecotypes perform similarly in a common garden, it lends hope that *B. gracilis* has a high degree of plasticity and populations from Canada can occupy the same climatic envelope as that from Mexico. If ecotypes or populations perform differently in a common garden, then land managers may consider options such as facilitated migration or restoration with drought tolerant genetic material to minimize impacts of increasing drought.

The performance of *B. gracilis* in a world of abundant CO<sub>2</sub>, high temperatures and variable precipitation presents a unique and sometimes contradictory set of challenges compared to current conditions. The 1930s illustrated the susceptibility of this ecosystem to catastrophic failure due to drought and is of increasing concern as climate models predict increasing severity and frequency of drought in the western United States. The stability and productivity of this ecosystem depends on the characteristics of *B. gracilis*. Understanding the role of local adaptation and plasticity in this species is essential to understanding the performance of these lands in the future.

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# THE ROLE OF PLASTICITY AND LOCAL ADAPTATION IN THE DROUGHT TOLERANCE OF *BOUTELOUA GRACILIS*

## INTRODUCTION

The productivity and stability of grasslands in arid and semi-arid regions of the United States under conditions of increasing severity and frequency of drought predicted by climate models (IPCC 2013, Cook 2013) will likely be driven by the performance of their dominant species (Smith and Knapp 2003, Hooper and Vitousek 1997, Evans et al. 2011). Declines in percent abundance or biomass of the dominant species may result in declines in ecosystem productivity, invasion resistance and the stability of carbon, nitrogen and water cycling. The ability of these species to survive in rapidly changing environmental conditions will depend on adequate genetic variation, phenotypic plasticity or potential for migration of the species (Jump and Peñuelas 2005, Nicotra 2010). Predicting the performance of these critical species will require an understanding of the limits of each of these potential responses to novel drought conditions and may identify appropriate action on the part of land managers or restoration practitioners.

Physiological and morphological traits are frequently used to model and quantify a species' resilience to climate change but variability in these trait values is often simplified to discrete values at the species or functional type level (Craine 2012, Pappas 2015, Anderegg 2015). Oversimplification of these parameters may fail to capture behavior at range edges, population level differences or plasticity that is necessary to make informed land management decisions (Jump and Peñuelas 2005) and there is increased interest in the variability of these parameters. Studies of the dominant

tallgrass prairie species *Andropogon gerardii* reveal significant local adaptation in leaf drought tolerance traits (Johnson et al. 2015, Maricle 2017) while studies on the Mongolian steppe suggest that populations of a dominant grass *Leymus chinensis* exhibit levels of plasticity in their water use efficiency that may be larger than differences due to climate of origin of the plant material (Liu 2015). The relative importance of local adaptation, plasticity or migration may be highly specific to the species in question and necessitates species specific research.

*B. gracilis* is a dominant or secondary grass in a widespread variety of grasslands spanning the entire longitudinal gradient of the Western United States (Riegel 1940, Sims 1978) and contributes up to ninety percent of the annual net primary productivity of all biomass in the shortgrass steppe ecosystems in the heart of *B. gracilis*'s range (Detling 1979, Milchunas 1989). Precipitation reduction experiments have shown that decreasing water availability affects the abundance of *B. gracilis* with concurrent reductions in ecosystem productivity and stability (Evans 2011, Byrne 2017, Epstein 1996). Rates of net photosynthesis ( $A_{net}$ ) in *B. gracilis* have been shown to be heavily dependent on soil moisture content, increase rapidly with increasing soil moisture (Thomey et al. 2014) and increase in proportion to stomatal conductance ( $g_s$ ) over the growing season (Monson 1986). What is uncertain is whether local adaptation to aridity results in different population level responses to soil moisture in *B. gracilis*.

The objective of this study is to quantify the variability in key drought tolerance traits in populations of *B. gracilis* along both an elevation-precipitation gradient in Boulder County, Colorado and a latitude-aridity gradient from New Mexico to South Dakota. The grasses were collected from field sites and grown in a greenhouse to

determine whether drought tolerance traits correlate with a gradient in site aridity and whether the sensitivity of these traits to changes in soil moisture was consistent across the gradient. Because plants from more arid environments endure a climate with a more negative water balance, we predicted that plants from more arid environments would 1) grow more slowly and have lower biomass over the course of the study, 2) have higher WUE with greater sensitivity in adjusting WUE under conditions of water limitation and 3) have leaf level physiological traits that indicate greater drought tolerance.

## MATERIALS AND METHODS

### Species and Population Selection

*Bouteloua gracilis* was selected as the study organism because it dominates ecosystems that cover a large climate gradient. Fifteen different populations of *B. gracilis* were identified and sampled, spanning two aridity gradients: ten populations were selected from an east-west elevational gradient in northern Colorado and five populations were selected from a north-south latitude gradient in New Mexico and South Dakota.

Sites were selected using a Growing Season Aridity Index (GSAI) generated using the 30 year normals for precipitation and temperature from the PRISM group at Oregon State University (PRISM Climate Group 2016). The GSAI of each potential site was calculated by dividing the total precipitation from April-September by potential evapotranspiration for the same period. Potential evapotranspiration (PET) for April-September was estimated using the Thornthwaite method (Thornthwaite 1948). The

normal precipitation from the PRISM data was then summed for the months of April through September providing the normal growing season precipitation for each site. Although the Thornthwaite equation underestimates PET (Cruff, 1967), we applied a correction factor for arid/semi-arid sites (Pruitt 1960) that resulted in a strong correlation between our calculations and values calculated using the CGIAR-CSI Global-PET database (Zomer et al. 2008, Figure S.1).

Where possible, sites were selected with less than five percent slope and soils belonging to Hydrologic Group B or C (well drained soil) as determined by USGS Web Soil Survey to minimize the effect of drainage and soil type on plant community structure (Soil Survey Staff NRCS 2016).

#### Plant Material

Living meristematic tissue (crown tissue) samples were collected along the elevational transect within two weeks in mid-June 2016. Samples from the latitudinal gradient were sampled from June 27, 2016 to July 22, 2017. The crown tissue of 20 individuals was sampled by removing 9 cm<sup>2</sup> of soil/crown tissue at the surface and extending 5cm of below the soil surface. Because *B. gracilis* is a rhizomatous perennial grass, identification of genetically distinct individuals of *B. gracilis* requires genetic analysis. Our sampling method utilized the same as previous studies that estimated that sampling >10m apart would result in genetically independent individuals (Butterfield 2015). Therefore, the selection of individuals was based on a series of transects, sampling every 10m on four 50m transects to sample 20 individuals.



Samples were placed immediately into plastic bags with 100mL water in a cooler to preserve tissue in transit. Each individual sampled was divided and transplanted into greenhouse containers (2.65 L pots, Stuewe and Sons CP413CH) filled with 1250 grams of fifty percent Green's Grade Turface and fifty percent Sun Pro potting mix on the day of sampling. Roots were rinsed free of soil but not sterilized. All pots were kept at pot holding capacity for a two-week establishment period to facilitate transplant success. All aboveground tissue was clipped to one centimeter above crown level after the establishment period to ensure all aboveground growth occurred during the experimental period. Plants were grown in a greenhouse at Colorado State University's Plant Growth Facility where temperatures were maintained between 23.3°C and 26.7°C and grown under high pressure sodium lights for sixteen hours per day for the duration of the study.

#### Water Limitation Treatment

At 20 days after transplant, pots from each individual were assigned to one of two watering treatments: water-limited (WL) or water-abundant (WA). Watering was stopped in WL pots until Volumetric Water Content (VWC) reached less than 10% (with the exception of one 50mL addition two days into the drying period to facilitate adjustment). Irrigation was supplied every two days such that VWC of pots in the WL treatment were brought up to 10% as determined based on a preliminary regression of mass by VWC (Appendix S.2). Pots assigned to the WA treatment were irrigated every two days so that VWC of pots were brought up to pot holding capacity, and this watering regime was maintained for the duration of the experiment (14 weeks).

## Osmotic Potential

Six leaves from each population by treatment sub-group were measured using a vapor pressure osmometer (Wescor Vapro 5520) to determine leaf osmotic potential ( $\Psi_{\text{osm}}$ ). Samples for  $\Psi_{\text{osm}}$  from the Northern Colorado elevation-aridity gradient were collected on October, 29 2016 and leaves from each of the populations on the latitude-aridity gradient were collected in accordance with date of transplant to minimize the effect of phenology on the measurements. Leaves for  $\Psi_{\text{osm}}$  were frozen at  $-80^{\circ}\text{C}$  to lyse cells, stored in a freezer at  $(-18^{\circ}\text{C})$  and measured during January of 2017 (Ocheltree *In Prep.*). Recent work indicates that the osmotic potential of a leaf at full hydration ( $\Psi_{\text{osm}}$ ) is correlated to  $\Psi_{\text{TLP}}$ , a common drought tolerance metric, between species and is considerably faster than several other drought assessment methods (Bartlett et al. 2012).

## Photosynthesis and Stomatal Conductance

Rates of photosynthesis ( $A_{\text{net}}$ ) and stomatal conductance ( $g_s$ ) were measured on six individuals from each population within each treatment using a Li-6400 Portable Photosynthesis System (Li-Cor Inc., Lincoln, NE, USA). Measurements were conducted on the second day after watering and approximately eight weeks after watering treatments began. Leaves were measured at ambient greenhouse conditions on sunny days with the greenhouse shade cloth drawn on or moderately cloudy days without the shade cloth (comparable PAR readings). Conditions inside the leaf chamber were controlled at the following settings: Carbon dioxide concentration = 400ppm, PAR =  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , relative humidity = 15-30%. Temperature was allowed to vary

inside the cuvette. Two to three leaves from each individual were placed in the cuvette for a single measurement, and leaf width was measured on each leaf to calculate leaf area within the cuvette.

#### Leaf Midday Water Potential

At approximately ten weeks after watering treatments were established, midday ( $\Psi_{\text{mid}}$ ) was measured using a Scholander-type pressure chamber (PMS Instrument Company Model 1000, Albany, OR, USA). Five individuals from each population within each treatment were sampled;  $\Psi_{\text{mid}}$  measurements were made between 12:00pm and 2:00pm on sunny days as outlined in the traits literature (Perez-Harguindeguy et al. 2016).

#### Leaf Mass per Area and Leaf Dry Matter Content

Leaf mass per area (LMA) and leaf dry matter content (LDMC) were assessed at the end of the experiment. All pots were saturated to pot holding capacity for two days to ensure full leaf hydration prior to measurement. Six leaves from each treatment and population were clipped pre-dawn to ensure maximum hydration and immediately weighed to obtain leaf saturated mass. Each leaf was then scanned at 600 dpi and analyzed for leaf area (Schindelin et al. 2012). Leaves were then dried for three days (55° C, VWR Signature Floor Model 1690) and weighed to determine the dry mass for each leaf. LMA was calculated by dividing the dry mass by the area of each leaf and LDMC was calculated by dividing the dry mass by the saturated mass of each leaf (Perez-Harguindeguy et al. 2016).

## Biomass

After all pots were brought to pot-holding capacity, all plants were allowed to remain in pots until completely desiccated or approximately three weeks had passed. Biomass for each plant was divided into the following categories: AboveGround Biomass (AGB), Flowering heads (F), Crown (C) and BelowGround Biomass (BGB). The crown was separated from aboveground biomass by clipping each plant at approximately one centimeter above the origin of the tiller, and from belowground biomass by clipping all root tissue away from the crown. Belowground biomass was then rinsed free of all soil using a low-pressure nozzle and fine roots lost during the rinsing process were captured using 1mm mesh screens. All biomass was placed in an oven (55° C, VWR Signature Floor Model 1690) for three days to dry before weighing. Sensitivity of biomass production is often calculated as the change in biomass relative to the change in precipitation inputs (Huxman and Smith 2004, Wilcox et al. 2015). However, since our watering treatments were the same for all populations, our metric of sensitivity includes only the change in biomass.

## Statistical Analyses

Means and standard errors for each population under both WA and WL conditions were calculated for each physiological metric using the *dplyr* package in R (R Core Team 2017). Multiple regression analyses were performed using the 'lm' function in R to investigate relationships between watering treatment, GSAI and quadratic terms for nonlinear relationships for each physiological metric. Final models for the inclusion of interaction and quadratic terms for each physiological metric were selected based on

regression diagnostics and selected the model with the lowest AIC. Relationships between measured parameters were analyzed using the Standardized Major Axis (SMA) regression package *smatr-3* (Warton 2012). SMA regression was used only when two measured variables were being compared to examine the the relationship between the two variables.

## RESULTS

### Site-Level Characteristics

Variability in GSAI was primarily explained by the Mean Annual Temperature (MAT) of each site when both factors were examined together (Table 1.a). Because both Mean Annual Precipitation (MAP) and MAT are highly correlated with GSAI in this study, patterns driven by GSAI require further study to determine which aspect of GSAI is relevant.

### Soil Moisture Treatment

During the study, low repetitions of measurements of VWC generated some variability in the mean VWC for each population. The pattern of variability was not consistent and VWC in either treatment did not indicate a significant relationship with GSAI (Table 1.b, Figure S.3).

Table 1. a. Results from regression analyses showing the relationship between Growing Season Aridity Index (GSAI) and relevant climatic variables (top) and b. Volumetric Water Content (VWC) of each treatment (bottom).

<b>Dependent Variable</b>	<b>Predictor Variable</b>	<b>p-value</b>	<b>Partial R<sup>2</sup> of complete model</b>
GSAI	Mean Annual Temperature	<0.001	0.74
	Mean Annual Precipitation	0.001	0.24

<b>Dependent Variable</b>	<b>Predictor Variable</b>	<b>p-value</b>	<b>R<sup>2</sup> of complete model</b>
VWC- WL	GSAI	0.255	0.032
VWC-WA	GSAI	0.383	-0.014

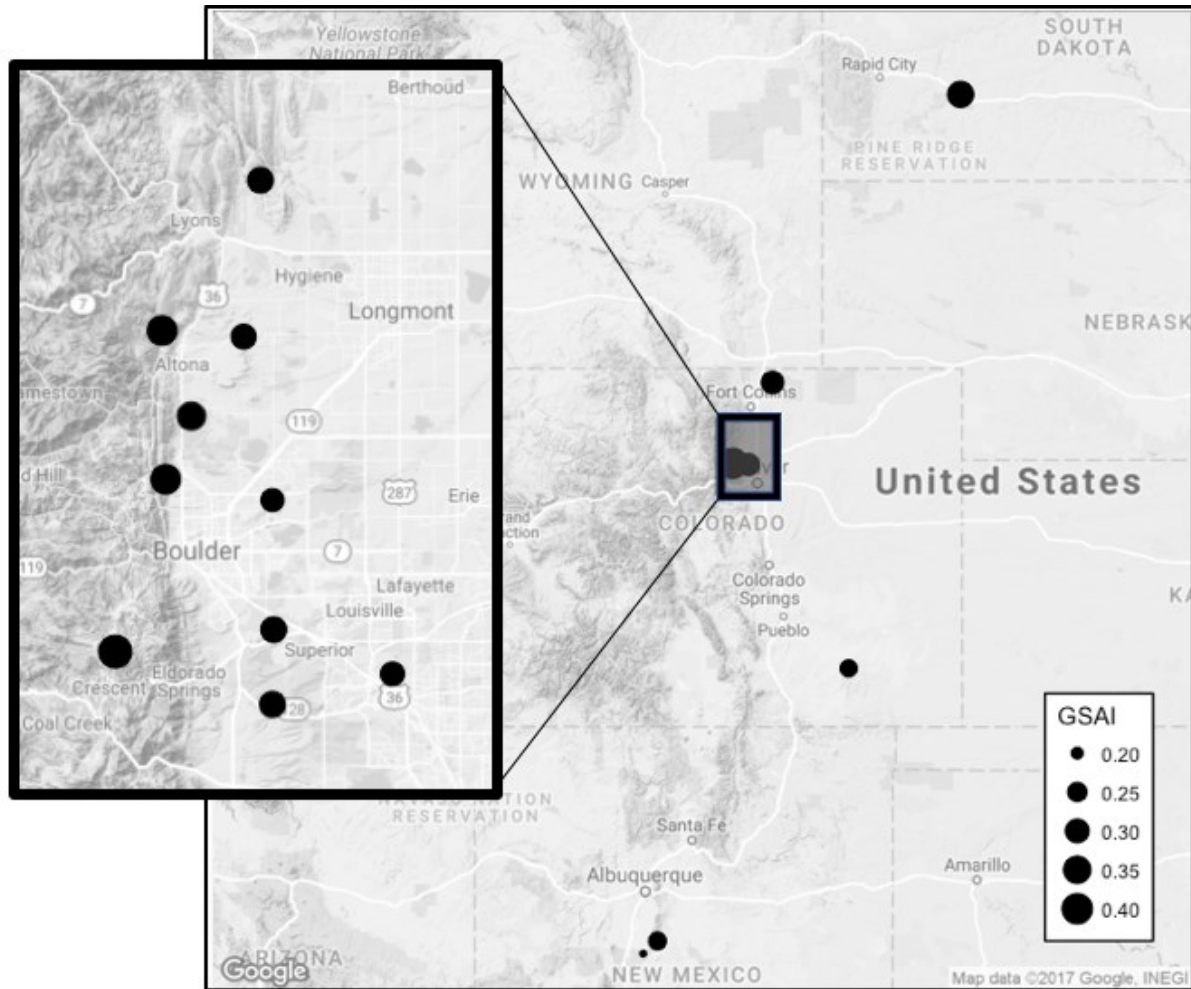


Figure 1. Map of site locations for elevational gradient (inset) and latitudinal gradient. Marker size designates Growing Season Aridity Index (GSAI) and is consistent across both maps. Some of the least arid sites come from high elevations in northern Colorado.

## Biomass

GSAI of origin and watering treatment were significant predictors for mean total biomass (AGB+BGB, Figure 2.a). A quadratic term for GSAI was significant and improved the fit of the model, suggesting that the relationship between total biomass and GSAI is nonlinear (Table 2).

The sensitivity of biomass production to changes in soil moisture was calculated as total biomass in the WA treatment minus the total biomass in the WL treatment (Figure 2.b). Regression of biomass sensitivity against revealed GSAI and a quadratic term for GSAI to be significant (Table 2) suggesting that plants from more arid populations respond more strongly to changes in soil moisture and that this relationship is nonlinear.

Table 2. Results from regression analyses showing the relationship between Biomass and Growing Season Aridity Index (GSAI) and Watering treatment (top row) and the relationship between the sensitivity of biomass to watering treatment with GSAI (bottom row).

<b>Dependent Variable</b>	<b>Predictor Variable</b>	<b>p-value</b>	<b>R<sup>2</sup> of complete model</b>
Biomass	GSAI	<0.001	0.70
	GSAI <sup>2</sup>	0.001	
	Watering Treatment	<0.001	
Sensitivity	GSAI	0.017	0.42
	GSAI <sup>2</sup>	0.027	



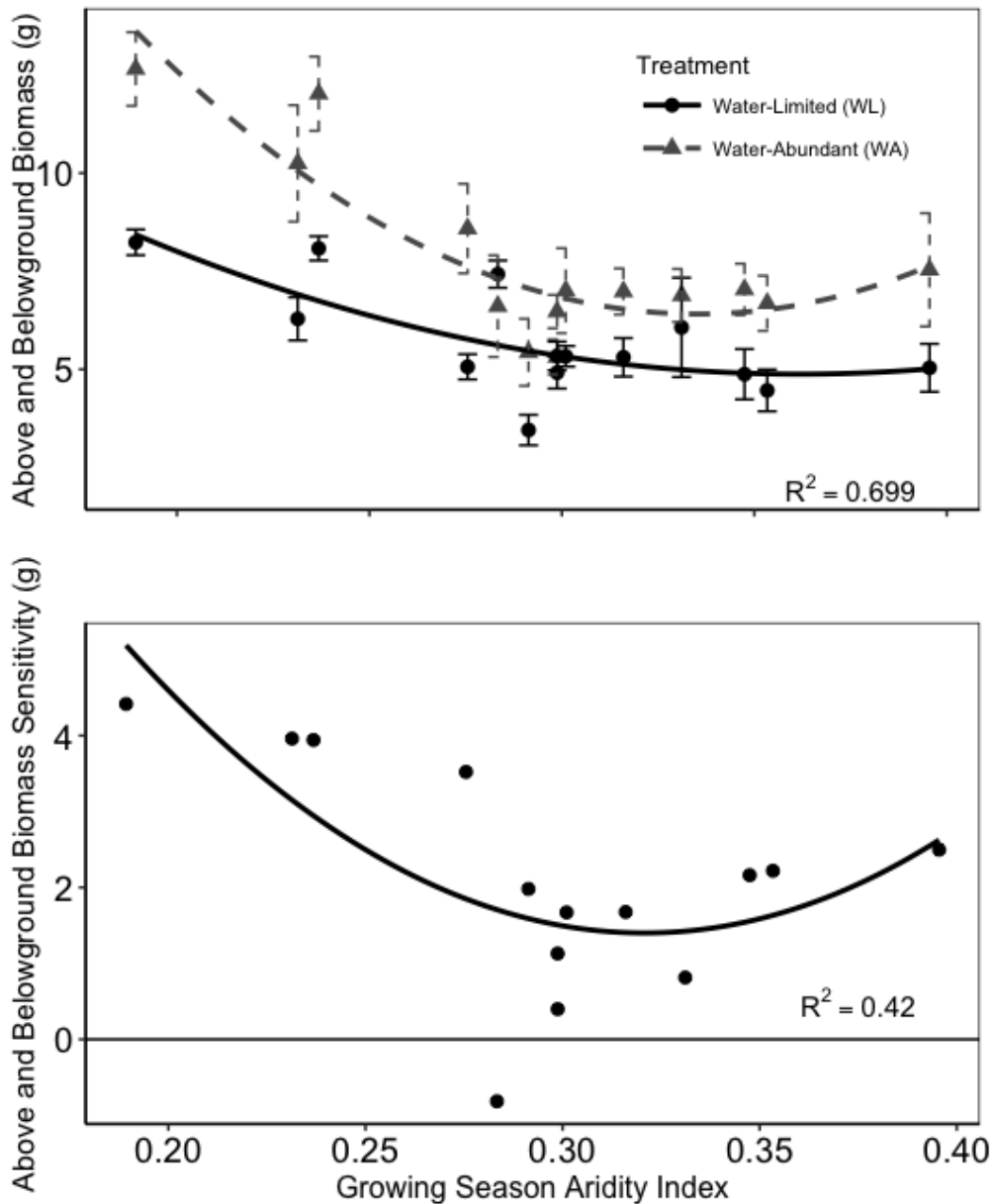


Figure 2. a) Mean total biomass for populations grown under water-abundant (grey triangles) and water-limited (black circles) treatments regressed against the Growing Season Aridity Index of the climate of origin (GSAI). GSAI, treatment and a quadratic term for GSAI were significant predictors of biomass ( $p < 0.001$ ,  $p < 0.001$ ,  $p = 0.001$ ). b). Total biomass sensitivity (WA Mean Biomass-WL Mean Biomass for each population) plotted by GSAI. GSAI was a significant predictor of biomass sensitivity ( $p = 0.0173$ ).

## Photosynthesis and Stomatal Conductance

Neither watering treatment nor GSAI explained a significant amount of variability in measured rates of  $A_{net}$  (Figure 3.a). Interaction between these two predictors did not explain any further variability (Table 3). GSAI alone did not predict rates of  $g_s$  for either watering treatments (Figure 3.b), but the significance of an interaction term between watering treatment and GSAI indicates that this relationship changed depending on watering treatment (Table 3). When plants were supplied with abundant soil moisture, GSAI was positively correlated with  $g_s$  ( $p = 0.009$ ,  $R^2_{adj} = 0.399$ ), but this relationship was not significant when soil moisture was limiting to plant growth. The populations from the most arid sites exhibited higher rates of  $g_s$  when soil moisture was limiting than when pots were watered to pot-holding capacity. None of the variability in instantaneous Water Use Efficiency (WUE) was explained by GSAI or watering treatment (Table 3).

Table 3. Results from regression analyses showing the relationship between Growing Season Aridity Index (GSAI), Watering treatment and mean net photosynthesis for populations ( $A_{net}$ ) (top row), mean stomatal conductance ( $g_s$ ) (middle row) and Water Use Efficiency (WUE) (bottom row).

<b>Dependent Variable</b>	<b>Predictor Variable</b>	<b>p-value</b>	<b>R<sup>2</sup> of complete model</b>
$A_{net}$	GSAI	0.700	0.031
	Watering Treatment	0.121	
	Interaction	0.141	
$g_s$	GSAI	0.966	0.20
	Watering Treatment	0.0473	
	Interaction	.0392	
WUE	GSAI	0.828	-0.0078
	Watering Treatment	0.647	
	Interaction	0.507	

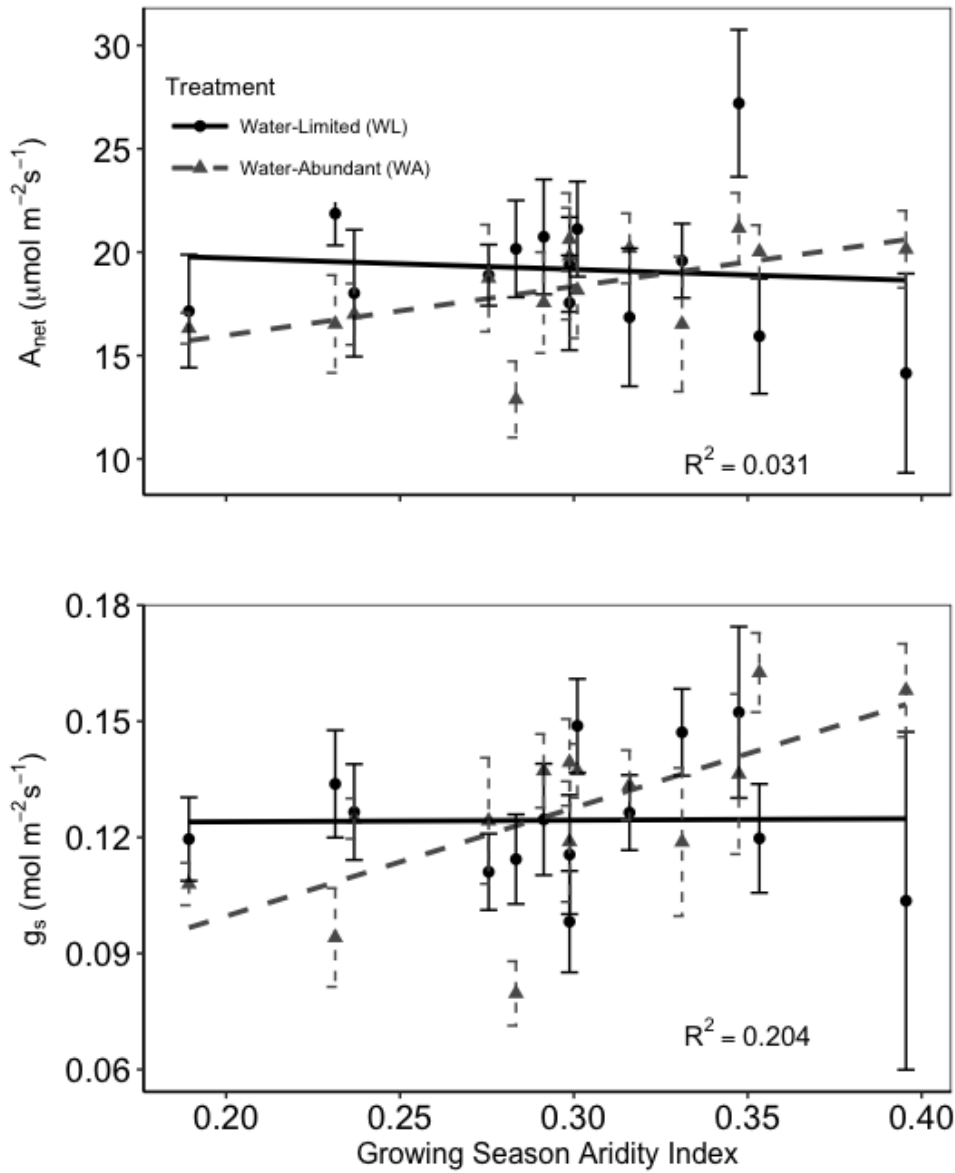


Figure 3 a) Mean photosynthetic rates ( $A$ ) for populations grown under water-abundant (grey triangles) and water-limited (black circles) treatments regressed against GSAI with no significant relationships ( $p > 0.12$ ). b) Stomatal conductance ( $g_s$ ) increased significantly with GSAI under WA (high soil moisture) conditions ( $p = 0.009$ ,  $R^2_{adj} = 0.399$ ) but not under WL (low soil moisture) conditions.

## Leaf Water and Osmotic Potential

Both GSAI and watering treatment were significant predictors of  $\Psi_{\text{osm}}$  (Figure 4, Table 4a), and the significance of a quadratic term indicated a nonlinear relationship between GSAI and  $\Psi_{\text{osm}}$  while there were no significant interactions between  $\Psi_{\text{osm}}$  and GSAI. Populations from more arid environments had less negative values of  $\Psi_{\text{osm}}$  than those from less arid sites.

Both GSAI and watering treatment were significant predictors for  $\Psi_{\text{mid}}$  (Figure 4, Table 4b). An interaction term was not significant ( $p= 0.122$ ) and was not included in the selected model, indicating that this level of water limitation did not significantly change the relationship between GSAI and  $\Psi_{\text{mid}}$  in this study.

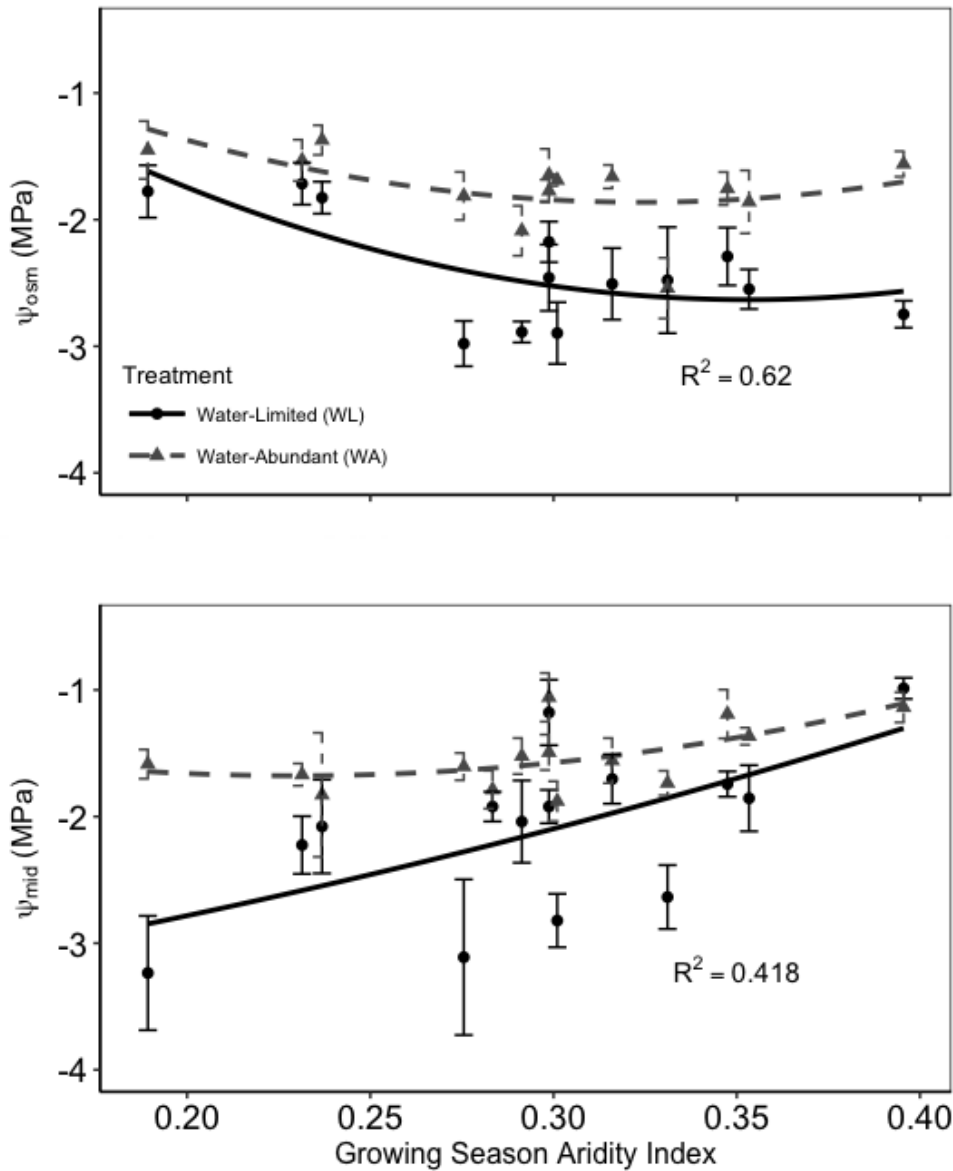


Figure 4.a) Osmotic potential ( $\Psi_{osc}$ ) for water-abundant (grey triangles) and water-limited (black circles) treatments regressed against GSAI. Both GSAI and watering treatment were significant predictors of  $\Psi_{osc}$  ( $p=0.024$ ,  $p<.001$ ,  $R^2_{adj} = .62$ ). More arid populations displayed less negative values of  $\Psi_{osc}$ . b) Midday water potential ( $\Psi_{mid}$ ) regressed against GSAI. Both GSAI and watering treatment were significant predictors of  $\Psi_{mid}$  ( $p=0.0039$ ,  $p=0.0016$ ,  $R^2_{adj} = .432$ ). More arid populations displayed more negative values of  $\Psi_{mid}$ .

$\Psi_{osm}$  showed a significant negative relationship with total biomass when analyzed using standardized major axis regression (Figure 5). This relationship was significant when data from all treatments was included ( $p < 0.001$ ,  $R^2_{adj} = .537$ ) but also within treatments (Figure 5). Total biomass was highest in populations with the least negative osmotic potential, indicating a possible tradeoff between negative osmotic potentials and increased biomass.

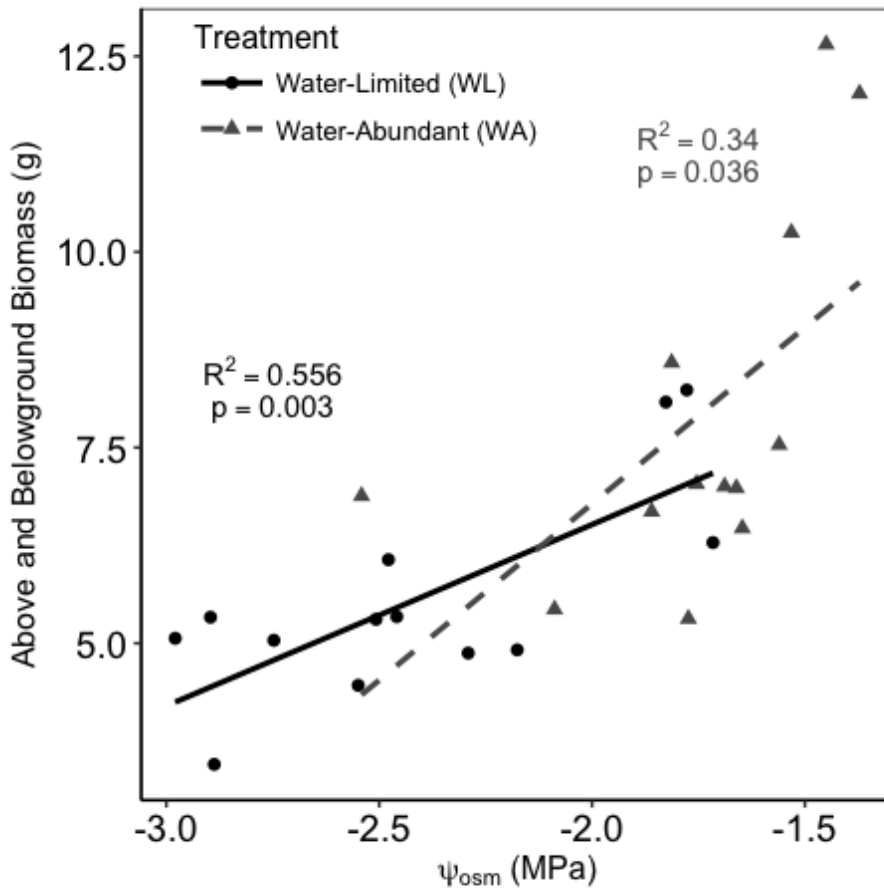


Figure 5. Mean osmotic potential for populations grown under water-abundant (grey triangles) and water-limited (black circles) treatments regressed against the mean population above and belowground biomass. A significant negative relationship ( $p < .0001$ ,  $R^2_{adj} = .537$ ) indicates a possible tradeoff between osmotic potential and growth rate.

## Leaf Traits

Variation in neither LMA nor LDMC was significantly explained by GSAI (Figure 6). A slight but non-significant trend can be seen as increasing LMA as aridity of origin decreases and there were no populations from arid environments with high LMA. Data was analyzed only under conditions of sufficient water (WA treatment) to assess differences in population level traits, rather than investigating the response of these traits to a treatment.

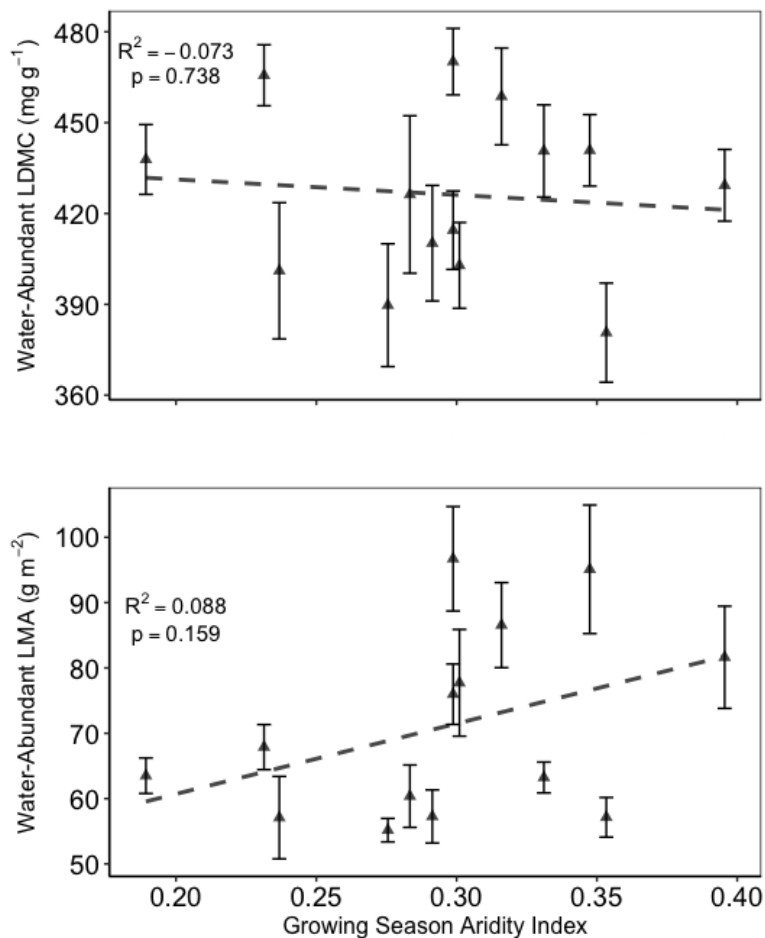


Figure 6. a) Mean Leaf Dry Matter Content (LDMC) for each population grown under water-abundant (grey triangles) conditions regressed against GSAI ( $R^2=-0.073$ ,  $p=0.738$ ). GSAI was not ( $p = 0.446$ ). b) Mean Leaf Mass per Area (LMA) for plants grown under water-abundant conditions regressed against GSAI ( $R^2=0.088$ ,  $p=0.159$ ).



## DISCUSSION

### Biomass and GSAI

While studies on other species have shown that, within a species, high competition in mesic sites favors fast growing individuals but arid environments favor individuals that emphasize reproductive fitness over rapid growth rates (Petru 2006), our data show a strong correlation between GSAI and total plant biomass that is contrary to the expected relationship between aridity and plant biomass. Plants from more arid sites had higher biomass than plants from more mesic sites regardless of watering treatment. Our results agree with earlier research comparing *B. gracilis* among populations along a latitudinal gradient; populations of *B. gracilis* from Arizona, Colorado and New Mexico showed the highest biomass per plant and tallest plant height compared to populations from Montana and Canada (Riegel 1940). Similar patterns were not observed in a reciprocal transplant study by Giuliani et al. (2014) that found that *B. gracilis* from a mesic, yet higher temperature, site in eastern Kansas exhibited higher biomass than *B. gracilis* from the shortgrass steppe in Colorado, regardless of water limitation. Differences were observed at the Kansas site but not at the lower temperature and drier site in Colorado. These results suggest that perhaps adaptation to temperature, rather than aridity, is the primary driver of the biomass response of *B. gracilis*. For example, individuals of *B. gracilis* grown at higher temperatures are more productive than that grown at lower temperatures (Kemp 1980, Read and Morgan 1996) and that productivity of *B. gracilis* increases with mean annual temperature when precipitation is held constant (Epstein 1996). This differs from another perennial C4 grasses, *A. gerardii*, that shows increased percent cover

(Johnson et al. 2015) or higher rates of photosynthesis when compared to populations from mesic sites (Maricle 2017). These differences are apparent under dry conditions but converge as water availability increases.

Although plants from arid sites produced the greatest total biomass, they were also most sensitive to reductions in soil moisture based on the difference in biomass produced between the two treatments. The difference between biomass produced under well-watered and water-limited conditions was used as an estimate of sensitivity, which was negatively correlated with GSAI and increased as the climate of origin became more arid (Fig 2b). This observed response is contrary to observations in other species that show that ecotypes from arid regions are less sensitive to changes in water availability and produce less biomass than those from more mesic regions (Beierkuhnlein 2011, Peuke 2002). Although these results are based on a single species, our observed relationship is consistent with other studies that show biomass production of entire communities is most sensitive to moisture-limitation in arid environments (Huxman Smith 2004).

#### $A_{net}$ and $g_s$

Differences in average biomass were not related to increases in midday  $A_{net}$  or increases in instantaneous  $WUE$  at the population level. Rates of  $g_s$  and  $A_{net}$  increased with GSAI when water was not limited, but were not associated with increases in plant biomass. When plants were grown under our water-limited treatment, there were no consistent patterns in the variability of  $A_{net}$  and  $g_s$  across the populations. The change in the relationship between the two treatments was driven by increases in  $A_{net}$  and  $g_s$  of

the most arid populations and decreases in the least arid populations when grown under water-limited compared to well-watered conditions. This increase in  $A_{net}$  and  $g_s$  could result from changes in biomass allocation of the individuals or, because these populations also exhibited the greatest reduction in biomass, they may have had higher nitrogen concentrations in their leaves (Adler et al. 2004).

Whatever the reason for the observed increase in  $A_{net}$  and  $g_s$  under water-limited conditions in some populations, the variability in gas exchange did not explain differences in biomass production among the populations. *B. gracilis* has been shown to have a distinct diurnal pattern of activity, with highest rates of leaf conductance occurring at or near dawn (Sala 1982) and a strong relationship with leaf temperature and the temperature of growth conditions (Read and Morgan 1996). Therefore, midday  $A_{net}$  may not have accurately captured differences in the photosynthetic or stomatal behavior of these populations.

#### $\Psi_{osm}$ and $\Psi_{mid}$

Plants that are physiologically active during dry seasons and have the same levels of physiological activity in arid climates must tolerate more extreme gradients in the soil-plant-atmosphere continuum. While annuals avoid these extreme climatic conditions by completing their life cycle before water limitation becomes severe, warm-season perennial plants are expected to tolerate more negative values of  $\Psi_{mid}$  to maintain daily carbon assimilation. Under field conditions,  $\Psi_{mid}$  has been shown to correlate with site aridity across a range of species in previous studies (Gleason 2013).  $\Psi_{mid}$  has also been shown to vary with genotype in a variety of crop species

(Sibounheuang 2006, Coupel-Ledru et al. 2014) and ecotype in native woody species (Al-ani et al. 1972, Kubiske and Abrams 1992). Our data supports the theory that populations from arid sites would exhibit lower values of  $\Psi_{mid}$  that may be driven by genetic differences. Because  $\Psi_{mid}$  was correlated with GSAI, but was independent of  $g_s$  ( $p=0.41$ , data not shown), this data suggests that differences in hydraulic conductance among the populations may be driving the differences in  $\Psi_{mid}$  in the populations.

Similarly,  $\Psi_{osm}$  in plants at arid sites should also decrease to facilitate the movement of water into leaf tissues from more negative than the soil water potentials. Studies in natural populations of *Phragmites australis* have demonstrated that local adaptation to high salt increases the population's ability to adjust  $\Psi_{osm}$  (Zhao 1999) potentially indicating that plasticity in  $\Psi_{osm}$  adjustment can be driven by environmental conditions. The level of adjustment in  $\Psi_{osm}$  has also been shown to vary with genotype in agricultural species (Jongdee 2002, Zhang 1999).  $\Psi_{TLP}$ , which is strongly correlated with  $\Psi_{osm}$ , has demonstrated plasticity in response to changing water availability in a number of species (Bartlett 2014, Burghardt 2008). Globally, plasticity in  $\Psi_{osm}$  and  $\Psi_{tip}$  are of less importance than local climate in predicting values of drought tolerance metrics and that previous exposure to drought may decrease the plasticity of  $\Psi_{tip}$  by causing the plant to maintain a lower turgor loss point during wet periods (Bartlett 2014). Our data show a relationship between  $\Psi_{osm}$  and GSAI that contradicts our hypothesis and previous research; populations from less arid sites showed greater osmotic adjustment and potentially higher plasticity in their ability to adjust  $\Psi_{osm}$ . The populations from arid sites did not adjust  $\Psi_{osm}$  and the lower values of  $\Psi_{mid}$  demonstrated by these

populations may actually signify increased water stress in the leaves due to decreased water transport to the leaves.

Under well-watered conditions there was little difference in  $\Psi_{\text{mid}}$  among populations. However, under water-limited conditions there was a positive correlation between site aridity index and  $\Psi_{\text{mid}}$ , suggesting that plants from more mesic sites maintained  $\Psi_{\text{mid}}$  at a more constant level than plants from more arid sites. Plants from the mesic sites lowered  $\Psi_{\text{osm}}$  when grown under water-limited conditions, which would allow them to maintain a higher  $\Psi_{\text{mid}}$ . Plants from the arid sites did not appear to adjust  $\Psi_{\text{osm}}$ , and therefore  $\Psi_{\text{mid}}$  was also lower under water-limited growth conditions.

The discrepancies in the behavior of  $\Psi_{\text{osm}}$  and  $\Psi_{\text{mid}}$  across the aridity gradient may indicate several different pathways of local adaptation in *B. gracilis*. If the relationship between  $\Psi_{\text{tip}}$  and  $\Psi_{\text{osm}}$  within this species is consistent with existing research, this data may indicate that populations from more arid sites are operating below  $\Psi_{\text{tip}}$  on a daily basis (Bartlett 2012). The ability to function at leaf water potentials below the turgor loss point has been recognized in other species in the shortgrass steppe (Ocheltree *In Review*) and may be an adaptation to water-limited environments. Arid populations may have other physiological properties such as more elastic cell walls providing maintenance of cell turgor at lower water potentials that allow the plant to function at lower levels of  $\Psi$  (Clifford et al. 1998). A variety of drought adaptations may explain the maintenance of lower values of  $\Psi_{\text{mid}}$  in arid populations and may warrant further study in light of these results.

Studies have shown that the ability of xylem to withstand lower values of  $\Psi$  without cavitation is related to water limitation in climate of origin in many species (Wortemann 2011, Schuldt 2015) and is globally linked to the mortality of trees (Anderegg 2015). If arid populations have xylem that are more resistant to cavitation, they may be able to forego potential carbon expenses associated with osmotic adjustment in favor of growth. If the water potential at which *B. gracilis* loses hydraulic conductivity ( $P_{50}$ ) varies in this species as it does in other species (Lamy 2014, Wortemann 2011), it is possible that  $\Psi_{osm}$  adjusts proportionally to protect major xylem elements from embolism. Therefore plants from mesic climates with a less negative  $P_{50}$  may adjust  $\Psi_{osm}$  more dramatically to maintain “safe” levels of xylem water potential, while arid populations may rely on more resistant xylem.

Finally, it is possible that triggering the osmotic response of *B. gracilis* has a soil moisture threshold that varies with aridity of origin, and that the soil moisture levels used in this study were not low enough to elicit a stress response in arid populations where more extreme water limitations would elicit an osmotic stress response.

In the context of this study,  $\Psi_{osm}$  was examined as a drought tolerance mechanism, but it can also provide freezing tolerance. Because GSAI is a metric that incorporates both temperature and precipitation, it may be the case that this relationship between  $\Psi_{osm}$  and GSAI is driven primarily by low temperatures associated with high GSAI. Other work has shown that freezing tolerance is associated with a reduction in growth rates (Savage and Cavender-Bares 2015) that may explain the lowered growth rate in our more mesic populations. This may be due to a trade-off in carbon investment between osmotic solutes and structural carbohydrates or may be due to the

activation of a suite of genes that promote both low stature and freezing tolerance (Lata and Prasad 2011). Research shows that the perennating structures *B. gracilis* from southern populations can increase tolerance of freezing temperatures over a period of months (Schwartz 1989), but the effect of this adjustment on other growth characteristics is unclear.

### Leaf Traits

LMA and LDMC are widely used to estimate growth rate (Wright 2004) but failed to adequately predict the performance of the populations in this study. Higher LMA values at the less arid sites would indicate greater investment in leaf tissue relative to leaf area, and may explain some decreases in growth rate in accordance with the well cited relationships of the leaf economic spectrum (Wright 2004). The general relationship between LMA and aridity, latitude or temperature is in agreement with much of literature (Maes et al. 2013, Wright 2004) but LMA has also been shown to increase with both drought stress and cold stress in other species (Wright et al. 2002, Gonzalez-Zurdo et al. 2015, Niinemets 2016) so it is unclear what drives changes in LMA within this species. The relationship between LMA and temperature observed in this study is, however, the inverse of the relationship observed in other populations of *B. gracilis* from the Colorado Plateau (Butterfield 2015). The relationship between aridity and LMA in our study was driven by a few sites in northern Colorado and the differences among populations were not significant ( $p=0.159$ ). The discrepancies seen between the two studies may be due to local gradients in soil fertility, genetic isolation of populations or different precipitation regimes.

We did not find a correlation between LDMC and environmental variables among the populations of *B. gracilis* that we examined. These results are in agreement with recent research on the same species but in different locations (Butterfield 2015). LDMC has been correlated with leaf-level drought tolerance in C<sub>4</sub> grasses (Ocheltree et al. 2016) and so our results would suggest that the populations examined in this study did not differ in their ability to tolerate drought in leaf tissue. The failure of LDMC to predict total biomass in our study indicates that these population level differences may not be occurring as shifts along the leaf economic spectrum as seen in other species (Mason 2015) and may be overlooked in an approach utilizing strictly morphological traits.

## Implications

Our data suggests that populations that originate from different aridity climates use different strategies when responding to lowered soil moisture levels and this may reflect complex adaptation to unique environments. This raises concerns for land managers that may have to manage unique populations of *B. gracilis* as one management strategy may not have the same effect on *B. gracilis* from all climates. Future drought, variability of precipitation and variability of temperature may affect these populations very differently. If future temperature and precipitation regimes become decoupled from other physiological drivers like day length, northern populations may respond inappropriately to novel conditions and land managers may need to consider utilizing better adapted genetic material.

The different responses to soil moisture limitation seen in this study may relate to differences in precipitation regimes seen across our aridity gradient. Populations from



the arid sites receive most of their precipitation in a monsoonal pattern (Thomey et al. 2014). Individuals from the arid sites may be adapted to respond quickly to increases in soil moisture to capitalize on water during a shorter period of availability and may not operate during periods of low soil moisture. Because this season is short and comes annually, these plants may be adopting strategies that mimic those of the annuals found in arid ecosystems (Monson 1979)- namely a period of rapid growth with senescence to dormancy instead of death. These populations may have adapted to grow quickly in response to increased soil moisture and may have adapted other mechanisms such as summer dormancy or leaf senescence to avoid drought in the dry season instead of drought tolerance mechanisms to continue physiological activity during the dry season (Vico et al. 2014).

In contrast to the drought experienced by arid populations, the growing season of mesic populations of *B. gracilis* may be limited by cold temperatures and respond more strongly to day length or temperature than soil moisture. These plants may be exposed to freezing temperatures more frequently during periods of water availability than those from monsoonal climates and must maintain the ability to tolerate freezing temperatures in active tissue. The adjustment of  $\Psi_{osm}$  may then indicate the presence or expression of a suite of genes that are required for survival of cold tolerance but are also activated during drought (Lata and Prasad 2011). The short stature of plants from our northern populations may be an adaptation to cold as well; plants with lower stature may maintain a larger boundary layer to insulate plants from cold temperatures (Sage and Sage 2002).

*B. gracilis* from these populations may also have faced heavier grazing pressures than those in the south, especially during periods of continental drought (Flores et al. 1991) that may have had an effect on the biomass partitioning of these populations causing more mesic populations to generate mat-type formations as opposed to vertical height. One study indicated that certain genotypes of *B. gracilis* may spread faster laterally than others, but that this does not necessarily result in a decrease in height or above ground biomass (Samuel 1985). This suggests that mat formation is not necessarily related to our observed differences in height in this species. While in previous studies, *B. gracilis* with significant variation in morphology or genotype all responded similarly to simulated grazing (Kotanen and Bergelson 2000), these studies have not been done with such a wide range of genetic sources and the effect of grazing on these populations in a common garden is unknown.

This data suggests that local adaptation may be important to this species' success and these different strategies should be considered when making decisions regarding restoration or facilitated migration. Increased emphasis on biomass production from southern populations may be beneficial to ranchers but disruption of community dynamics as well as unknown differences in cold and grazing tolerance must be considered before introducing novel seed sources to managed lands. The contrasting responses to reduced soil moisture in *B. gracilis* indicates that populations are locally adapted and display unexpected differences in the plasticity of their drought tolerance traits.

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## CONCLUSION

The results of this study indicate significant differences in the response of different populations of *B. gracilis* to soil moisture levels. Biomass sensitivity to the change in soil moisture was significantly related to the aridity of the climate of origin. The observed biomass was highly negatively correlated with the osmotic response of each population and this response was the opposite of the expected response. Arid populations exhibited almost no osmotic adjustment yet displayed increased growth and lowered midday water potentials. Further research is necessary to explain the pattern of responses to soil moisture reduction seen in *B. gracilis*.

The importance of daylength on the development and phenology of *B. gracilis* was noted in several early common garden studies (Riegel 1944, McMillan 1956). These studies indicated that *B. gracilis* from the north and west populations flowered earlier than those from the south and the east. McMillan stated that a 15.5 hour day length was not long enough to induce flowering in southern populations of *B. gracilis*, but produced flowering in the northern populations. Given our 16 hour day length, it is possible that the northern populations in our study were adjusting phenologically to a more advanced stage of growth characterized by reproduction and the slowing of growth. Riegel noted that northern populations showed rapid early growth, slowed, and were overtaken by southern populations as the season progressed. These studies also noted that the flowering date of *B. gracilis* was sensitive to water availability and the interaction of day-length of origin and water relations is unexamined. Other variations in local adaptation of the optimal temperature of photosynthesis, diurnal patterning, root characteristics or nitrogen use efficiency may be important in understanding the

observed differences in population behavior. These characteristics may even be governed by day length and the phenology of the plant as Morgan et al. (1998) observed that both photosynthetic and water relations of *B. gracilis* are related to the ontogeny of the plant.

An expansion of the number of populations used would assist in identification of the mechanisms driving differences in  $\Psi_{\text{osm}}$  and  $\Psi_{\text{mid}}$ . Specifically, an examination of the relationship between xylem resistance to cavitation and climate of origin may explain the ability of arid populations to tolerate more negative values of  $\Psi_{\text{mid}}$ . Additionally, further study is required to examine the effect of field conditions and intense, sustained drought on these populations to determine if the physiology of the arid populations begins to resemble that of the mesic populations under more extreme water limitation. Field studies are also needed to examine the effect of ecotype on competition prior to large restoration efforts. Differences in local adaptation may yield unexpected results in local ecosystems. The generation of an ultra-competitive monoculture that is not frost resistant or a poorly competitive dominant species that allows for invasion would be highly detrimental to ecosystem functioning.

The prospect of future severe drought has renewed interest in the understanding of this drought tolerant and productive species that dominates so much of the shortgrass steppe. This body of research indicates that the intraspecific differences in *B. gracilis* are significant and may run contrary to our expectations. Because the performance of this species is so vital in the functioning of widespread ecosystems, it is critical that we further examine these responses.

## APPENDIX

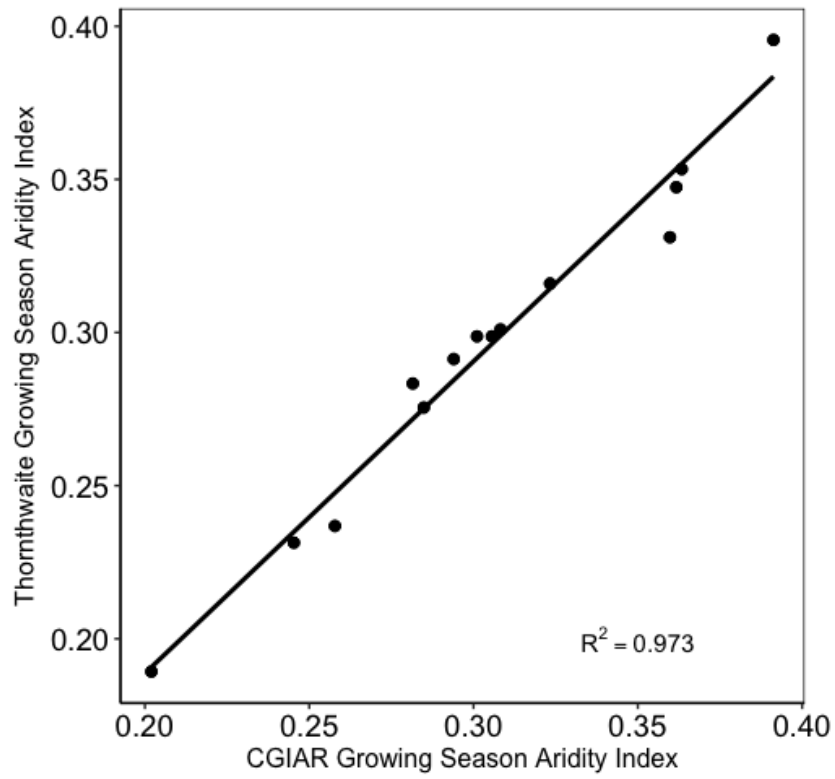


Figure S.1. The relationship between growing season aridity index calculated from the CGIAR-PET database and the growing season aridity index using the adjusted Thornthwaite method.



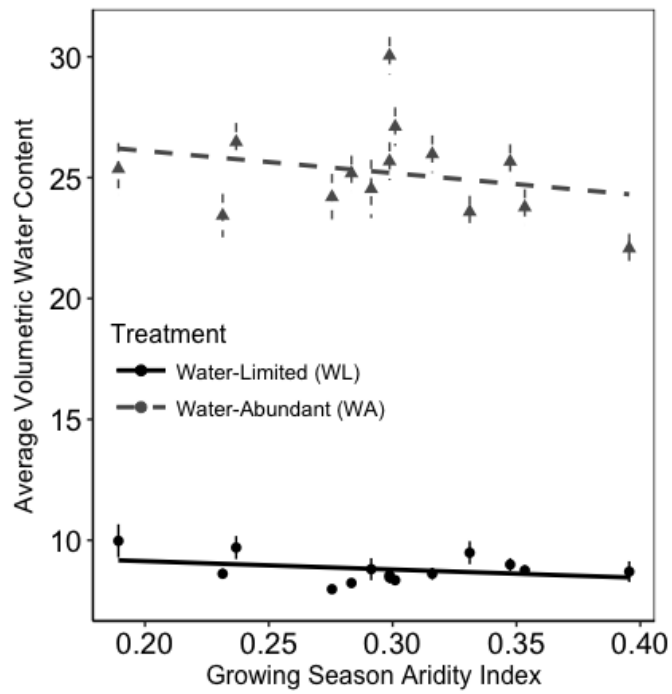


Figure S.3. Volumetric water content and Growing Season Aridity Index. Linear regressions illustrating non-significance are shown in Table 1.