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Effects of precipitation on photosynthesis and water potential in *Andropogon gerardii* and *Schizachyrium scoparium* in a southern mixed grass prairie

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ABSTRACT

In grassland ecosystems, spatial and temporal variability in precipitation is a key driver of species distributions and population dynamics. We experimentally manipulated precipitation to understand the physiological basis for differences in responses of species to water availability in a southern mixed grass prairie. We focused on the performance of two dominant C_4 grasses, *Andropogon gerardii* Vitman and *Schizachyrium scoparium* (Michx.) Nash, in treatments that received ambient rainfall, half of ambient rainfall ("drought" treatment), or approximately double ambient rainfall ("irrigated" treatment). Water potentials of *S. scoparium* were lower than *A. gerardii*, suggesting superior ability to adjust to water deficit in *S. scoparium*. Additionally, drought reduced photosynthesis to a greater extent in *A. gerardii* compared to *S. scoparium*. Leaf-level photosynthesis rates were similar in ambient and irrigated treatments, but were significantly lower in the drought treatment. Although stomatal conductance was reduced by drought, this was not limiting for photosynthesis. Leaf $\delta^{13}C$ values were decreased by drought, caused by an increase in C_i/C_a. Chlorophyll fluorescence measures indicated light-harvesting rates were highest in irrigated treatments, and were lower in ambient and drought treatments. Moreover, drought resulted in a greater proportion of absorbed photon energy being lost via thermal pathways. Reductions in photosynthesis came as a result of non-stomatal limitations in the C_4 cycle. Our results provide mechanistic support for the hypothesis that *S. scoparium* is more drought tolerant than *A. gerardii*.

1. Introduction

In the central grassland region of North America, regional variation in plant community composition is driven largely by precipitation (Hartnett and Fay, 1998; Lauenroth et al., 1999). Shortgrass steppe occupies the western, semi-arid portion of the Great Plains, while tallgrass prairie, the most productive vegetation type, occupies the eastern, mesic portion of the region. The mixed grass prairie, where tallgrass prairie species of the east intermingle with drought-tolerant shortgrass species of the west (Lauenroth et al., 1999), is intermediate in location, productivity, and precipitation.

Species-specific differences in drought tolerance illustrated by the west-to-east variation in species composition are reinforced by responses to temporal variation in precipitation within the mixed grass prairie. During the Great Drought of the 1930s, tallgrass species decreased in abundance much faster than shortgrass species and recovered more slowly, or persisted in swales but disappeared from ridge-tops (Albertson and Weaver, 1946; Albertson and Tomanek, 1965). More specifically, *Andropogon gerardii* Vitman (big bluestem) suffered much larger decreases in abundance than *Schizachyrium scoparium* (Michx.) Nash (little bluestem) (Weaver and Albertson, 1943).

The historical interpretation that *S. scoparium* is more drought tolerant than *A. gerardii* (Weaver and Fitzpatrick, 1932) has been confirmed by subsequent physiological measurements in field studies (e.g., Hake et al., 1984) and greenhouse studies (Heckathorn and DeLucia, 1995; Heckathorn et al., 1997). However, we lack

Abbreviations: C_i, internal (intercellular) concentration of CO_2 (ppm); C_i/C_a, ratio of intercellular to ambient CO_2 concentrations; D, proportion of absorbed photon energy lost to thermal dissipation; ETR, electron transport rate through PSII ($\mu mol\ m^{-2}\ s^{-1}$); F_0, minimal fluorescence from PSII following dark adaption; F_M, maximum fluorescence yield from PSII following saturating pulse of light in a dark-adapted plant; F_V, variable fluorescence = $F_M - F_0$; F_s, steady-state yield of PSII fluorescence in the light; F'_M, maximum fluorescence yield from PSII following a saturating pulse of light in a light-adapted plant; F'_0, minimum light-adjusted fluorescence after a brief dark period in a light-adapted plant; g_s, stomatal conductance to water vapor ($mol\ m^{-2}\ s^{-1}$); J_{O_2}, gross rate of O_2 evolution from PSII ($\mu mol\ m^{-2}\ s^{-1}$) calculated from fluorescence parameters; P, proportion of absorbed photon energy that was allocated to photochemistry; P_{max}, maximum rate of CO_2 fixation; PPFD, photosynthetic photon flux density (400–700 nm); PSII, photosystem II; q_P, photochemical quenching, a measure of the proportion of oxidized electron carriers in thylakoids; $\delta^{13}C$, ratio of $^{13}C/^{12}C$ in organic material relative to a standard (‰); θ, volumetric water content of soil ($m^3\ m^{-3}$); Φ_{PSII}, quantum yield of PSII calculated as $(F'_M - F_s)/F'_M$.

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an understanding of the physiological responses to drought in these dominant species. A mechanistic understanding of drought responses will be especially important if we hope to forecast effects of expected changes in precipitation and temperature on the abundance and distribution of dominant grassland species (Clark et al., 2001).

A number of distinct physiological processes can reduce photosynthesis rates during drought. Photosynthetic limitations during drought sometimes result from stomatal closure (Ruiz-Sánchez et al., 2007). Stomatal limitations on photosynthesis manifest themselves as a limitation of CO_2 availability, observable as a decrease in the internal (intercellular) concentration of CO_2, C_i (Farquhar and Sharkey, 1982). Photosynthesis in droughted C_3 plants is often limited by reduced stomatal conductance (Ripley et al., 2010). However, in C_4 species, which dominate the central grasslands of North America (Sage et al., 1999), a high affinity for inorganic carbon means C_i might not be reduced enough to decrease photosynthesis rates during times of decreased stomatal conductance (Lal and Edwards, 1996). Consequently, photosynthesis in droughted C_4 plants is normally reduced by non-stomatal limitations (Ripley et al., 2010).

One form of non-stomatal limitation in droughted C_4 grasses involves decreased activities of photosynthetic enzymes, which can occur in the C_3 cycle enzyme Rubisco (Soares-Cordeiro et al., 2009) or in the C_4 cycle enzymes PEP carboxylase (Soares-Cordeiro et al., 2009) or pyruvate P_i dikinase (Du et al., 1996). A second cause of decreased photosynthesis during drought is low foliar nitrogen content (Heckathorn et al., 1997). Since the majority of leaf nitrogen relates to photosynthesis (e.g., photosynthetic enzymes, chlorophyll, and light-harvesting complexes; Heckathorn et al., 1997), low foliar nitrogen can result in decreased carboxylation ability (Turner et al., 2008). The sensitivity of chloroplast light harvesting to environmental stress (Baker, 2008) suggests a third mechanism by which drought can limit photosynthesis. Photodamage is common in water-stressed plants (e.g., Resco et al., 2008; Mehta et al., 2010). An increase in absorbed photon energy, paired with a decrease in CO_2 fixation, results in a surplus of excitation energy that can be damaging for the photosynthetic apparatus in chloroplasts (Demmig-Adams et al., 1996). As a result, drought tolerance in plants might be influenced by an ability to dissipate absorbed energy safely before damage occurs in chloroplasts.

Standard gas exchange measures can distinguish between stomatal and non-stomatal limitations on photosynthesis in droughted plants. Whereas stomatal limitations are indicated by a decrease in C_i, non-stomatal limitations on photosynthesis are observable by unchanged or increased C_i (Farquhar and Sharkey, 1982), often resulting in higher C_i/C_a. Although the C_i/C_a ratio can be measured directly for short periods, leaf $\delta^{13}C$ provides an indirect measure of C_i/C_a that integrates over longer periods.

Carbon isotope ratios ($\delta^{13}C$) in C_3 plants have long been known to relate linearly with C_i/C_a (Farquhar et al., 1982), in turn dependent on g_s and photosynthesis rates. Carbon isotope discrimination occurs in different steps in a C_4 plant, owing to different metabolic pathways compared to C_3 plants (Farquhar, 1983). $\delta^{13}C$ in C_4 plants depends not only on C_i/C_a, but also on the proportion of CO_2 that leaks out of bundle sheath cells after having been pumped in by the C_4 cycle (Henderson et al., 1992). Thus, $\delta^{13}C$ values in C_4 plants vary based on changes in bundle sheath cell leakage or C_i/C_a.

If gas exchange measurements indicate stomatal limitation is not the cause of decreased photosynthesis, a second set of physiological measurements can provide inference about the important non-stomatal mechanisms. To detect reduced enzyme activity, extraction and assays of enzymes are necessary. Alternatively, inferences about photosynthetic carbon fixation can be made from measures of chlorophyll fluorescence (e.g., Edwards and Baker, 1993), where chloroplast light-harvesting processes relate very closely to CO_2 assimilation in C_4 leaves. To detect reduced foliar N content, one can either make direct measures of absolute N content (e.g., Kjeldahl digestion; Heckathorn et al., 1997), or perform relative measures of N from a C/N ratio by an elemental analyzer. Finally, any conditions that influence chloroplast electron transport or PSII should be discernable with chlorophyll fluorescence measures (Baker, 2008). An increase in minimal dark-adapted fluorescence (F_0) or a decrease in maximal dark-adapted fluorescence (F_M) would indicate an inability to transport electrons beyond PSII, and potential damage to the reaction center complex (Misra et al., 2001).

Although effects of drought have been well studied in C_3 plants (e.g., Lawlor, 2002; Flexas et al., 2006), much remains unknown with respect to droughted C_4 plants (Ghannoum, 2009). We experimentally manipulated precipitation in a southern mixed grass prairie to investigate mechanisms of drought tolerance in two dominant C_4 grass species, *A. gerardii* (big bluestem) and *S. scoparium* (little bluestem). Based on previous comparisons of drought tolerance (e.g., Heckathorn and DeLucia, 1995; Heckathorn et al., 1997), we hypothesized *S. scoparium* would be better able to maintain photosynthesis under water stress compared to *A. gerardii* and that drought-induced effects on photosynthesis in these species would be non-stomatal, typical of C_4 plants (Ripley et al., 2010). To test this hypothesis, we distinguished both between stomatal and non-stomatal limitations on photosynthesis and among three mechanisms that might explain this difference in drought tolerance: (1) higher stomatal conductance under drought, (2) an increased ability to dissipate excess light energy, and (3) an increased ability to lower water potential. We evaluated these mechanisms based on differences in plant photosynthetic rates, as well as plant water potential and leaf $\delta^{13}C$.

2. Materials and methods

2.1. Study site and experimental conditions

The study site was southern mixed grass prairie, on an upland site dominated by *A. gerardii*, *S. scoparium*, *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama), and *Bouteloua hirsuta* Lag. (hairy grama) (Albertson and Tomanek, 1965; Adler et al., 2006). Soil was a silty clay loam (Albertson, 1937) over limestone bedrock (Albertson and Tomanek, 1965).

Hays, KS, USA is near the dividing line between semi-arid and dry subhumid climates (Lauenroth et al., 1999). Average rainfall in Hays is 580 mm yr^{-1}, and about 80% of this precipitation occurs from April through September (Adler and HilleRisLambers, 2008). During 2009, 594 mm of rain were recorded. In the present study, experimental treatments were established to manipulate rainfall totals. Nine 2 m × 8 m plots were established on a prairie hillside 3.5 km west of Hays (38.9°N, 99.4°W). There were three replicate plots of each of three precipitation treatments. Three plots received ambient rainfall, three plots were irrigated once weekly to approximately double ambient rainfall (ambient plus average rainfall), and three plots were covered with rainout shelters to reduce ambient rainfall by 50% (Adler et al., 2009). Volumetric water content of soil (θ, $m^3\ m^{-3}$) was measured in representative plots with EC-5 soil moisture sensors (Decagon Devices, Inc., Pullman, WA, USA) at 5 cm depth. Ambient rainfall measures are from a CoCoRaHS station (www.cocorahs.org) ca. 2.4 km northwest of the site.

2.2. Photosynthesis measures

Measures of photosynthesis, chlorophyll fluorescence, and water potential were performed between the hours of 10:00 and 15:00 on sunny days during June and July 2009. Photo-

synthesis rates were measured on young, fully expanded leaves in field plants using an LI-6400 open IRGA system (Li-Cor Biosciences, Inc., Lincoln, NE, USA) in differential mode. Fluorescence light curves were constructed for individual *A. gerardii* and *S. scoparium* plants, including measures of CO_2 fixation rates and light-adapted chlorophyll fluorescence at irradiances (PPFD) from 0 to 2000 μmol quanta m^{-2} s^{-1}. Gas flow through the leaf chamber was 400 μmol s^{-1}, CO_2 concentration was maintained at 385 μmol mol^{-1}, and temperature and relative humidity were maintained near ambient levels. Net photosynthesis was measured at each irradiance, as was stomatal conductance to water vapor (g_s), intercellular (substomatal) CO_2 concentration (C_i), steady-state fluorescence (F_s), maximum light-adjusted fluorescence after a saturating pulse of light (F'_M), and minimum light-adjusted fluorescence after a brief dark period (F'_0). Chlorophyll fluorescence parameters allowed calculation of many processes involved in light harvesting and photochemistry. The quantum yield of PSII (Φ_{PSII}) was calculated after Genty et al. (1989) at each irradiance as $(F'_M - F_s)/F'_M$. Φ_{PSII} was multiplied by the PPFD, then by 0.84 (amount of incident radiation absorbed by the leaf, Björkman and Demmig, 1987), then by 0.5 (assuming half of photons are absorbed by PSII), giving the electron transport rate (ETR) per leaf area. ETR was divided by 4 (4 electrons transported per O_2 evolved) to arrive at a measure of the gross photosynthesis rate (J_{O_2}) (μmol O_2 evolved m^{-2} s^{-1}) (Krall and Edwards, 1992). Photochemical quenching (q_P), a measure of the proportion of oxidized electron carriers in thylakoids, was calculated after Hichem et al. (2009) as:

$$q_P = \frac{F'_M - F_s}{F'_M - F'_0} \quad (1)$$

Several parameters were obtained from fluorescent light curves. The maximum measured rate of CO_2 fixation was taken as P_{max}. Initial slopes of light response curves (i.e., efficiency under limiting light) were taken as the quantum efficiency of CO_2 fixation.

The proportion of absorbed photon energy allocated to photochemistry (P) or thermal dissipation (D) was calculated after Demmig-Adams et al. (1996) as:

$$P = \frac{F'_M - F_s}{F'_M} \times q_P \quad (2)$$

$$D = 1 - \frac{F'_M - F_s}{F'_M} \quad (3)$$

$$X = \frac{F'_M - F_s}{F'_M} \times (1 - q_P) \quad (4)$$

where X is the proportion of absorbed radiant energy in excess of P and D (Demmig-Adams et al., 1996).

2.3. Water potential measures

Mid-day water potentials were measured on plant tillers in the study with a Scholander pressure chamber (Scholander et al., 1965) (model 1000; PMS Instrument Company, Albany, OR, USA).

2.4. Stable isotope measures

Entire leaves were sampled from all individuals following photosynthesis and water potential measures. Leaf carbon isotope ratios ($\delta^{13}C$) were determined relative to VPDB (Vienna Pee Dee Belemnite) (Ehleringer and Osmond, 1989). Leaves were dried at 60 °C for 24 h, and were milled to powder in a Wiley Mill (model 3383-L10; Thomas Scientific; Swedesboro, NJ, USA). 1.0 mg samples (±0.1 mg) were placed in tin capsules and were later combusted in a Eurovector elemental analyzer. The resulting CO_2 gas entered the inlet of a Micromass Isoprime isotope ratio mass spectrometer for determination of $^{13}C/^{12}C$ ratios (R). $\delta^{13}C$ values were determined as:

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (5)$$

Routine precision for the instrument was ±0.002‰ for $\delta^{13}C$, calculated as the standard deviation of three duplicate standards. Use of the elemental analyzer also provided C/N ratios in leaf material.

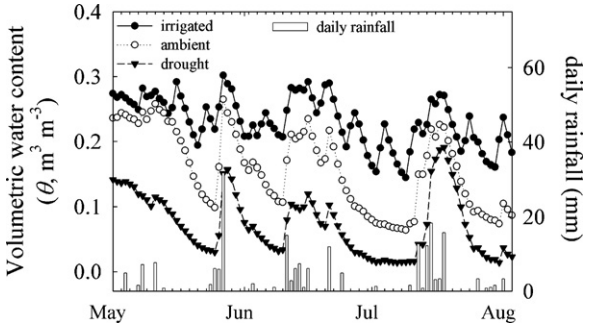

Fig. 1. Volumetric water content (θ; left axis, in m^3 m^{-3}) of representative plots in the study. Filled circles are measurements from an irrigated plot receiving approximately double the ambient rainfall. Open circles are from a plot receiving ambient rainfall. Filled triangles are measurements from a droughted plot receiving half the ambient rainfall. Histogram bars overlaid on the plot show daily ambient rainfall totals (right axis, in mm) during the study.

2.5. Data analysis

Data were analyzed using analysis of variance (ANOVA), with species, treatments, and months as factors. Data from fluorescent light curves were analyzed with repeated measures analysis of variance (ANOVAR), with species, treatments, and months as factors, and PPFD as the repeated effect (StatView 5.0, 1998 SAS Institute, Inc., Cary, North Carolina, USA). Post-hoc comparisons of means were performed with Fisher's protected least significant difference. All analyses were performed at $\alpha = 0.05$.

3. Results

3.1. Precipitation treatments and soil moisture

Volumetric water content (θ) of soil in irrigated treatments ranged from 0.145 to 0.304 m^3 m^{-3} (Fig. 1). θ in ambient treatments ranged from 0.060 to 0.265 m^3 m^{-3}, and θ in drought treatments ranged from 0.006 to 0.240 m^3 m^{-3} (Fig. 1). There was no overlap of θ between treatments. θ fluctuated with rain events, with increases following precipitation. θ in ambient treatments appeared to have the largest fluctuations between precipitation inputs.

3.2. Water potential measures

Mid-day water potentials of plants ranged from −0.43 MPa in irrigated *A. gerardii* during June to −1.92 MPa in droughted *S. scoparium* during July (Fig. 2). Plants growing in droughted plots had significantly lower water potentials compared to ambient or irrigated treatments (ANOVA, $p < 0.0001$). Mid-day water potentials in irrigated plants were not different from plants in ambient treatments (ANOVA, $p = 0.918$). Additionally, plant water potentials were significantly lower in July compared to June (ANOVA, $p = 0.0004$). Water potentials in *S. scoparium* were significantly lower than in *A. gerardii* (ANOVA, $p = 0.0002$).

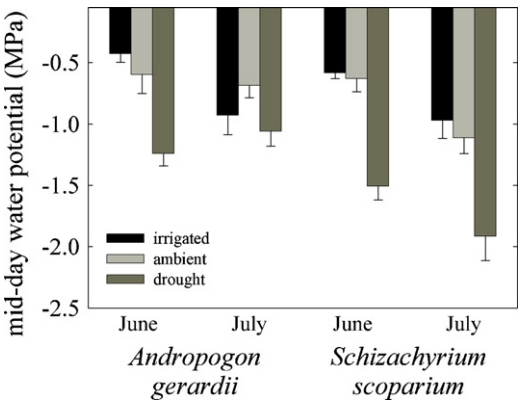

Fig. 2. Mid-day water potentials of plants (MPa) during June and July 2009. Bars are grouped by species and months, and are arranged by treatment. Bars are means of 20–22 replicates ± SE.

3.3. Gas exchange measures

Net rates of CO_2 fixation followed a hyperbolic relationship with respect to irradiance. Photosynthesis rates saturated at a PPFD near 1000 μmol m^{-2} s^{-1} in June, but saturated near a PPFD of 500 μmol m^{-2} s^{-1} in July (Figs. 3 and 4). Net photosynthesis rates were not different between species (ANOVAR, $p = 0.409$), but were significantly lower in July compared to June (ANOVAR, $p = 0.0280$). Maximum rates of photosynthesis (P_{max}) were as high as 18.8 μmol CO_2 m^{-2} s^{-1} in June and as low as 3.9 μmol CO_2 m^{-2} s^{-1} in July (Figs. 3 and 4). Precipitation had a much greater influence on photosynthesis measures compared to species differences. Plants in ambient and irrigated treatments had significantly higher photosynthesis rates compared to plants in drought treatments (ANOVAR, $p < 0.0001$). A significant treatment × month interaction ($p < 0.0001$) indicated lower rates of photosynthesis in drought treatments in June (Fig. 3), but this difference became less in July (Fig. 4).

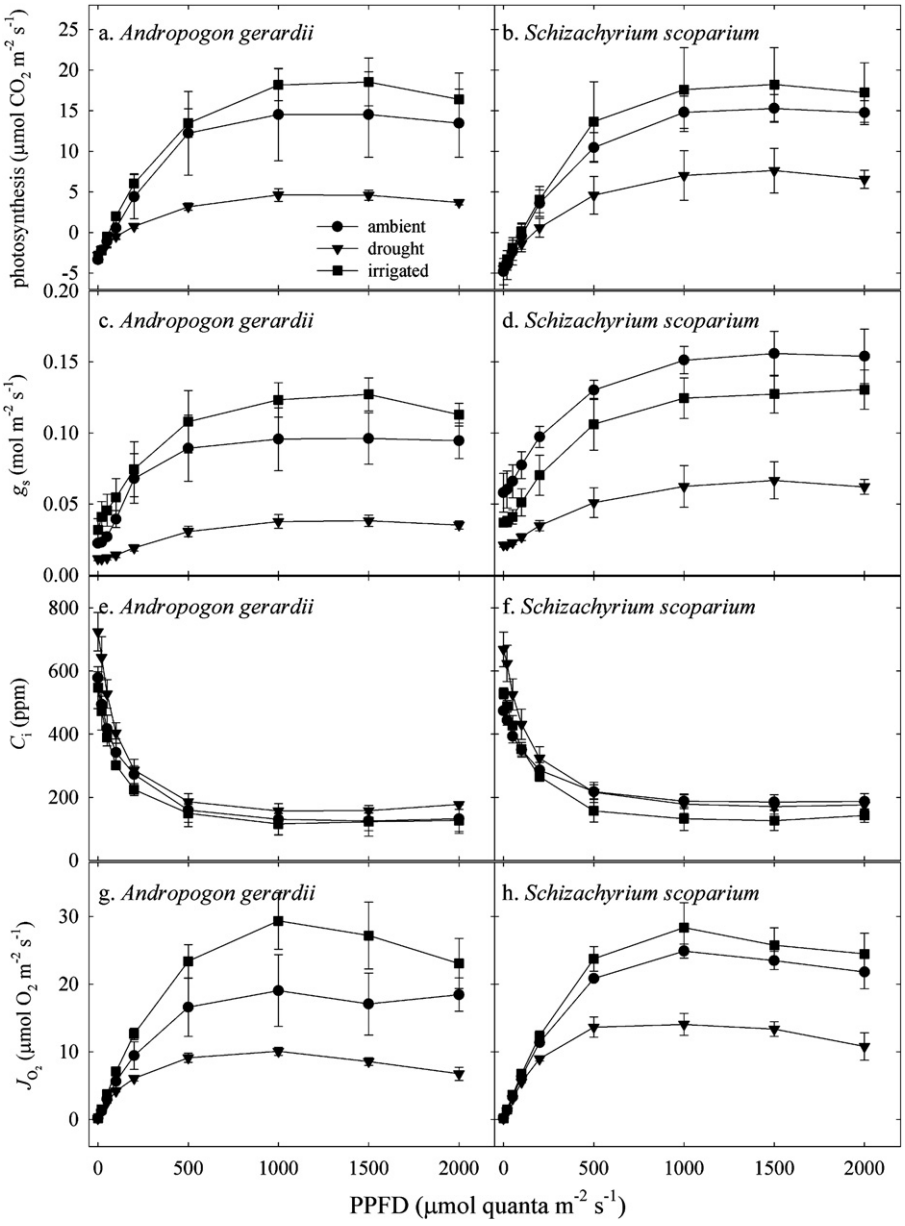

Fig. 3. Photosynthesis rates, stomatal conductance (g_s), intercellular CO_2 concentrations (C_i), and gross rates of O_2 evolution (J_{O_2}, calculated from chlorophyll fluorescence data) collected during June 2009. Circles represent ambient rainfall, triangles are drought treatments, and squares represent irrigated treatments. Points are means of 3–4 replicates ± SE.

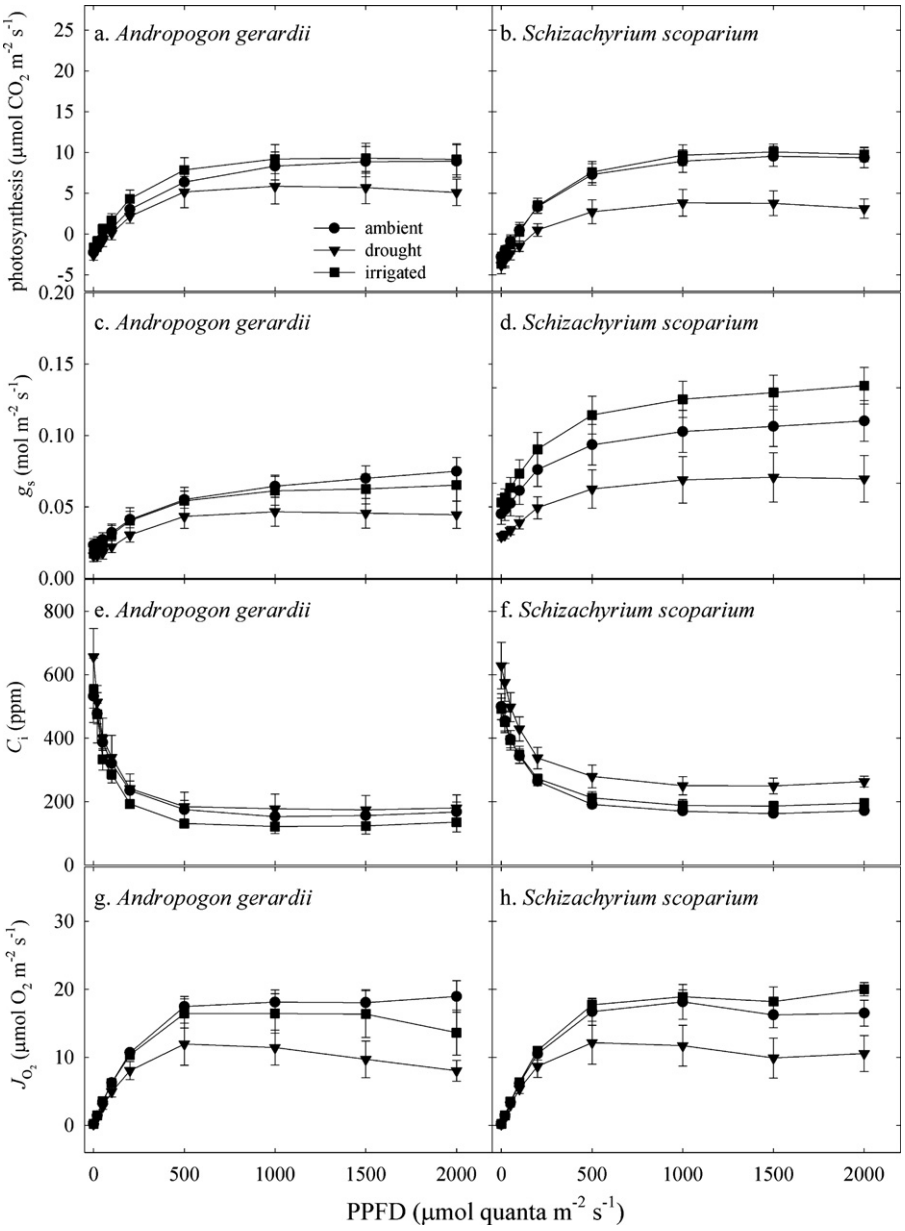

Fig. 4. Photosynthesis rates, g_s, C_i, and J_{O_2} collected during July 2009. Points are labeled as in Fig. 3 and are means of 3–6 replicates ± SE.

Stomatal conductance to water vapor (g_s) had similar patterns to net photosynthesis rates. At high PPFD, g_s ranged from 0.035 to 0.154 mol m^{-2} s^{-1} (Figs. 3 and 4). There were significant effects of species, month, and treatment. g_s in *S. scoparium* was significantly greater than in *A. gerardii* (ANOVAR, $p < 0.0001$). g_s was also significantly greater in irrigated and ambient treatments compared to drought treatments (ANOVAR, $p < 0.0001$). Moreover, g_s in June was significantly greater than in July (ANOVAR, $p < 0.0001$).

Intercellular CO_2 concentrations (C_i) decreased with increasing irradiance. At high PPFD, C_i ranged from 127 to 263 μmol mol^{-1} (Figs. 3 and 4). C_i was not different between species or months (ANOVAR, $p \geq 0.142$). C_i was significantly higher in the drought treatment compared to ambient or irrigated treatments (ANOVAR, $p < 0.0001$).

Net quantum efficiency of CO_2 fixation, measured under limiting light, ranged from 0.025 to 0.051 CO_2 $photon^{-1}$ across species and treatments (Fig. 5). There were no significant differences between species, treatments, or months (ANOVA, $p \geq 0.079$). There was a significant treatment × month interaction ($p = 0.010$), indicating a changing influence of month on net quantum efficiencies.

3.4. Carbon isotope values

Leaf $\delta^{13}C$ ranged from −13.5‰ in droughted *S. scoparium* to −12.1‰ in irrigated *A. gerardii* (Fig. 6). There was no significant difference in $\delta^{13}C$ between months (ANOVA, $p = 0.126$). $\delta^{13}C$ was significantly higher in *A. gerardii* compared to *S. scoparium* (ANOVA, $p < 0.0001$), and $\delta^{13}C$ became significantly lower with decreasing precipitation. Leaf $\delta^{13}C$ was highest in irrigated treatments, which were significantly higher than in ambient plots, in turn significantly higher than in drought treatments (ANOVA, $p \leq 0.0116$).

Leaf C/N ratios ranged from 29.4 in droughted *A. gerardii* during June to 45.8 in ambient-treatment *S. scoparium* during July (Fig. 7). There were no significant differences between

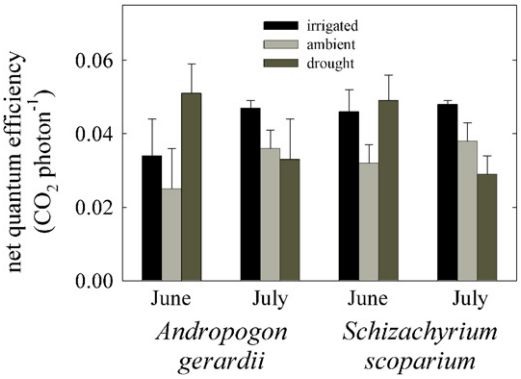

Fig. 5. Net quantum efficiency of CO_2 fixation (CO_2 photon^{-1}), as calculated from CO_2 fixation under limiting light. Bars are means of 3–6 replicates ± SE.

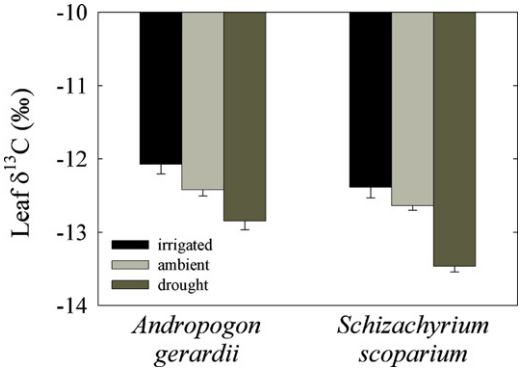

Fig. 6. Leaf carbon isotope ratios ($\delta^{13}C$, in ‰) of plants during the study. Since values for June and July were not significantly different, they have been grouped. Bars are means of 7–9 replicates ± SE.

species or treatments (ANOVA, $p \geq 0.096$), but leaf C/N ratios were significantly higher in July compared to June (ANOVA, $p < 0.0001$).

3.5. Chlorophyll fluorescence measures

Rates of gross O_2 evolution (J_{O_2}), calculated from thylakoid electron transport measures from chlorophyll fluorescence, were higher than net rates of CO_2 fixation and showed similar patterns with relation to irradiance and treatments. Maximum J_{O_2} ranged from 10.4 to 29.6 μmol O_2 m^{-2} s^{-1} across species and treatments (Figs. 3 and 4). Treatment had a much greater effect on chlorophyll fluorescence parameters than differences between species. J_{O_2} was not different between species or months (ANOVAR, $p \geq 0.169$). Decreasing precipitation decreased J_{O_2} (Figs. 3 and 4). J_{O_2} in drought treatments was significantly lower than irrigated and ambient treatments, which were not different from one another (ANOVAR, $p < 0.0001$).

Steady-state fluorescence in the light (F_s) was as high as 643 units in irrigated *A. gerardii* during June and as low as 446 units in droughted *A. gerardii* during June (Supplementary Figs. 1 and 2). There was a significant reduction of F_s with increasing light (ANOVAR, $p < 0.0001$). F_s was not different between species (ANOVAR, $p = 0.831$), but F_s was significantly reduced by drought. F_s in the irrigated treatment was significantly higher than in the ambient treatment, which was in turn significantly higher than in the drought treatment (ANOVAR, $p \leq 0.0106$). Additionally, F_s was significantly higher in June compared to July (ANOVAR, $p < 0.0001$).

Maximum fluorescence in the light (F'_M) ranged from 2301 units in irrigated *A. gerardii* during June to 476 units in droughted *A. gerardii* during July (Supplementary Figs. 1 and 2). F'_M was strongly reduced with increasing light (ANOVAR, $p < 0.0001$). F'_M was not different between species (ANOVAR, $p = 0.662$), but F'_M was significantly reduced by drought. F'_M in ambient and irrigated treatments was significantly higher than in the drought treatment (ANOVAR, $p < 0.0001$). Moreover, F'_M was significantly higher in June compared to July (ANOVAR, $p = 0.0287$).

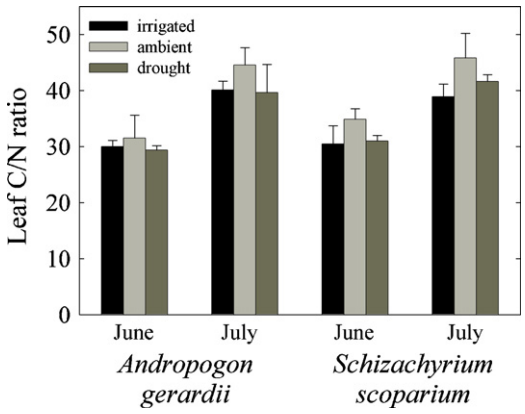

Fig. 7. Leaf C/N ratios of plants during June and July 2009. Bars are grouped by species and months, and are arranged by treatment. Bars are means of 3–6 replicates ± SE.

Minimum fluorescence in the light (F'_0) was as high as 569 units in irrigated *A. gerardii* during June and as low as 378 units in droughted *S. scoparium* during July (Supplementary Figs. 1 and 2). F'_0 was reduced with increasing light (ANOVAR, $p < 0.0001$), but F'_0 was not different between species (ANOVAR, $p = 0.972$). F'_0 was significantly reduced by drought. F'_0 in the irrigated treatment was significantly higher than in the ambient treatment, which in turn was significantly higher than in the drought treatment (ANOVAR, $p \leq 0.0177$). F'_0 was significantly higher in June compared to July (ANOVAR, $p < 0.0001$).

Photochemical quenching (q_P), a measure of the proportion of oxidized electron carriers in thylakoids, was as high as 1.015 in the dark, but was reduced to 0.180 under high light (Supplementary Figs. 1 and 2). q_P was not different between species (ANOVAR, $p = 0.481$), but q_P was significantly reduced by drought. q_P in ambient and irrigated treatments was significantly higher than in the drought treatment (ANOVAR, $p \leq 0.0043$). Moreover, q_P was significantly higher in June compared to July (ANOVAR, $p = 0.0269$).

The proportion of absorbed energy that was allocated to photochemistry (P) ranged from 0.012 to 0.747 (i.e., from 1.2 to 74.7%) across species and treatments (Supplementary Figs. 3 and 4). P was reduced by increasing PPFD (ANOVAR, $p < 0.0001$), but P was not different between species or months (ANOVAR, $p \geq 0.224$). P was significantly reduced by drought. P in ambient and irrigated treatments was significantly higher than in the drought treatment (ANOVAR, $p \leq 0.0084$).

The proportion of absorbed energy that was lost as heat (D) ranged from 0.264 to 0.968 (i.e., from 26.4 to 96.8%) across species and treatments (Supplementary Figs. 3 and 4). D increased with increasing PPFD (ANOVAR, $p < 0.0001$), but D was not different between species or months (ANOVAR, $p \geq 0.184$). D was significantly increased by drought. D in the drought treatment was significantly higher than in ambient and irrigated treatments (ANOVAR, $p \leq 0.0049$).

The proportion of absorbed energy in excess of P or D (X) ranged from −0.005 to 0.083 (i.e., from −0.5 to 8.3%) across species and treatments (data not shown). X was not different between species, treatments, or months (ANOVAR, $p \geq 0.333$).

4. Discussion

4.1. Drought tolerance in A. gerardii and S. scoparium

S. scoparium has been regarded as more drought tolerant than *A. gerardii* (Weaver and Fitzpatrick, 1932; Heckathorn and DeLucia, 1995; Heckathorn et al., 1997). Many of our results support this conclusion. Experimental drought reduced photosynthesis to a greater extent in *A. gerardii* compared to *S. scoparium*, and *S. scoparium* was able to maintain higher P_{max} and higher g_s under drought compared to *A. gerardii*. Similarly, water potentials of *S. scoparium* were lower than *A. gerardii*, suggesting superior osmotic adjustment in *S. scoparium*. However, chlorophyll fluorescence analysis indicates both species were able to resist photoinhibition.

Most photosynthesis and gas exchange parameters decreased in July compared to June in both *A. gerardii* and *S. scoparium*. Drier soil conditions and higher vapor pressure deficit led to increased evaporative stress on the plants, similar to measures from a tallgrass prairie by Hake et al. (1984). The dominant C_4 grasses in Kansas have been shown to take up most of their water from the top 30 cm of soil (Nippert and Knapp, 2007a). Thus, performance of these shallow-rooted grasses would be expected to be closely related to rainfall conditions.

Photosynthesis rates in plants were closely related to plant water potential. As expected, plant water potentials in the present study were lower than previously published values from grasses in tallgrass prairie settings. Mid-day plant water potentials from plants in irrigated treatments were similar to results from tallgrass prairie settings presented by Knapp et al. (1998) and Nippert et al. (2009) for *A. gerardii* and Nippert and Knapp (2007b) for *A. gerardii* and *S. scoparium*. By contrast, water potentials for plants in our study were slightly higher than droughted field *A. gerardii* and *S. scoparium* plants measured by Hake et al. (1984). Decreasing water status in plants is commonly linked to decreased photosynthesis and decreased production (Lawlor, 2002; Ghannoum, 2009), consistent with leaf- and plant-level measures in the present study. The significant decrease in plant water potential from June to July is consistent with decreasing soil moisture amounts, and similar to seasonal trends observed by Hake et al. (1984).

4.2. Effects of drought on carbon fixation and stomatal conductance

In this study, a decrease in photosynthesis was closely related to soil moisture in both *A. gerardii* and *S. scoparium* (Figs. 3 and 4), a good indication of short-term water stress (Resco et al., 2008). Our results are consistent with previous work in tallgrass prairie settings. Specifically, photosynthesis and stomatal conductance (g_s) in ambient-treatment grasses in the present study are similar to the tallgrass prairie drought treatments of Nippert et al. (2009) and lower than tallgrass irrigated treatments of Swemmer et al. (2006). Moreover, measures in irrigated treatments in the present study are similar to previous measures of plants under ambient treatments in tallgrass prairie settings (McAllister et al., 1998; Nippert et al., 2009).

4.3. Non-stomatal limitations on photosynthesis

Decreasing water supply caused a decrease in photosynthesis and g_s in both *A. gerardii* and *S. scoparium*, whereas decreasing water led to an increase in C_i (Figs. 3 and 4). Increased C_i indicates stomata were not limiting for photosynthesis. Rather, decreased rates of photosynthesis were caused by non-stomatal limitations (Farquhar and Sharkey, 1982). Early stages of drought induce stomatal closure in plants (Lawlor, 2002), but photosynthesis in droughted C_4 plants is more commonly limited by non-stomatal factors (Ripley et al., 2010). Non-stomatal limitations on C_4 photosynthesis can result from a decrease in Rubisco activity (Carmo-Silva et al., 2007; Soares-Cordeiro et al., 2009), from limitations imposed by C_4 cycle enzymes like PEP carboxylase or pyruvate P_i dikinase (Du et al., 1996; Soares-Cordeiro et al., 2009), from decreased leaf nitrogen content (Ghannoum, 2009), or increased photodamage to light-harvesting machinery (Demmig-Adams et al., 1996). Non-stomatal limitations result in higher C_i/C_a, which can be discerned with leaf $\delta^{13}C$.

Further evidence for the importance of non-stomatal limitations comes from decreases of leaf $\delta^{13}C$ under drought in both *A. gerardii* and *S. scoparium* (Fig. 6). Decreases in $\delta^{13}C$ can result from increases in leakiness of CO_2 from bundle sheath cells (Farquhar, 1983) or from increases in C_i/C_a (if bundle sheath leakage is >0.32; Sandquist and Ehleringer, 1995). Increases in bundle sheath cell leakiness will cause a decrease in the quantum efficiency of CO_2 fixation since extra light energy will be needed to recycle leaked CO_2 (Farquhar, 1983). Quantum efficiency was not different between treatments (Fig. 5). Instead, C_i/C_a increased with drought in both *A. gerardii* and *S. scoparium* (Figs. 3 and 4), indicating the decrease in $\delta^{13}C$ was a result of increasing C_i/C_a, consistent with previous work in some drought-affected C_4 species (Fravolini et al., 2002).

Unchanged bundle sheath cell leakiness in these plants suggests Rubisco activity was not limiting for photosynthesis under drought. Bundle sheath leakiness is an indication of how much C_4 cycle activity exceeds Rubisco activity (Henderson et al., 1998). A review of C_4 plants under drought indicated water stress commonly reduced C_3 cycle enzymes to a greater extent than C_4 cycle enzymes (Ghannoum, 2009). Our results indicate a high level of bundle sheath cell leakage in these grasses, but this value did not change with drought. Instead, drought caused an increase in C_i/C_a in *A. gerardii* and *S. scoparium*. Increasing C_i values, paired with unchanged bundle sheath cell leakage, indicate a decrease in carboxylation capacity, specifically a decrease in effectiveness of the C_4 cycle. The high bundle sheath leakage indicated by $\delta^{13}C$ values would necessitate high levels of internal cycling of CO_2 by the C_4 cycle. Perhaps additional cycling of CO_2 by the C_4 cycle could be a mechanism for dissipating excess light energy under water stress.

In some cases, drought is accompanied by lower leaf N content in C_4 plants (Ghannoum, 2009), but we found no treatment effect on leaf C/N ratios (Fig. 7). Most nitrogen in leaves is in the form of photosynthetic enzymes, chlorophyll, and light-harvesting complexes (Heckathorn et al., 1997), which all relate to photosynthesis. Consequently, decreased foliar N results in a decreased ability for mesophyll cells to assimilate CO_2 (Turner et al., 2008). Both *A. gerardii* and *S. scoparium* had increased C/N ratios in July compared to June. However, there was no difference in leaf $\delta^{13}C$ between months (Fig. 6). Therefore, the increase in C_i was not caused by low levels of leaf N. The affinity of C_4 plants for inorganic carbon is apparently high enough to overcome any effects of decreased N assimilation.

Differences in drought tolerance between *A. gerardii* and *S. scoparium* do not result from differences in photochemistry or excess energy dissipation. Chlorophyll fluorescence measures can be used to assess environmental stress on light-harvesting processes in chloroplasts (Baker, 2008). In the present study, there were no differences between species in any parameter derived from chlorophyll fluorescence measures. By contrast, drought had a strong effect on chlorophyll fluorescence parameters. Gross rates of photosynthesis (J_{O_2}), measured by chlorophyll fluorescence analysis, were reduced by drought in the present study. J_{O_2} was higher than net rates of CO_2 fixation (Figs. 3 and 4). This was expected owing to respiratory losses of CO_2 and electron sinks other than CO_2 reduction (e.g., nitrate assimilation or Mehler reaction). Similarly, all light-adjusted fluorescence parameters (F_s, F'_M, F'_0, and q_P) decreased with drought (Supplementary Figs. 1 and 2). Decreases in chlorophyll fluorescence parameters can indicate damage to PSII or

down-regulation of light-harvesting machinery (Krause and Weis, 1991). Moreover, F_s, F'_M, F'_0, and q_P were significantly higher in June compared to July. Drier soils in July or normal seasonal maturation of these grasses could lead to increased photoprotection. Such changes can be expected when large thylakoid proton gradients are established (Crofts and Horton, 1991), e.g., during environmental conditions where CO_2 fixation is decreased relative to light harvesting, as might be expected in droughted plants.

The proportion of absorbed light energy lost by thermal dissipation (D) increased with drought (Supplementary Figs. 3 and 4), similar to results presented by Hichem et al. (2009) for *Zea mays*. Increases in D offset decreases of light energy allocated to photochemistry (P), suggesting thermal dissipation becomes important as a photoprotection mechanism during drought in *A. gerardii* and *S. scoparium*. Photodamage is common in water-stressed plants (e.g., Resco et al., 2008; Mehta et al., 2010). An increase in absorbed photon energy, paired with a decrease in CO_2 fixation, results in a surplus of excitation energy that can be damaging for the photosynthetic apparatus in chloroplasts (Demmig-Adams et al., 1996). As a result, drought tolerance in plants might be influenced by an ability to dissipate absorbed energy safely before damage occurs in chloroplasts. Both *A. gerardii* and *S. scoparium* responded to drought with down-regulation of photochemistry to avoid damage to the photosynthetic machinery. J_{O_2} that is higher than net rates of CO_2 fixation indicates photodamage was not limiting for photosynthesis under drought.

5. Conclusions

This study is the first to report physiological measures of plant responses to precipitation treatments in a mixed grass prairie. *S. scoparium* was more drought tolerant than *A. gerardii* owing to lower limitations on photosynthesis and a greater ability to lower its water potential. Even though stomatal conductance was reduced with drought, g_s was not limiting for photosynthesis. We were also able to rule out limitations caused by C_3 cycle activity, leaf N content, or increased photodamage, indicating that limitations on photosynthesis in droughted *A. gerardii* and *S. scoparium* came from non-stomatal limitations in the C_4 cycle. The specific enzymes involved will be an interesting area for future research. Tolerant isozymes or activation states in C_4 enzymes (e.g., PEP carboxylase or pyruvate P_i dikinase) might be important in determining shifts of species in relation to water availability. Drought exerts physiological effects on C_4 grasses through changes in the C_4 cycle. Much of the central plains of North America is dominated by C_4 grasses, and many models forecast changes in precipitation. With expected increases in aridity due to global climate change (Ghannoum, 2009), a mechanistic understanding of the drought responses of dominant prairie grasses is clearly important.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2011.03.011.

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