

## RESEARCH ARTICLE

# Save or spend? Diverging water-use strategies of grasses and encroaching clonal shrubs

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## Abstract

1. Shrub encroachment is one of the primary threats to mesic grasslands around the world. This dramatic shift in plant cover has the potential to alter ecosystem-scale water budgets and responses to novel rainfall regimes. Understanding divergent water-use strategies among encroaching shrubs and the grasses they replace is critical for predicting shifts in ecosystem-scale water dynamics as a result of shrub encroachment, particularly if drought events become more frequent and/or severe in the future.
2. In this study, we assessed how water-use traits of a rapidly encroaching clonal shrub (*Cornus drummondii*) and a dominant C<sub>4</sub> grass (*Andropogon gerardii*) impact responses to changes in water availability in tallgrass prairie. We assessed intra-annual change in depth of water uptake, turgor loss point and stomatal regulation in each species. Sampling took place at Konza Prairie Biological Station (north-eastern KS, USA) during the 2021 and 2022 growing seasons.
3. *Cornus drummondii* shifted from shallow to deep soil water sources across the 2021 and 2022 growing seasons. This plasticity in depth of water uptake facilitated a 'wasteful' water-use strategy in *C. drummondii*, where stomatal conductance and transpiration rates continued to increase even when no further gain in photosynthetic rate occurred. *A. gerardii* photosynthetic rates and stomatal conductance were more variable through time and were more responsive to changes in leaf water potential than *C. drummondii*. However, intra-annual adjustment of turgor loss point was more pronounced in *C. drummondii* ( $\Delta\pi_{\text{LP}} = -0.48 \text{ MPa} \pm 0.15 \text{ SD}$ ) than in *A. gerardii* ( $\Delta\pi_{\text{LP}} = -0.29 \text{ MPa} \pm 0.19 \text{ SD}$ ).
4. *Synthesis*. These results suggest that *C. drummondii* is highly resilient to changes in water availability in surface soils and will likely remain unaffected by future droughts unless they are severe enough to reduce the availability of deep soil water. Given that clonal shrubs are key invaders of grasslands world-wide, increased leaf-level water loss is expected to accelerate ecosystem-level drying as clonal shrub encroachment proceeds in mesic grasslands.

## KEYWORDS

ecophysiology, grassland ecohydrology, plant water stress, shrub encroachment, stable isotopes, tallgrass prairie, woody encroachment

## 1 | INTRODUCTION

Coexistence between grasses and woody species is a hallmark of grassland and savanna ecosystems around the world (Bond, 2019). Complex feedbacks including disturbance and herbivory typically keep these systems in a 'stable' state, limiting the spread of woody vegetation and maintaining grass dominance (Holdo & Nippert, 2022; Ratajczak et al., 2011; Ratajczak, Nippert, & Ocheltree, 2014; Sankaran et al., 2004). These coexisting functional types often vary substantially in morphology and physiology as well as in the ways they access and consume resources. As a result, grasses and woody plants typically occupy different ecohydrological niches, which facilitates their coexistence in grassland and savanna systems (O'Keefe et al., 2019; Silvertown et al., 2015; Ward et al., 2013; Weaver, 1968). One prominent hypothesis for tree- or shrub-grass coexistence is the two-layer hypothesis, originally proposed by Walter (1971), which states that trees and grasses have different functional rooting depths and rely on water from different portions of the soil profile. Grasses primarily take up water from surface soils while trees and shrubs have deeper rooting systems that can access deeper portions of the soil profile (Case et al., 2020; Keen et al., 2022; Kulmatiski & Beard, 2013; Nippert & Knapp, 2007; O'Keefe & Nippert, 2017; Ratajczak et al., 2011), theoretically reducing competition for water between functional groups, particularly in surface soils (Walter, 1971).

Physiological and morphological differences between grasses and woody plants dictate their water-use strategies and impact the way species respond to fluctuations in precipitation, temperature and other environmental conditions. Shrubs and grasses vary dramatically in canopy architecture, with woody plants typically having greater leaf area compared with grasses (Canadell et al., 1996). In addition, dominant grasses use the  $C_4$  photosynthetic pathway in most tropical and mid-latitude grasslands and savannas (Edwards et al., 2010) while woody shrubs and trees use the  $C_3$  photosynthetic pathway (Ehleringer & Cerling, 2002). Specialized anatomical structures in the leaves of  $C_4$  species allow for the concentration of  $CO_2$  around rubisco, nearly eliminating photorespiration and generally increasing water-use efficiency (WUE: the ratio of carbon gained via photosynthesis to water lost via transpiration) compared to  $C_3$  plants (Ehleringer & Cerling, 2002; Sage, 2004). These anatomical differences also typically result in higher photosynthetic rates in  $C_4$  grasses (Ehleringer & Cerling, 2002; Sage, 2004) and greater rates of transpiration in  $C_3$  woody plants (O'Keefe et al., 2020).

In addition to these well-established functional-type differences, the degree of stomatal regulation of gas exchange often varies between species and can have large impacts on survival and productivity when drought events occur (McDowell et al., 2008; Roman et al., 2015). Some species decrease stomatal conductance in response to declines in soil water potential to avoid excess water loss when conditions are dry, prioritizing hydraulic safety but risking carbon starvation during periods of extended drought (Klein, 2014; McDowell et al., 2008). On the opposite end of the spectrum, other

species maintain relatively high stomatal conductance when soil moisture declines in order to continue fixing carbon. These species prioritize carbon gain but are more at risk of hydraulic failure if drought conditions persist (Klein, 2014; McDowell et al., 2008). We currently lack an understanding of where grasses and encroaching clonal shrubs fall along this spectrum, and how potential growth form differences might mediate their responses to changes in water availability in mesic grasslands.

Dynamics of tree- and shrub-grass coexistence are shifting in grasslands and savannas world-wide due to woody encroachment, a phenomenon in which tree and shrub cover increases at the expense of historically dominant grass species (Gibbens et al., 2005; Knapp, Briggs, et al., 2008; Ratajczak, Nippert, Briggs, et al., 2014; Ratajczak, Nippert, & Ocheltree, 2014; Stevens et al., 2017; Van Auken, 2000). Consequences of this shift from grass to woody dominance include reduced plant biodiversity (Eldridge et al., 2011; Ratajczak et al., 2012), lower forage availability for grazing livestock (Anadón et al., 2014), and population decreases or local extinction of grassland-obligate species (Fuhlendorf et al., 2002; Grant et al., 2004). Increasing woody cover in grasslands and savannas has been attributed to multiple drivers, including reduced fire frequency and intensity (Ratajczak, Nippert, Briggs, et al., 2014; Twidwell et al., 2016), rising atmospheric  $CO_2$  concentrations (Higgins & Scheiter, 2012; O'Connor et al., 2022), overgrazing and loss of native browsers (O'Connor et al., 2020; Staver & Bond, 2014), and the spread of seed and propagule sources for woody species (Woods et al., 2019). In mesic grasslands of the Great Plains (central United States), the primary driver of woody encroachment is reduced fire frequency (Briggs et al., 2005; Ratajczak, Nippert, & Ocheltree, 2014). In this region, trees and woody shrubs have been expanding over the last century, with the most severe encroachers being of the genus *Juniperus* in the southern and western Great Plains (Briggs et al., 2002; Engle et al., 1996; Knapp, McCarron, et al., 2008) and clonal shrub species in the northern and eastern Great Plains (Briggs et al., 2005; Knapp, Briggs, et al., 2008; Ratajczak, Nippert, Briggs, et al., 2014). Compared with grass-dominated systems, shrub-dominated systems typically have higher leaf area index (Currey et al., 2022; Knapp, Briggs, et al., 2008; Ratajczak et al., 2011; Tooley et al., 2022), higher ratios of above- to below-ground biomass (Ma et al., 2021; Zhou et al., 2022), higher canopy transpiration rates (O'Keefe et al., 2020), and greater coarse root biomass (Jackson et al., 1996; McKinley et al., 2008; Pinno & Wilson, 2011), in addition to having greater vegetation access to deeper soil water or even groundwater sources (Keen et al., 2022; Kulmatiski & Beard, 2013; Nippert & Knapp, 2007; Ratajczak et al., 2011). Shifts from grass to shrub dominance at the landscape-scale, therefore, can have major impacts on water, carbon and nutrient cycling through these ecosystems (Archer et al., 2001, 2017; Barger et al., 2011; Hibbard et al., 2001; Huxman et al., 2005; Zhou et al., 2018).

In addition to changes in vegetation cover due to woody encroachment, climate change is altering precipitation regimes in many grassland and savanna ecosystems (Easterling et al., 2017; Garbrecht et al., 2004). In many mesic grasslands, precipitation variability is

projected to increase, and total annual precipitation is expected to stay the same or even increase in the future (Easterling et al., 2000; IPCC, 2021; Jones, 2019; USGCRP, 2017). Increased precipitation variability that leads to longer dry periods punctuated by fewer, but larger rainfall events has been shown to reduce soil moisture and grassland productivity nearly as much as an overall reduction in growing season precipitation (Fay et al., 2002, 2003). Shifts in the patterns of water supply to grasslands have the potential to modify ecohydrological niches and disrupt or alter the dynamics of coexistence between grasses and woody vegetation in open ecosystems.

In this study, we assessed how water-use traits of an encroaching clonal shrub (*Cornus drummondii*) and a dominant  $C_4$  grass (*Andropogon gerardii*) impact responses to intra-annual changes in water availability in tallgrass prairie. In this system, *C. drummondii* is known to maintain remarkably stable rates of carbon assimilation and growth—both within and across growing seasons—despite fluctuations in resource availability and environmental conditions (Muench et al., 2016; Nippert et al., 2013; Wedel et al., 2021). However, the mechanisms supporting this level of stability are currently undescribed. We assessed the degree of intra-annual plasticity of key water-use variables as well as leaf-level responses to seasonal variation in water availability in co-existing grasses and shrubs in situ during the 2021 and 2022 growing seasons. More specifically, we asked (1) Does *C. drummondii* adjust depth of water uptake within individual growing seasons to access deeper soil water and avoid competition for surface water with grasses? (2) Is *C. drummondii* capable of osmotic adjustment in response to changes in soil water availability, and how does this compare to adjustment in *A. gerardii*? (3) Do these species differ in degree of stomatal regulation in response to decreasing soil moisture and leaf water potential, and how does this regulation impact carbon fixation? We expected to see shifts to deeper water use by *C. drummondii* within individual growing seasons, particularly during periods of low rainfall, to avoid competition with grasses for surface soil water. Additionally, we expected to see a greater degree of osmotic adjustment and stomatal regulation in *A. gerardii*, as  $C_4$  grasses are known to be physiologically drought tolerant and responsive to changes in water availability (Fay et al., 2008).

## 2 | MATERIALS AND METHODS

### 2.1 | Site description and experimental design

Konza Prairie Biological Station (KPBS) is a 3487-ha tallgrass prairie site in northeastern Kansas (USA; 39.1°N, 96.9°W) that is divided into replicated experimental watersheds with varying fire frequencies (1-, 2-, 4- and 20-year burn frequencies) and grazing regimes (no grazing, bison grazing and cattle grazing). This site is dominated by  $C_4$  grasses (primarily *A. gerardii*, *Panicum virgatum*, *Sorghastrum nutans* and *Schizachyrium scoparium*) but contains a high diversity of subdominant grasses ( $C_3$  and  $C_4$ ) and forbs (Collins & Calabrese, 2012). In the last several decades, native clonal shrubs (primarily *C.*

*drummondii*, *Rhus glabra* and *Prunus americana*) have rapidly increased in cover in areas where fire return intervals are >2–3 years (Briggs et al., 2005; Ratajczak, Nippert, & Ocheltree, 2014). Historically, woody cover was low and confined to hardwood gallery forests along stream corridors (Abrams, 1986). When fire frequency is reduced, shrubs typically establish first in lowland positions or along limestone-shale contact zones on hillslopes where water availability is high (Ratajczak, Nippert, Briggs, et al., 2014), then spread into the surrounding watershed (primarily via vegetative clonal reproduction; Benson & Hartnett, 2006; Ratajczak et al., 2011). Grasses are shaded out as shrub patches expand and canopies become more dense (Tooley et al., 2022), which reduces fuel loads and protects shrubs against future fires (Ratajczak et al., 2011). KPBS is located in the Flint Hills, where the geology is characterized by alternating limestone and shale layers. Upland soils are shallow, rocky and relatively dry, while lowland soils are deeper (often >2m), and wetter (Ransom et al., 1998).

This study took place at the ShRaMPs (Shrub Rainout Manipulation Plots) experimental site at KPBS. At this site, 6 × 6 m drought shelters were built over intact shrub–grass communities on neighbouring watersheds with different burn frequencies (K1B, 1-year burn; K4A, 4-year burn). This study took advantage of the infrastructure at ShRaMPs to establish a broader range of soil moisture conditions and provide a wider parameter space for assessing differences in water-use strategies between an encroaching shrub (*C. drummondii*) and a dominant  $C_4$  grass (*A. gerardii*). In this study, our goal was to assess continuous independent physiological responses between species across a gradient of water availability and plant water stress, rather than an explicit factorial test for differences between drought or fire treatments. Additional information on the experimental design at ShRaMPs is available in Appendix 1. Daily precipitation, air temperature ( $T_{air}$ ) and relative humidity (RH) data were collected at KPBS headquarters, 2.6 km away from ShRaMPs (Nippert, 2023). Precipitation data were collected using an Ott Pluvio2 rain gauge;  $T_{air}$  and RH were collected using a Campbell HMP35C temperature and relative humidity probe. Volumetric water content (VWC) was measured at three depths (10, 15 and 30 cm) in each shelter using time-domain reflectometry (TDR) probes (Campbell Scientific). Measurements were recorded at 30-min intervals on Campbell Scientific data loggers (CR1000X).

### 2.2 | Plasticity of source water use

#### 2.2.1 | Soil and xylem water sampling

Non-photosynthetic tissue from *C. drummondii* and *A. gerardii* was collected from each shelter 4–5 times during each of the 2021 and 2022 growing seasons. For *C. drummondii*, 10–15 cm of stem tissue from 3 to 4 stems were collected, bark and phloem were removed, and samples were combined and sealed in an exetainer vial (LabCo Ltd, UK). For *A. gerardii*, non-photosynthetic crown tissue was collected from 5 to 6 individuals and stored in the same way (Table S1).

All samples were immediately placed on ice in a cooler, then stored long term at 1–2°C.

Surface soil was collected at the same time as vegetation samples during each sampling round using a standard soil corer (5-cm diameter). Under each shelter, root-free soil samples were collected at 0, 10 and 20 cm depths and were immediately sealed in exetainer vials upon collection. In addition, deep soil cores (1 m depth) were collected from each watershed in 2021 (August) and 2022 (July and September; [Table S1](#)). In 2021, only one set of deep cores could be collected due to COVID restraints. During each round of sampling, deep soil cores were collected from the 1-year burn ( $n=2$ ) and the 4-year burn ( $n=2$ ) watersheds at ShRaMPs. Deep soil cores were collected outside, but adjacent to, the shelters to avoid damaging vegetation communities. These cores were collected to determine soil water isotopic signatures from deeper soil layers that have longer residence times and are less variable over the course of a year compared to surface soil (Gazis & Feng, 2004). These cores were extracted with a hydraulic-push corer (540MT Geoprobe Systems, Salina, KS, USA) and immediately stored in sealed plastic coring tubes. Cores were subsampled at 0, 10, 20 and 30 cm, then every 25 cm for the remainder of the core. At each depth, root-free soil was immediately placed in a sealed exetainer vial. All soil samples in exetainer vials were stored at 1–2°C until extraction.

Xylem and soil water were extracted using cryogenic vacuum distillation at Kansas State University (Ehleringer & Osmond, 1989; Nippert & Knapp, 2007). All extracted water samples (soil and vegetation) were analysed for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  on a Picarro WS-CRDS isotopic water analyser in the Stable Isotope Mass Spectrometry Laboratory (SIMSL) at Kansas State University. ChemCorrect software was used to identify organic contamination in extracted samples. Contamination was primarily an issue for *C. drummondii* samples, many of which had high methanol concentrations that interfered with hydrogen and oxygen stable isotope measurements (Brand et al., 2009; Totschnig et al., 2000; West et al., 2010). To correct for this, all contaminated samples and a subset of non-contaminated samples were re-analysed using a Los Gatos Liquid Water Isotope Analyzer (DLT-100; Mountain View, CA, USA) at the University of Pennsylvania, which allowed for more accurate measurement of methanol concentrations in each sample.  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for non-contaminated samples were similar between machines, but substantial offsets occurred for samples flagged for methanol contamination ([Figure S1](#)). To correct these samples, varying concentrations of methanol (15–400 ppm) were added to in-house water standards with known isotopic signatures and analysed on the same Los Gatos water analyser. Offsets caused by increasing methanol concentrations were calculated and used to establish a calibration curve, which was applied to all contaminated samples ([Figure S2](#)). All isotopic ratios (‰) were expressed relative to V-SMOW (Vienna Standard Mean Ocean Water), the international standard for oxygen and hydrogen. Long-term precision of both the SIMSL Picarro analyser and the Los Gatos analyser using in-house standards was <0.3‰ for  $\delta^2\text{H}$  and <0.15‰ for  $\delta^{18}\text{O}$ . Additional information regarding sample processing and stable isotope analyses can be found in [Appendix 2](#).

## 2.2.2 | Stable isotope mixing model

Xylem and soil water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  were used in a Bayesian stable isotope mixing model (*simmr*; Parnell, 2016) to assess changes in proportional use of water from different soil depths across individual growing seasons. Sources included water from surface soils collected at 4–5 time points during each growing season, and water from deep (1-m) soil cores collected at 1–2 time points during each growing season. Since deep soil cores were not collected as frequently as surface soils, and because deep soil water (>30 cm) isotope values are less variable across the growing season compared with surface soils (Gazis & Feng, 2004; [Figure S5](#)), we averaged the isotopic values from deep cores at each depth >30 cm from each growing season and used those values to represent deep soil water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  at each time point in the isotope mixing models (profiles for each time point are shown in [Figure S4](#)). Potential water sources for the model initially included surface soil depths collected at each sampling interval (0, 10 and 20 cm) as well as 'deep' soil water (averaged across depths >30 cm). There was substantial overlap between the 20 cm source and either the 10 cm or >30 cm source for most sampling intervals ([Figure S3](#)), so the 20 cm source was excluded to avoid source overlap in the mixing model. For each *simmr* run, a posterior distribution consisting of 10,000 Markov Chain Monte Carlo iterations was produced that showed the best estimates of proportional source water use from each depth for each species. Model summaries included means, standard deviations and credible intervals for each source. Separate *simmr* models were performed for each sampling interval rather than averaging across the growing season so that changes in surface soil signatures through time were incorporated into each model.

## 2.3 | Intra-annual adjustment of turgor loss point ( $\pi_{\text{TLP}}$ )

### 2.3.1 | Turgor loss point ( $\pi_{\text{TLP}}$ )

In 2022 only, *C. drummondii* and *A. gerardii* samples were collected at five points during the growing season and osmolarity was measured using a VAPRO® Vapor Pressure Osmometer (Model 5600; Logan, Utah, USA). At each time point, one sample per species was collected from each shelter (14 shelters total; [Table S1](#)). Measurements of osmotic potential at full turgor were conducted following the methods in Bartlett et al. (2012) and Griffin-Nolan et al. (2019). After sample collection, stems were re-cut underwater and rehydrated overnight (8–10 h) in a dark, cool room to minimize transpiration. A disc was cut from each leaf using a 5-mm tissue biopsy punch, and the leaf disc was immediately wrapped in aluminium foil and submerged in liquid nitrogen for 60 s to lyse leaf cells. Leaf discs were then removed from foil and punctured 15–20 times using forceps before being placed in the sealed osmometer measurement chamber. Samples were allowed to equilibrate for 10 min (Bartlett et al., 2012; Griffin-Nolan et al., 2019) prior to measurement. Osmolarity values were

converted to osmotic potential at full turgor ( $\pi_o$ ) using the following equation:

$$\pi_o = \text{osmolarity} \times -2.3958 / 1000.$$

$\pi_o$  was then used to estimate leaf turgor loss point ( $\pi_{\text{TLP}}$ ) using the following equations for *A. gerardii* (herbaceous; Griffin-Nolan et al., 2019) and *C. drummondii* (woody; Bartlett et al., 2012):

$$\textit{A. gerardii: } \pi_{\text{TLP}} = 0.944 \times \pi_o - 0.611,$$

$$\textit{C. drummondii: } \pi_{\text{TLP}} = 0.832 \times \pi_o - 0.631.$$

### 2.3.2 | Statistical analysis

Mixed effects models were performed to assess changes in  $\pi_{\text{TLP}}$  for each species throughout the 2022 growing season using the *lmer* function from the *lmerTest* package (Kuznetsova et al., 2017) and the *Anova* function from the *car* package (Fox et al., 2012) in R version 4.1.0 (R Core Team, 2021). Fixed effects included species and day of year and shelter was included as a random effect.

## 2.4 | Gas exchange and leaf water potential

### 2.4.1 | Leaf water potential

Midday leaf water potential ( $\Psi_{\text{leaf}}$ ) measurements were conducted five times during each growing season (2021–2022). Midday measurements were conducted between the hours of 12:00 and 13:00. Three replicates per species (*C. drummondii* and *A. gerardii*) were measured from each shelter during each sampling round (Table S1). Measurements were taken on a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA). The youngest fully expanded leaf or leaves were collected for each sample and sealed in a moist, high  $[\text{CO}_2]$  bag, then placed in a dark cooler to encourage stomatal closure and limit transpirative water loss. Samples were allowed to equilibrate for ~1 h prior to measurement (Rodriguez-Dominguez et al., 2022).

### 2.4.2 | Gas exchange

Measurements of net photosynthetic rate ( $A_{\text{net}}$ ), transpiration rate ( $E$ ) and stomatal conductance ( $g_s$ ) were conducted every 3–4 weeks throughout each growing season using a LI-COR 6400XT or 6800 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). Two individuals per species were measured in each shelter during each sampling round (Table S1). Reference  $\text{CO}_2$  concentration was set to  $400 \mu\text{mol mol}^{-1}$ , PAR was set to  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and relative humidity was maintained between 50% and 65% during measurements. Measurements were taken between the hours of 10:00 and 13:00 on clear, sunny days. For grass leaves that were smaller than the measurement chamber ( $3 \times 3 \text{ cm}$ ), leaf area was measured and

adjusted manually. *C. drummondii* leaves covered the entirety of the chamber head, and so did not require any area corrections. Intrinsic water-use efficiency (iWUE) was calculated using the following equation, where  $A_{\text{net}}$  is net photosynthetic rate and  $g_s$  is stomatal conductance (Farquhar et al., 1989):

$$\text{iWUE} = \frac{A_{\text{net}}}{g_s}.$$

### 2.4.3 | Statistical analysis

Mixed effects models were used to assess whether changes in stomatal conductance or transpiration rates occurred during the 2021 and 2022 growing seasons for each species using the *lmer* function from the *lmerTest* package (Kuznetsova et al., 2017) and the *Anova* function from the *car* package (Fox et al., 2012) in R version 4.1.0 (R Core Team, 2021). In these models, species and day of year were included as fixed effects and replicate nested within shelter was included as a random effect. Separate models were performed for each growing season (2021 and 2022). Stomatal conductance data was log-transformed to meet model assumptions of normality.

## 3 | RESULTS

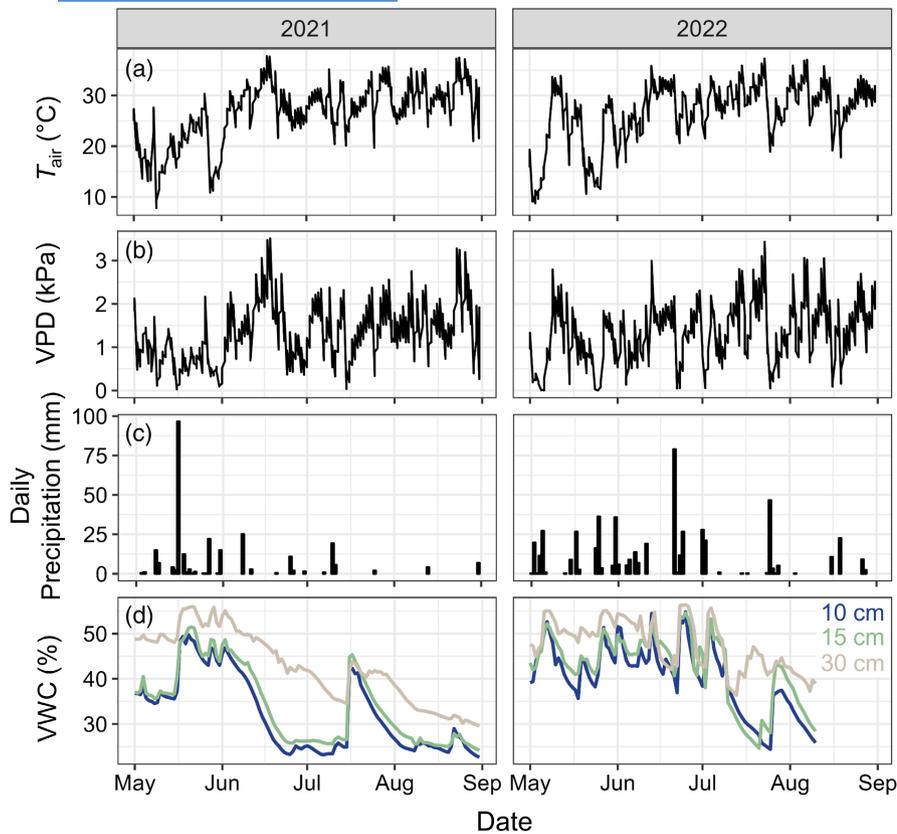
### 3.1 | Environmental conditions

Long-term (1982–2022) mean annual precipitation (MAP) for this site is 830.3 mm, with ~73% (604.9 mm) of total annual precipitation occurring during the growing season, on average. In 2021, MAP was below-average at 632.2 mm, primarily driven by a reduction in growing season precipitation (423.6 mm; Figure 1) compared to the long-term average. In 2022, MAP was 825.7 mm with 601.7 mm occurring during the growing season. Soil VWC was lower overall in 2021 compared with 2022, particularly later in the growing season (July and August).

### 3.2 | Plasticity of source water use

#### 3.2.1 | Soil water profiles

At all sampling dates,  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values were highest at the surface (0 cm) and declined until 30 or 40 cm depth ( $p < 0.001$ ; Figure 2; Figure S4).  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values were largely constant at depths  $\geq 40 \text{ cm}$ , and deep soil cores (30–100 cm depth) had very little variation between sampling dates (Figure S5)—deeper soil water is outside of the zone of evaporative enrichment in surface soil, has higher clay content and is also largely outside of the rooting zone of grasses, resulting in very little intra-annual variation in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  compared to surface soil layers (Gazis & Feng, 2004). Surface soil  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  changed throughout each growing season (Figure 2; Figure S5; Table S3) in response to the timing and isotopic signature of precipitation inputs.



**FIGURE 1** (a) Air temperature ( $T_{air}$ ), (b) vapour pressure deficit (VPD), (c) daily precipitation, and (d) volumetric water content (VWC; 10, 15, and 30 cm depth) during the 2021 and 2022 growing seasons. Values for  $T_{air}$ , RH, and VPD (a–c) represent daytime averages for the hours 10:00–13:00 (the time range during which gas exchange measurements were performed at each sampling date).

### 3.2.2 | Stable isotope mixing model (simmr)

Xylem water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for both species fell within the range of source values at every time point (Figure 2). Results from the stable isotope mixing model indicated that *A. gerardii* primarily used water from the 10 cm depth at most time points, and water use from the >30 cm depth made up the lowest proportion of overall water use at all time points except the end of the 2022 growing season (Figure 3a; Table S2). In contrast, *C. drummondii* primarily used water from the 10 cm depth early in both growing seasons, but reliance on the >30 cm source increased substantially over the course of each season. By the last sampling date of both growing seasons, deeper soil water (>30 cm) made up  $\geq 75\%$  of total water use by *C. drummondii* (Figure 3b; Table S2). This shift toward greater reliance on deeper soil water corresponded with a decline in precipitation and water availability throughout the growing season, particularly in 2021 (Figure 3c).

### 3.3 | Intra-annual adjustment of turgor loss point ( $\pi_{TLP}$ )

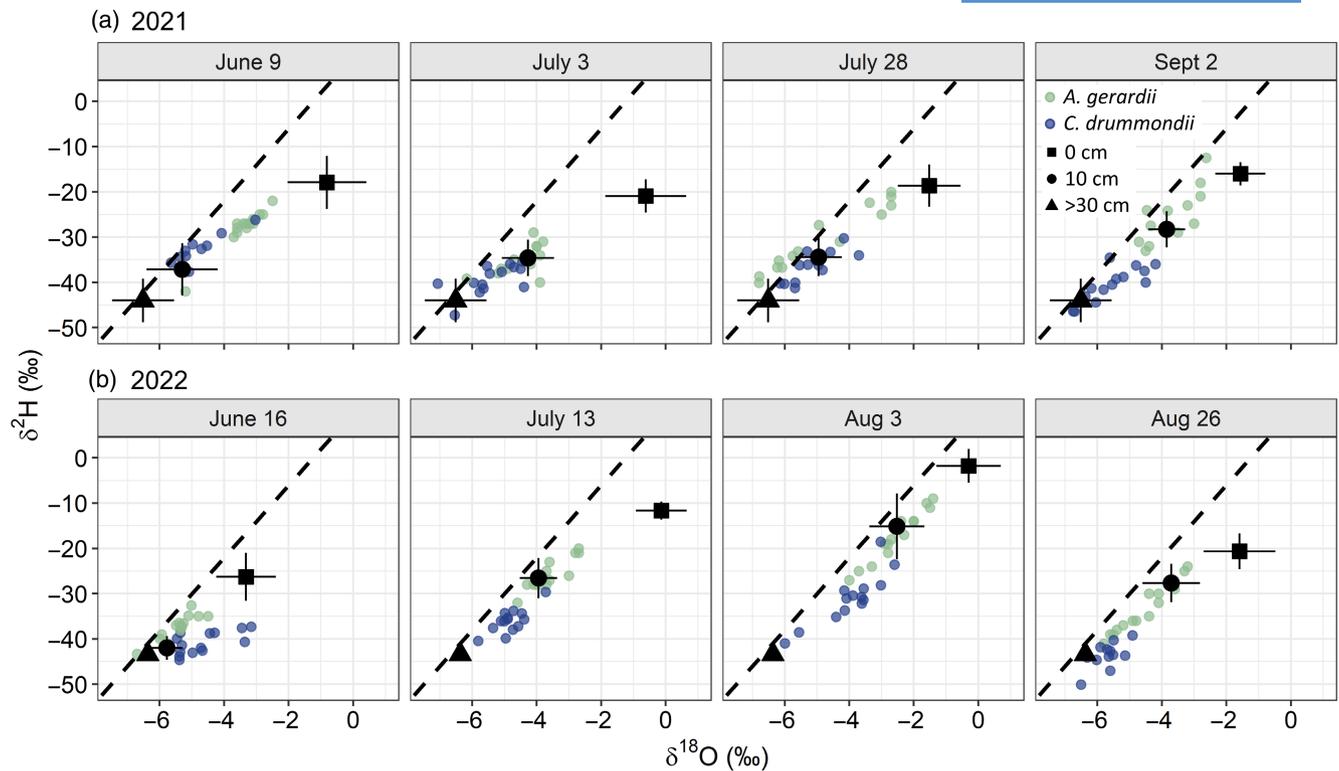
$\pi_{TLP}$  declined significantly through the 2022 growing season for both *A. gerardii* ( $p < 0.001$ ) and *C. drummondii* ( $p < 0.001$ ). *A. gerardii*  $\pi_{TLP}$  declined by 0.29 MPa ( $\pm 0.19$  SD), and *C. drummondii*  $\pi_{TLP}$  declined by 0.48 MPa ( $\pm 0.15$  SD; Figure 4). There was a significant species  $\times$  day of year interaction, where  $\pi_{TLP}$  values were similar

between species early in the growing season (DOY 154 and 165), but *C. drummondii* had significantly lower  $\pi_{TLP}$  values compared with *A. gerardii* during the mid- to late-growing season (DOY 188, 210 and 239; Figure 4).

## 3.4 | Gas exchange and leaf water potential

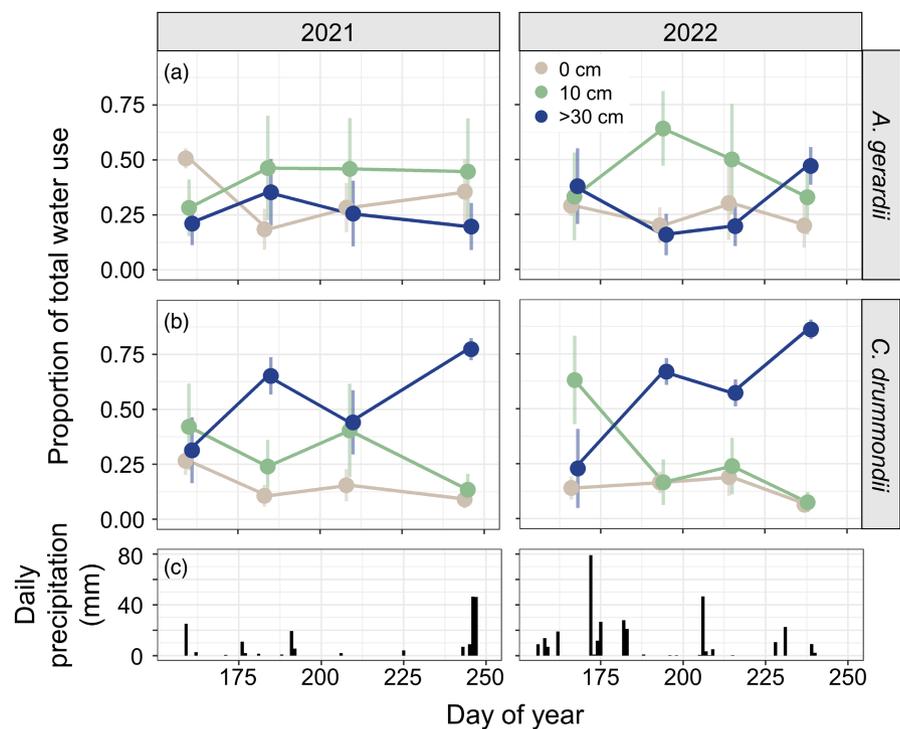
### 3.4.1 | Stomatal regulation and WUE

For both species,  $g_s$  was generally lower in 2021 (drier year) compared with 2022 (Figure S6B). Despite these lower  $g_s$  values,  $E$  was higher in 2021 for both species, particularly during the first half of the growing season (Figure S6C).  $g_s$  and  $E$  generally declined throughout each growing season for both species (Figure S6B,C). *A. gerardii* iWUE was higher in the mid- to late-growing season (July and early August) in 2021 (when precipitation rates were particularly low; Figure 3c), compared to 2022. In contrast, *C. drummondii* iWUE varied very little between years, despite the substantial difference in growing season precipitation (Figure S6C). *A. gerardii* exhibited significant increases in  $A_{net}$  with increasing  $g_s$  during both years (2021,  $p < 0.001$ ; 2022,  $p < 0.001$ ). In contrast, the relationship between  $A_{net}$  and  $g_s$  was weaker for *C. drummondii*—the increase in  $A_{net}$  in response to increasing  $g_s$  was significant in 2021 ( $p < 0.001$ ) but only marginally significant in 2022 ( $p = 0.058$ ) (Figure 5a,b).  $E$  increased significantly with increasing  $g_s$  for both *A. gerardii* (2021,  $p < 0.001$ ; 2022,  $p < 0.001$ ) and *C. drummondii* (2021,  $p < 0.001$ ; 2022,  $p < 0.001$ ) (Figure 5c,d).



**FIGURE 2** Stable isotope values for soil water sources and xylem water samples. Black shapes represent soil water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for each soil depth (0 cm, squares; 10 cm, circles; >30 cm, triangles)  $\pm 1\text{SD}$ , and coloured circles represent *Andropogon gerardii* (green) and *Cornus drummondii* (blue) xylem water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  from each sampling date in the 2021 (a) and 2022 (b) growing seasons. The dashed black line represents the global meteoric water line.

**FIGURE 3** Stable isotope mixing model (simmr) estimates of proportional use of soil water sources (0, 10 and >30 cm depths) across the 2021 and 2022 growing seasons for *Andropogon gerardii* (a) and *Cornus drummondii* (b). Points represent mean values  $\pm 1$  standard deviation. Daily precipitation values for each growing season are shown in panel (c).



### 3.4.2 | Impacts of water potential on leaf gas exchange

*A. gerardii* had a significant positive relationship between  $A_{\text{net}}$  and  $\Psi_{\text{leaf}}$  during both years (2021,  $p < 0.001$ ; 2022,  $p < 0.001$ ). In contrast, the relationship between  $A_{\text{net}}$  and  $\Psi_{\text{leaf}}$  for *C. drummondii* was not significant during either year (2021,  $p = 0.432$ ; 2022,  $p = 0.454$ ; Figure 6a,b). Both species had significant positive relationships between midday  $\Psi_{\text{leaf}}$  and both  $g_s$  and  $E$  (Figure 6c–f). *C. drummondii*  $g_s$  and  $E$  were higher overall compared with *A. gerardii* during both growing seasons ( $p < 0.001$  in all cases). Significant interactions between species and midday  $\Psi_{\text{leaf}}$  occurred only for  $E$  in 2021 ( $p = 0.037$ ). In this case, *A. gerardii*  $E$  declined more rapidly with decreasing midday  $\Psi_{\text{leaf}}$  compared with *C. drummondii*. The relationships between midday  $\Psi_{\text{leaf}}$  and both  $g_s$  and  $E$  were weaker overall for *C. drummondii* compared to *A. gerardii* in 2021 (Figure 6c–f). Both species had significant negative relationships between iWUE and

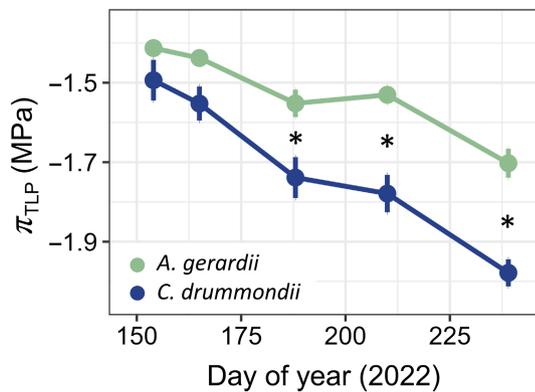


FIGURE 4 Turgor loss point ( $\pi_{\text{TLP}}$ ) measured at five time points during the 2022 growing season for *Andropogon gerardii* (green) and *Cornus drummondii* (blue). Points represent mean values  $\pm 1$  standard error. Stars represent significant differences between species ( $p < 0.05$ ).

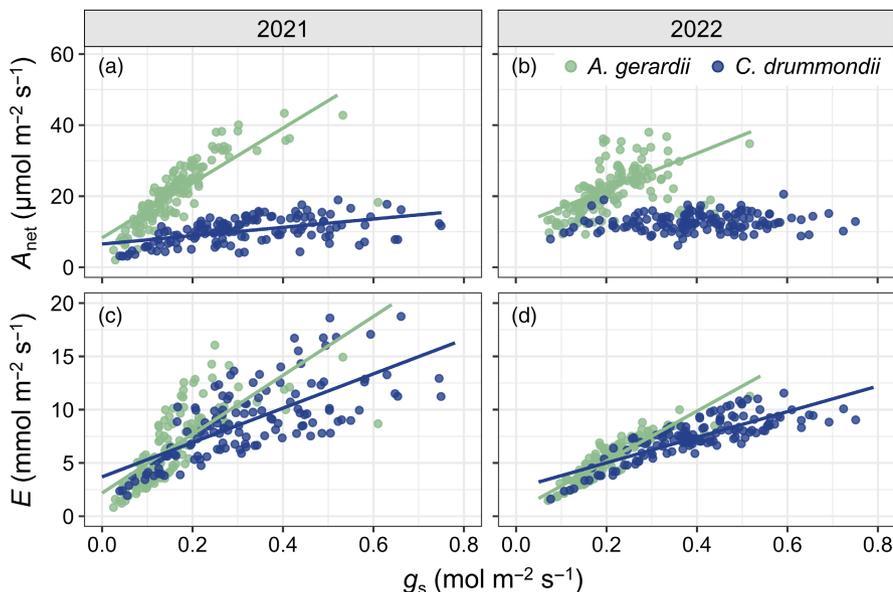


FIGURE 5 (a, b) Changes in net photosynthetic rates ( $A_{\text{net}}$ ) and (c, d) transpiration rates ( $E$ ) in response to changes in stomatal conductance ( $g_s$ ) values during the 2021 and 2022 growing seasons for *Andropogon gerardii* (green) and *Cornus drummondii* (blue).

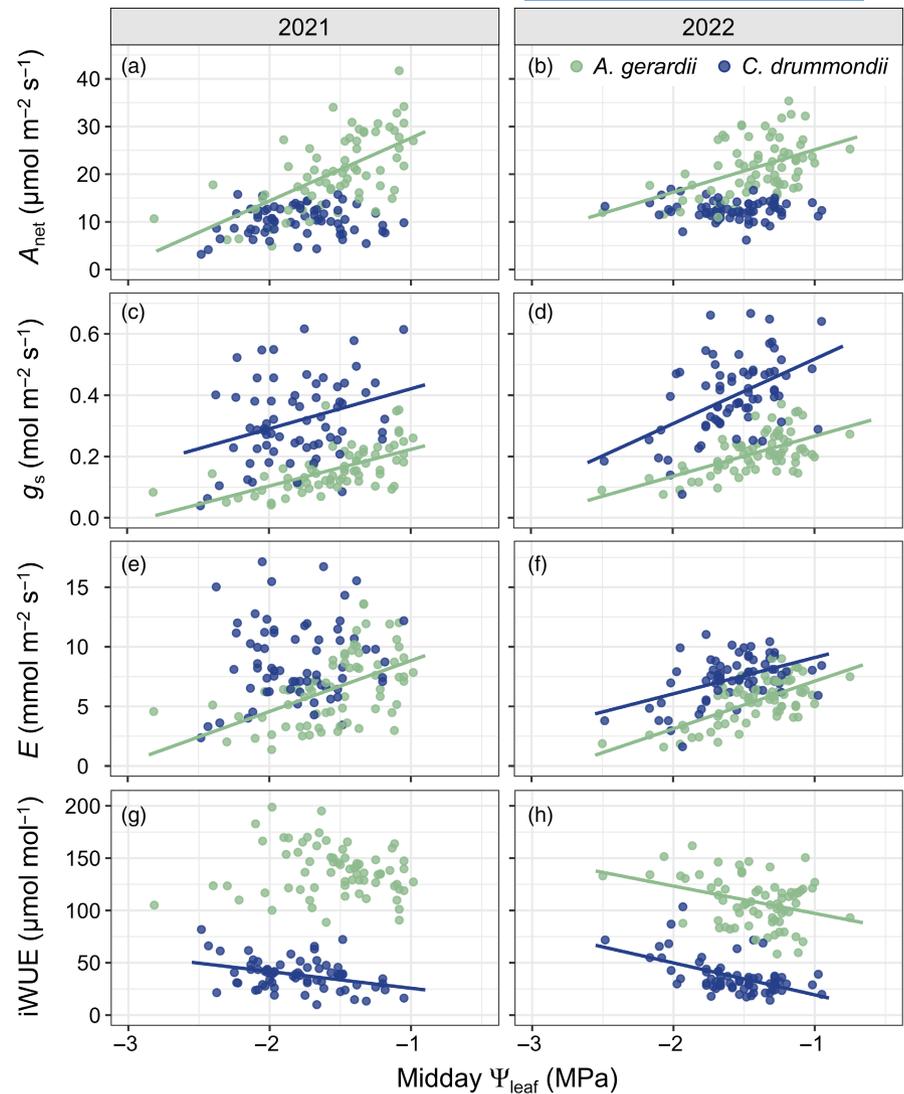
$\Psi_{\text{leaf}}$  overall (Table 1), but this relationship was not significant for *A. gerardii* in 2021 ( $p = 0.368$ ) (Figure 6g).  $C_i$  was positively related to  $\Psi_{\text{leaf}}$  during both growing seasons for *C. drummondii* and in 2022 for *A. gerardii*, but there was no relationship between  $C_i$  and  $\Psi_{\text{leaf}}$  for *A. gerardii* in 2021 when conditions were drier (Figure S7).

## 4 | DISCUSSION

In this study, we assessed differences in water-use strategies of coexisting clonal shrubs (*C. drummondii*) and dominant grasses (*A. gerardii*) in tallgrass prairie to better understand how fluctuations in water availability may impact encroached and non-encroached communities differently. *C. drummondii* showed evidence of using shallow soil water when it was available early in the growing season and shifting to deeper water uptake during drier portions of the growing season (Figure 3). This trend has been observed in sub-shrub and forb species in tallgrass prairie (Nippert & Knapp, 2007) as well as in coniferous species (*Juniperus virginiana* and *Pinus ponderosa*) in semi-arid grasslands (Eggemeier et al., 2009) and savanna trees and seedlings in South Africa (Kulmatiski & Beard, 2013). Previous studies specifically assessing *C. drummondii* water-use patterns have either shown uniform deep-water uptake throughout the growing season (Ratajczak et al., 2011) or did not sample frequently enough to assess intra-annual plasticity (Keen et al., 2022; O'Keefe & Nippert, 2017). While *C. drummondii* is known to have deep, extensive coarse root systems (E.G. Tooley, unpublished), these shrubs still maintain relatively high fine root biomass and root hydraulic conductivity in surface soils (Figure S8; O'Keefe et al., 2022), facilitating efficient surface water uptake when it is available.

*A. gerardii*, like many other dominant grass species, has been shown to have almost complete reliance on water in surface soil layers (<30 cm)—grasses concentrate fine root biomass in the top ~20 cm of soil (Jackson et al., 1996; Nippert et al., 2012; Weaver & Darland, 1949), making them highly effective at taking advantage

**FIGURE 6** Changes in (a, b) net photosynthetic rates ( $A_{\text{net}}$ ), (c, d) stomatal conductance ( $g_s$ ), (e, f) transpiration rates ( $E$ ), and (g, h) intrinsic water-use efficiency (iWUE) in response to changes in midday leaf water potential ( $\Psi_{\text{leaf}}$ ) during the 2021 and 2022 growing seasons for *Andropogon gerardii* (green) and *Cornus drummondii* (blue).



**TABLE 1** Summary of mixed effects model results comparing gas exchange parameters ( $A_{\text{net}}$ ,  $g_s$ , and  $E$ ) and intrinsic water-use efficiency (iWUE) to midday  $\Psi_{\text{leaf}}$  for *Andropogon gerardii* and *Cornus drummondii* in 2021 and 2022. Bolded values with asterisks (\*) represent significant effects ( $p < 0.05$ ).

Predictor	$A_{\text{net}}$		$g_s$		$E$		iWUE	
	2021	2022	2021	2022	2021	2022	2021	2022
Midday $\Psi_{\text{leaf}}$	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	0.039*	<b>&lt;0.001*</b>
Species	<b>&lt;0.001*</b>							
Midday $\Psi_{\text{leaf}} \times$ species	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	0.304	0.792	<b>0.037*</b>	0.308	0.313	0.684

of precipitation inputs when they occur during the growing season (Holdo, 2013). Low rooting density as well as low root hydraulic conductivity at greater soil depths in grasses limits deeper soil water uptake (Nippert et al., 2012; O'Keefe et al., 2022). However, our data indicate that *A. gerardii* does shift depth of water uptake, at least within the top 30 cm of soil, throughout individual growing seasons in response to precipitation timing. *C. drummondii* shifts in depth of water uptake were more unidirectional, however, with the deepest water uptake consistently occurring at the end of each growing season (Figure 3). Below ~30 cm depth, soil water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  stayed

consistent down to 1 m (Figure S5). As a result, we were not able to ascertain the absolute depth of uptake by *C. drummondii* late in the growing season.

Shifting to deeper soil water sources when surface soils dry is typically considered a 'drought avoidance' strategy, but *C. drummondii* also showed substantial adjustment of  $\pi_{\text{TLP}}$  during the 2022 growing season, which is typically considered a 'drought tolerance' strategy (Fang & Xiong, 2015; Ilyas et al., 2021). *C. drummondii*  $\pi_{\text{TLP}}$  declined by 0.48 MPa ( $\pm 0.15$  SD), which was greater than the decline in  $\pi_{\text{TLP}}$  in *A. gerardii* over the same time period (0.29 MPa  $\pm 0.19$  SD)

(Figure 4). Across species,  $\pi_{\text{TLP}}$ —the water potential at which leaf cell turgor is lost (Bartlett et al., 2014; Cheung et al., 1975)—is highly correlated with water availability and commonly used as an indicator of drought tolerance (Bartlett et al., 2012; Zhou et al., 2018). Within species, reductions in  $\pi_{\text{TLP}}$  through time indicate that osmotic adjustment is occurring, where increased cellular solute concentrations decrease osmotic potential at full turgor, allowing the plant to maintain turgor and physiological functioning at lower soil water potentials (Bartlett et al., 2012; Sanders & Arndt, 2012).

The relatively large shift in *C. drummondii*  $\pi_{\text{TLP}}$  in 2022 suggests that this species was actively responding and adjusting to changes in water availability rather than simply avoiding water stress altogether. It should be noted that 2022 was not a particularly dry year—both MAP and growing season (April–August) precipitation were near the long-term averages for this site. The decline in  $\pi_{\text{TLP}}$  in *C. drummondii* during an average precipitation year was therefore larger than the average decline for woody species during drought globally (0.44 MPa; Bartlett et al., 2014). We expected to see greater physiological adjustment to seasonal changes in moisture availability in *A. gerardii*, as  $C_4$  grasses are known to be drought tolerant and highly responsive to timing of precipitation inputs during the growing season (Fay et al., 2008), largely due to their reliance on surface soil moisture (Nippert & Knapp, 2007). *A. gerardii* has been known to maintain positive carbon fixation rates even at very low (<−6 MPa) leaf water potentials, and substantial osmotic adjustment has been recorded for this species during drought years (Knapp, 1984, 1985). *A. gerardii* may have had lower adjustment of  $\pi_{\text{TLP}}$  than expected due to the fact that overall plant water stress was likely low in 2022.

Both species experienced general declines in  $g_s$  and  $E$  throughout the 2021 and 2022 growing seasons (Figure S6) and in response to reductions in  $\Psi_{\text{leaf}}$  (Figure 5). However, these relationships were stronger for *A. gerardii* compared with *C. drummondii*, particularly in 2021 when conditions were drier (Figure 6). Reductions in *A. gerardii*  $g_s$  were accompanied by reductions in  $A_{\text{net}}$  as  $\Psi_{\text{leaf}}$  declined, while *C. drummondii*  $A_{\text{net}}$  did not decline with  $\Psi_{\text{leaf}}$  (Figure 6). This reduction in *A. gerardii*  $A_{\text{net}}$  could be caused directly by stomatal closure, or by damage to photosynthetic structures as a result of water stress (Powles, 1984; Salvi et al., 2021; Tezara et al., 1999). In 2022, stomatal closure appears to be the primary driver of reductions in  $A_{\text{net}}$ , as  $C_i$  (leaf intercellular  $\text{CO}_2$  concentration) also declined with stomatal closure in response to declining  $\Psi_{\text{leaf}}$  (Figure S7). However, in 2021,  $C_i$  did not decline alongside  $A_{\text{net}}$  and  $g_s$  in *A. gerardii*, suggesting that drier conditions during the 2021 growing season may have resulted in an inability to use  $\text{CO}_2$  inside the leaf after stomatal closure (Powles, 1984; Salvi et al., 2021; Tezara et al., 1999).

iWUE values were substantially lower for *C. drummondii* compared with *A. gerardii* (Figure 6), even as  $\Psi_{\text{leaf}}$  declined, but this general trend is expected based on physiological differences between  $C_3$  and  $C_4$  species (Huxman & Monson, 2003; Kocacinar et al., 2008; Way et al., 2014). The ability of  $C_4$  species to concentrate  $\text{CO}_2$  in bundle sheath cells allows for the maintenance of higher  $A_{\text{net}}$  values for a given  $g_s$  compared with  $C_3$  species (Osborne & Sack, 2012).

However, *C. drummondii* continued to increase rates of water use ( $g_s$  and  $E$ ) as water stress decreased, even when no further increase in  $A_{\text{net}}$  occurred (Figure 5). This seemingly 'wasteful' water-use strategy is contrary to the idea that stomatal regulation tends to maximize carbon gain based on the amount of available water (stomatal optimization theory; Cowan & Farquhar, 1977; Sperry et al., 2017; Way et al., 2014; Wolf et al., 2016). In arid environments, 'wasteful' water-use has been hypothesized to be a competitive strategy, where plants maximize soil water uptake to prevent neighbours from accessing resources (Cohen, 1970; Ehleringer, 1993; Robinson et al., 1999). This hypothesis has also been suggested to explain high rates of nocturnal transpiration in  $C_4$  grasses (O'Keefe & Nippert, 2018).

## 5 | CONCLUSIONS

Overall, our results suggest that *C. drummondii* is resilient to changes in water availability in tallgrass prairie. This resilience is facilitated by (1) shifting to deeper soil water sources as the growing season progresses and surface soils dry out and (2) adjusting  $\pi_{\text{TLP}}$  to maintain cell turgor. Both of these strategies support the maintenance of consistent rates of carbon fixation, even as soil moisture and leaf water potentials decline. When dominated by  $C_4$  grasses, tallgrass prairie ecosystems are highly responsive to inter- and intra-annual variability in precipitation (Fay et al., 2003; Heisler-White et al., 2009; Nippert et al., 2006). We found that rates of carbon fixation and water-use by *A. gerardii* were mediated by water availability and leaf water potential and that increasing water-stress led to reductions in stomatal conductance and rates of carbon fixation (Figures 5 and 6). While *C. drummondii* water-use also generally declined with declining leaf water potential, higher water availability led to increased  $g_s$  and  $E$ , even when no additional increase in carbon fixation occurred (Figure 6a–f). Climate warming is expected to increase precipitation variability in many grassland and savanna ecosystems, leading to longer dry periods punctuated by fewer, but larger rainfall events (Easterling et al., 2000; IPCC, 2021; Jones, 2019; USGCRP, 2017). These conditions are largely expected to favour deeply rooted woody species over grasses (Kulmatiski & Beard, 2013)—larger rainfall events typically increase soil moisture to greater depths than smaller rainfall events, recharging deeper soil layers that woody vegetation has preferential access to (Kulmatiski & Beard, 2013). Longer dry periods are expected to primarily impact surface soils, as evaporation and grass water uptake in surface soils are higher than at deeper soil layers (Fay et al., 2002, 2003). Overall, the higher rates of water use by shrubs compared to grasses (O'Keefe et al., 2020), in addition to the 'wasteful' water-use strategy of *C. drummondii*, will likely lead to substantial changes in ecosystem-scale water cycling (including increased ecosystem-scale ET and depletion of deep soil water pools; Craine & Nippert, 2014; Keen et al., 2022) as shrub cover increases and changing climate conditions alter growing season environmental dynamics in tallgrass prairie.

## AUTHOR CONTRIBUTIONS

Rachel M. Keen collected data and performed laboratory analyses with assistance from Jesse B. Nippert and Brent R. Helliker. Rachel M. Keen analysed the data with substantial input from Jesse B. Nippert, Brent R. Helliker and Kate A. McCulloh. Rachel M. Keen led the writing process, and all authors provided intellectual input, contributed to manuscript preparation and gave final approval for publication.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14276>.

## DATA AVAILABILITY STATEMENT

Gas exchange, leaf water potential, turgor loss point and plant xylem and soil water isotope data for ShRaMPs are available through the KPBS Long-Term Ecological Research (LTER) and the Environmental Data Initiative (EDI) data portal: <https://doi.org/10.6073/pasta/d77b1696b198e152e12b87e8e8996777> (Keen & Nippert, 2024).

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## REFERENCES

- Abrams, M. D. (1986). Historical development of gallery forests in north-east Kansas. *Vegetatio*, 65(1), 29–37.
- Anadón, J. D., Sala, O. E., Turner, B. L., & Bennett, E. M. (2014). Effect of woody-plant encroachment on livestock production in north and South America. *Proceedings of the National Academy of Sciences of the United States of America*, 111(35), 12948–12953. <https://doi.org/10.1073/pnas.1320585111>
- Archer, S., Boutton, T. W., & Hibbard, K. A. (2001). Trees in grasslands: Biogeochemical consequences of woody plant expansion. In E. D. Schulze, M. Heimann, S. Harrison, E. Holland, J. Lloyd, I. C. Prentice, & D. S. Schimel (Eds.), *Global biogeochemical cycles in the climate system* (pp. 115–137). Academic Press.
- Archer, S. R., Andersen, E. M., Predick, K. I., Schwinning, S., Steidl, R. J., & Woods, S. R. (2017). Woody plant encroachment: Causes and consequences. In D. D. Briske (Ed.), *Rangeland systems: Processes, management and challenges* (pp. 25–84). Springer Nature.
- Barger, N. N., Archer, S. R., Campbell, J. L., Huang, C. Y., Morton, J. A., & Knapp, A. K. (2011). Woody plant proliferation in North American drylands: A synthesis of impacts on ecosystem carbon balance. *Journal of Geophysical Research: Biogeosciences*, 116(G4), 1–17.
- Bartlett, M. K., Scoffoni, C., & Sack, L. (2012). The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: A global meta-analysis. *Ecology Letters*, 15(5), 393–405.
- Bartlett, M. K., Zhang, Y., Kreidler, N., Sun, S., Ardy, R., Cao, K., & Sack, L. (2014). Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecology Letters*, 17(12), 1580–1590.
- Benson, E. J., & Hartnett, D. C. (2006). The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology*, 187, 163–178.
- Bond, W. J. (2019). *Open ecosystems: Ecology and evolution beyond the forest edge*. Oxford University Press.
- Brand, W. A., Geilmann, H., Crosson, E. R., & Rella, C. W. (2009). Cavity ring-down spectroscopy versus high-temperature conversion isotope ratio mass spectrometry; a case study on delta (2)H and delta (18)O of pure water samples and alcohol/water mixtures. *Rapid Communications in Mass Spectrometry*, 23(12), 1879–1884.
- Briggs, J. M., Knapp, A. K., Blair, J. M., Heisler, J. M., Hoch, G. A., Lett, M. S., & McCarron, J. K. (2005). An ecosystem in transition. Causes and consequences of the conversion of mesic grassland to shrubland. *Bioscience*, 55, 243–254.
- Briggs, J. M., Knapp, A. K., & Brock, B. L. (2002). Expansion of woody plants in tallgrass prairie: A fifteen-year study of fire and fire-grazing interactions. *The American Midland Naturalist*, 147(2), 287–294.
- Canadell, J., Jackson, R. B., Ehleringer, J. B., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). Maximum rooting depth of vegetation types at the global scale. *Oecologia*, 108, 583–595.
- Case, M. F., Nippert, J. B., Holdo, R. M., & Staver, A. C. (2020). Root-niche separation between savanna trees and grasses is greater on sandier soils. *Journal of Ecology*, 108(6), 2298–2308.
- Cheung, Y. N. S., Tyree, M. T., & Dainty, J. (1975). Water relations parameters on single leaves obtained in a pressure bomb and some ecological interpretations. *Canadian Journal of Botany*, 53(13), 1342–1346.
- Cohen, D. (1970). The expected efficiency of water utilization in plants under different competition and selection regimes. *Israel Journal of Botany*, 19(1), 50–54.
- Collins, S. L., & Calabrese, L. B. (2012). Effects of fire, grazing, and topographic variation on vegetation structure in tallgrass prairie. *Journal of Vegetation Science*, 23, 563–575.
- Cowan, I. R., & Farquhar, G. D. (1977). Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology*, 31, 471–505.
- Craine, J. M., & Nippert, J. B. (2014). Cessation of burning dries soils long term in a tallgrass prairie. *Ecosystems*, 17(1), 54–65.
- Currey, B., McWethy, D. B., Fox, N. R., & Brookshire, E. J. (2022). Large contribution of woody plant expansion to recent vegetative greening of the Northern Great Plains. *Journal of Biogeography*, 49(8), 1443–1454.
- Easterling, D. R., Kunkel, K. E., Arnold, J. R., Knutson, T., LeGrande, A. N., Leung, L. R., Vose, R. S., Waliser, D., & Wehner, M. (2017). Precipitation change in the United States. In D. Wuebbles, D. W. Fahey, K. Hibbard, D. J. Dokken, B. C. Stewart, & T. K. Maycock (Eds.), *Climate Science Special Report: Fourth National Climate Assessment, Volume I* (pp. 207–230). U.S. Global Change Research Program. <https://doi.org/10.7930/JOH993CC>
- Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., & Mearns, L. O. (2000). Climate extremes: Observations, modeling, and impacts. *Science*, 289, 2068–2074.

- Edwards, E. J., Osborne, C. P., Strömberg, C. A., Smith, S. A., C4 Grasses Consortium, Bond, W. J., Christin, P. A., Cousins, A. B., Duvall, M. R., Fox, D. L., & Freckleton, R. P. (2010). The origins of C4 grasslands: Integrating evolutionary and ecosystem science. *Science*, 328(5978), 587–591.
- Eggemeyer, K. D., Awada, T., Harvey, F. E., Wedin, D. A., Zhou, X., & Zanner, C. W. (2009). Seasonal changes in depth of water uptake for encroaching trees *Juniperus virginiana* and *Pinus ponderosa* and two dominant C4 grasses in a semiarid grassland. *Tree Physiology*, 29(2), 157–169.
- Ehleringer, J. R. (1993). Carbon and water relations in desert plants: An isotopic perspective. In B. Saugier, J. R. Ehleringer, A. E. Hall, & G. D. Farquhar (Eds.), *Stable isotopes and plant carbon-water relations* (pp. 155–172). Academic Press.
- Ehleringer, J. R., & Cerling, T. E. (2002). C3 and C4 photosynthesis. *Encyclopedia of Global Environmental Change*, 2(4), 186–190.
- Ehleringer, J. R., & Osmond, C. B. (1989). Stable isotopes. In C. B. Field, J. T. Ball, & J. A. Berry (Eds.), *Plant physiological ecology: Field methods and instrumentation* (pp. 281–300). Springer.
- Eldridge, D. J., Bowker, M. A., Maestre, F. T., Roger, E., Reynolds, J. F., & Whitford, W. G. (2011). Impacts of shrub encroachment on ecosystem structure and functioning: Towards a global synthesis. *Ecology Letters*, 14(7), 709–722. <https://doi.org/10.1111/j.1461-0248.2011.01630.x>
- Engle, D. M., Bidwell, T. G., & Moseley, M. E. (1996). *Invasion of Oklahoma rangelands and forests by eastern redcedar and Ashe juniper*. Oklahoma Cooperative Extension Service, Division of Agricultural Sciences and Natural Resources, Oklahoma State University.
- Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences*, 72, 673–689.
- Farquhar, G., Ehleringer, J., & Hubick, K. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, 503–537.
- Fay, P. A., Carlisle, J. D., Danner, B. T., Lett, M. S., McCarron, J. K., Stewart, C., Knapp, A. K., Blair, J. M., & Collins, S. L. (2002). Altered rainfall patterns, gas exchange, and growth in grasses and forbs. *International Journal of Plant Sciences*, 163(4), 549–557.
- Fay, P. A., Carlisle, J. D., Knapp, A. K., Blair, J. M., & Collins, S. L. (2003). Productivity responses to altered rainfall patterns in a C4-dominated grassland. *Oecologia*, 137(2), 245–251.
- Fay, P. A., Kaufman, D. M., Nippert, J. B., Carlisle, J. D., & Harper, C. W. (2008). Changes in grassland ecosystem function due to extreme rainfall events: Implications for responses to climate change. *Global Change Biology*, 14(7), 1600–1608.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., Firth, D., Friendly, M., Gorjanc, G., Graves, S., & Heiberger, R. (2012). *Package 'car'* (p. 16). R Foundation for Statistical Computing.
- Fuhlendorf, S. D., Woodward, A. J., Leslie, D. M., & Shackford, J. S. (2002). Multi-scale effects of habitat loss and fragmentation on lesser prairie-chicken populations of the US southern Great Plains. *Landscape Ecology*, 17, 617–628.
- Garbrecht, J., Van Liew, M., & Brown, G. O. (2004). Trends in precipitation, streamflow, and evapotranspiration in the Great Plains of the United States. *Journal of Hydrologic Engineering*, 9(5), 360–367.
- Gazis, C., & Feng, X. (2004). A stable isotope study of soil water: Evidence for mixing and preferential flow paths. *Geoderma*, 119(1–2), 97–111.
- Gibbens, R. P., McNeely, R. P., Havstad, K. M., Beck, R. F., & Nolen, B. (2005). Vegetation changes in the Jornada Basin from 1858 to 1998. *Journal of Arid Environments*, 61(4), 651–668.
- Grant, T. A., Madden, E., & Berkey, G. B. (2004). Tree and shrub invasion in northern mixed-grass prairie: Implications for breeding grassland birds. *Wildlife Society Bulletin*, 32(3), 807–818.
- Griffin-Nolan, R. J., Ocheltree, T. W., Mueller, K. E., Blumenthal, D. M., Kray, J. A., & Knapp, A. K. (2019). Extending the osmometer method for assessing drought tolerance in herbaceous species. *Oecologia*, 189, 353–363.
- Heisler-White, J. L., Blair, J. M., Kelly, E. F., Harmony, K., & Knapp, A. K. (2009). Contingent productivity responses to more extreme rainfall regimes across a grassland biome. *Global Change Biology*, 15(12), 2894–2904.
- Hibbard, K. A., Archer, S., Schimel, D. S., & Valentine, D. W. (2001). Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. *Ecology*, 82(7), 1999–2011.
- Higgins, S. I., & Scheiter, S. (2012). Atmospheric CO<sub>2</sub> forces abrupt vegetation shifts locally, but not globally. *Nature*, 488(7410), 209–212.
- Holdo, R. M. (2013). Revisiting the two-layer hypothesis: Coexistence of alternative functional rooting strategies in savannas. *PLoS One*, 8, e69625.
- Holdo, R. M., & Nippert, J. B. (2022). Linking resource-and disturbance-based models to explain tree-grass coexistence in savannas. *New Phytologist*, 237, 1966–1979.
- Huxman, T. E., & Monson, R. K. (2003). Stomatal responses of C3, C3–C4 and C4 *Flaveria* species to light and intercellular CO<sub>2</sub> concentration: Implications for the evolution of stomatal behavior. *Plant, Cell and Environment*, 26, 313–322.
- Huxman, T. E., Wilcox, B. P., Breshears, D. D., Scott, R. L., Snyder, K. A., Small, E. E., Hultine, K., Pockman, W. T., & Jackson, R. B. (2005). Ecohydrological implications of woody plant encroachment. *Ecology*, 86(2), 308–319.
- Ilyas, M., Nisar, M., Khan, N., Hazrat, A., Khan, A. H., Hayat, K., Fahad, S., Khan, A., & Ullah, A. (2021). Drought tolerance strategies in plants: A mechanistic approach. *Journal of Plant Growth Regulation*, 40, 926–944.
- IPCC. (2021). *Climate change 2021: The physical science basis*. In V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, & M. Huang (Eds.), *Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change* (pp. 1–31). Cambridge University Press. <https://www.ipcc.ch/report/ar6/wg1/chapter/summary-for-policymakers/>
- Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, 108, 389–411.
- Jones, M. B. (2019). Projected climate change and the global distribution of grasslands. In D. J. Gibson & J. A. Newman (Eds.), *Grasslands and climate change* (pp. 67–81). Cambridge University Press.
- Keen, R. M., & Nippert, J. B. (2024). *SRM01 ShRaMPs (Shrub Rainout Manipulation Plots): Interactive effects of drought and fire on grass and shrub physiology and productivity at Konza Prairie*. Environmental Data Initiative. <https://doi.org/10.6073/pasta/d77b1696b198e152e12b87e8e8996777>
- Keen, R. M., Nippert, J. B., Sullivan, P. L., Ratajczak, Z., Ritchey, B., O'Keefe, K., & Dodds, W. K. (2022). Impacts of riparian and non-riparian woody encroachment on tallgrass prairie ecohydrology. *Ecosystems*, 26, 1–12.
- Klein, T. (2014). The variability of stomatal sensitivity to leaf water potential across tree species indicates a continuum between isohydric and anisohydric behaviours. *Functional Ecology*, 28(6), 1313–1320.
- Knapp, A. K. (1984). Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia*, 65(1), 35–43.
- Knapp, A. K. (1985). Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a tallgrass prairie. *Ecology*, 66(4), 1309–1320.
- Knapp, A. K., Briggs, J. M., Collins, S. L., Archer, S. R., Bret-Harte, M. S., Ewers, B. E., Peters, D. P., Young, D. R., Shaver, G. R., Pendall, E., & Cleary, M. B. (2008). Shrub encroachment in North American grasslands: Shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology*, 14(3), 615–623.
- Knapp, A. K., McCarron, J. K., Silletti, G. A., Hoch, G. L., Heisler, M. S., Lett, J. M., Blair, J. M., Briggs, J. M., & Smith, M. D. (2008).

- Ecological consequences of the replacement of native grassland by *Juniperus virginiana* and other woody plants. In O. W. Van Auken (Ed.), *Western North American Juniperus Communities: A Dynamic Vegetation Type* (pp. 156–169). Springer.
- Kocacinar, F., Mckown, A. D., Sage, T. L., & Sage, R. F. (2008). Photosynthetic pathway influences xylem structure and function in *Flaveria* (Asteraceae). *Plant, Cell & Environment*, 31(10), 1363–1376.
- Kulmatiski, A., & Beard, K. H. (2013). Woody plant encroachment facilitated by increased precipitation intensity. *Nature Climate Change*, 3, 833–837. <https://doi.org/10.1038/nclimate1904>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82(13). <https://doi.org/10.18637/jss.v082.i13>
- Ma, H., Mo, L., Crowther, T. W., Maynard, D. S., van den Hoogen, J., Stocker, B. D., Terrer, C., & Zohner, C. M. (2021). The global distribution and environmental drivers of aboveground versus belowground plant biomass. *Nature Ecology & Evolution*, 5(8), 1110–1122.
- McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D. G., & Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist*, 178(4), 719–739.
- McKinley, D. C., Morris, M. D., Blair, J. M., & Johnson, L. C. (2008). Altered ecosystem processes as a consequence of *Juniperus virginiana* L. encroachment into North American tallgrass prairie. In O. W. Van Auken (Ed.), *Western North American Juniperus Communities: A Dynamic Vegetation Type* (pp. 170–187). Springer.
- Muench, A. T., O'Keefe, K., & Nippert, J. B. (2016). Comparative ecohydrology between *Cornus drummondii* and *Solidago canadensis* in upland tallgrass prairie. *Plant Ecology*, 217(3), 267–276.
- Nippert, J. (2023). APT01 daily precipitation amounts measured at multiple sites across Konza prairie. Environmental Data Initiative. <https://doi.org/10.6073/pasta/9474bf7f9e506a36ae664db049f5a4a7>
- Nippert, J. B., & Knapp, A. K. (2007). Linking water uptake with rooting patterns in grassland species. *Oecologia*, 153(2), 261–272.
- Nippert, J. B., Knapp, A. K., & Briggs, J. M. (2006). Intra-annual rainfall variability and grassland productivity: Can the past predict the future? *Plant Ecology*, 184, 65–74.
- Nippert, J. B., Ocheltree, T. W., Orozco, G. L., Ratajczak, Z., Ling, B., & Skibbe, A. M. (2013). Evidence of physiological decoupling from grassland ecosystem drivers by an encroaching woody shrub. *PLoS One*, 8(12), e81630.
- Nippert, J. B., Wieme, R. A., Ocheltree, T. W., & Craine, J. M. (2012). Root characteristics of C4 grasses limit reliance on deep soil water in tallgrass prairie. *Plant and Soil*, 355(1), 385–394.
- O'Connor, R. C., Blumenthal, D. M., Ocheltree, T. W., & Nippert, J. B. (2022). Elevated CO<sub>2</sub> counteracts effects of water stress on woody rangeland-encroaching species. *Tree Physiology*, tpac150. <https://doi.org/10.1093/treephys/tpac150>
- O'Connor, R. C., Taylor, J. H., & Nippert, J. B. (2020). Browsing and fire decreases dominance of a resprouting shrub in woody encroached grassland. *Ecology*, 101(2), e02935.
- O'Keefe, K., Bachle, S., Keen, R., Tooley, E. G., & Nippert, J. B. (2022). Root traits reveal safety and efficiency differences in grasses and shrubs exposed to different fire regimes. *Functional Ecology*, 36(2), 368–379.
- O'Keefe, K., Bell, D. M., McCulloh, K. A., & Nippert, J. B. (2020). Bridging the flux gap: Sap flow measurements reveal species-specific patterns of water use in a tallgrass prairie. *Journal of Geophysical Research: Biogeosciences*, 125(2), e2019JG005446.
- O'Keefe, K., & Nippert, J. B. (2017). Grazing by bison is a stronger driver of plant ecohydrology in tallgrass prairie than fire history. *Plant and Soil*, 411, 423–436.
- O'Keefe, K., & Nippert, J. B. (2018). Drivers of nocturnal water flux in a tallgrass prairie. *Functional Ecology*, 32(5), 1155–1167.
- O'Keefe, K., Nippert, J. B., & McCulloh, K. A. (2019). Plant water uptake along a diversity gradient provides evidence for complementarity in hydrological niches. *Oikos*, 128(12), 1748–1760.
- Osborne, C. P., & Sack, L. (2012). Evolution of C4 plants: A new hypothesis for an interaction of CO<sub>2</sub> and water relations mediated by plant hydraulics. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 367(1588), 583–600.
- Parnell, A. (2016). simmr: A stable isotope mixing model. R package version 0.3. <https://CRAN.R-project.org/package=simmr>
- Pinno, B. D., & Wilson, S. D. (2011). Ecosystem carbon changes with woody encroachment of grassland in the northern Great Plains. *Ecoscience*, 18(2), 157–163.
- Powles, S. B. (1984). Photoinhibition of photosynthesis induced by visible light. *Annual Review of Plant Physiology*, 35, 15–44.
- R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org>
- Ransom, M. D., Rice, C. W., Todd, T. C., & Wehmueller, W. A. (1998). Soils and soil biota. In A. K. Knapp, J. M. Briggs, D. C. Hartnett, & S. L. Collins (Eds.), *Grassland dynamics—Long-term ecological research in tallgrass prairie* (pp. 48–66). Oxford University Press.
- Ratajczak, Z., Nippert, J. B., Briggs, J. M., & Blair, J. M. (2014). Fire dynamics distinguish grasslands, shrublands and woodlands as alternative attractors in the Central Great Plains of North America. *Journal of Ecology*, 102, 1374–1385.
- Ratajczak, Z., Nippert, J. B., & Collins, S. L. (2012). Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology*, 93(4), 697–703.
- Ratajczak, Z., Nippert, J. B., Hartman, J. C., & Ocheltree, T. W. (2011). Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. *Ecosphere*, 2, art121.
- Ratajczak, Z., Nippert, J. B., & Ocheltree, T. W. (2014). Abrupt transition of mesic grassland to shrubland: Evidence for thresholds, alternative attractors, and regime shifts. *Ecology*, 95(9), 2633–2645.
- Robinson, D., Hodge, A., Griffiths, B. S., & Fitter, A. H. (1999). Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 266(1418), 431–435.
- Rodriguez-Dominguez, C. M., Forner, A., Martorell, S., Choat, B., Lopez, R., Peters, J. M., Pfautsch, S., Mayr, S., Carins-Murphy, M. R., McAdam, S. A., & Richardson, F. (2022). Leaf water potential measurements using the pressure chamber: Synthetic testing of assumptions towards best practices for precision and accuracy. *Plant, Cell & Environment*, 45(7), 2037–2061.
- Roman, D. T., Novick, K. A., Brzostek, E. R., Dragoni, D., Rahman, F., & Phillips, R. P. (2015). The role of isohydric and anisohydric species in determining ecosystem-scale response to severe drought. *Oecologia*, 179, 641–654.
- Sage, R. F. (2004). The evolution of C4 photosynthesis. *New Phytologist*, 161(2), 341–370.
- Salvi, A. M., Smith, D. D., Adams, M. A., McCulloh, K. A., & Givnish, T. J. (2021). Mesophyll photosynthetic sensitivity to leaf water potential in Eucalyptus: A new dimension of plant adaptation to native moisture supply. *New Phytologist*, 230(5), 1844–1855.
- Sanders, G. J., & Arndt, S. K. (2012). Osmotic adjustment under drought conditions. In R. Aroca (Ed.), *Plant responses to drought stress* (pp. 199–230). Springer.
- Sankaran, M., Ratnam, J., & Hanan, N. P. (2004). Tree–grass coexistence in savannas revisited—insights from an examination of assumptions and mechanisms invoked in existing models. *Ecology Letters*, 7(6), 480–490.
- Silvertown, J., Araya, Y., & Gowing, D. (2015). Hydrological niches in terrestrial plant communities: A review. *Journal of Ecology*, 103(1), 93–108.

- Sperry, J. S., Venturas, M. D., Anderegg, W. R., Mencuccini, M., Mackay, D. S., Wang, Y., & Love, D. M. (2017). Predicting stomatal responses to the environment from the optimization of photosynthetic gain and hydraulic cost. *Plant, Cell & Environment*, 40(6), 816–830.
- Staver, A. C., & Bond, W. J. (2014). Is there a 'browse trap'? Dynamics of herbivore impacts on trees and grasses in an African savanna. *Journal of Ecology*, 102(3), 595–602.
- Stevens, N., Lehmann, C. E., Murphy, B. P., & Durigan, G. (2017). Savanna woody encroachment is widespread across three continents. *Global Change Biology*, 23(1), 235–244.
- Tezara, W. M. V. J., Mitchell, V. J., Driscoll, S. D., & Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, 401(6756), 914–917.
- Tooley, E. G., Nippert, J. B., Bachle, S., & Keen, R. M. (2022). Intra-canopy leaf trait variation facilitates high leaf area index and compensatory growth in a clonal woody encroaching shrub. *Tree Physiology*, 42(11), 2186–2202.
- Totschnig, G., Baer, D. S., Wang, J., Winter, F., Hofbauer, H., & Hanson, R. K. (2000). Multiplexed continuous-wave diode-laser cavity ring-down measurements of multiple species. *Applied Optics*, 39(12), 2009–2016.
- Twidwell, D., Rogers, W. E., Wonkka, C. L., Taylor, C. A., Jr., & Kreuter, U. P. (2016). Extreme prescribed fire during drought reduces survival and density of woody resprouters. *Journal of Applied Ecology*, 53(5), 1585–1596.
- USGCRP. (2017). *Climate science special report—Fourth National Climate Assessment* (Vol. I, D. J. Wuebbles, D. W. Fahey, K. A. Hibbard, Eds.). U.S. Global Change Research Program.
- Van Auken, O. W. (2000). Shrub invasions of north American semiarid grasslands. *Annual Review of Ecology and Systematics*, 31(1), 197–215.
- Walter, H. (1971). *Ecology of tropical and subtropical vegetation*. Oliver and Boyd.
- Ward, D., Wiegand, K., & Getzin, S. (2013). Walter's two-layer hypothesis revisited: Back to the roots! *Oecologia*, 172, 617–630.
- Way, D. A., Katul, G. G., Manzoni, S., & Vico, G. (2014). Increasing water use efficiency along the C3 to C4 evolutionary pathway: A stomatal optimization perspective. *Journal of Experimental Botany*, 65(13), 3683–3693.
- Weaver, J., & Darland, R. (1949). Soil-root relationships of certain native grasses in various soil types. *Ecological Monographs*, 19, 303–338.
- Weaver, J. E. (1968). *Prairie plants and their environment: A fifty-year study in the Midwest*. University of Nebraska Press.
- Wedel, E. R., O'Keefe, K., Nippert, J. B., Hoch, B., & O'Connor, R. C. (2021). Spatio-temporal differences in leaf physiology are associated with fire, not drought, in a clonally integrated shrub. *AoB Plants*, 13(4), plab037.
- West, A. G., Goldsmith, G. R., Brooks, P. D., & Dawson, T. E. (2010). Discrepancies between isotope ratio infrared spectroscopy and isotope ratio mass spectrometry for the stable isotope analysis of plant and soil waters. *Rapid Communications in Mass Spectrometry*, 24(14), 1948–1954.
- Wolf, A., Anderegg, W. R., & Pacala, S. W. (2016). Optimal stomatal behavior with competition for water and risk of hydraulic impairment. *Proceedings of the National Academy of Sciences*, 113(46), E7222–E7230.
- Woods, N. N., Dows, B. L., Goldstein, E. B., Moore, L. J., Young, D. R., & Zinnert, J. C. (2019). Interaction of seed dispersal and environmental filtering affects woody encroachment patterns in coastal grassland. *Ecosphere*, 10(7), e02818.
- Zhou, Y., Boutton, T. W., & Wu, X. B. (2018). Soil phosphorus does not keep pace with soil carbon and nitrogen accumulation following woody encroachment. *Global Change Biology*, 24(5), 1992–2007.
- Zhou, Y., Singh, J., Butnor, J. R., Coetsee, C., Boucher, P. B., Case, M. F., Hockridge, E. G., Davies, A. B., & Staver, A. C. (2022). Limited increases in savanna carbon stocks over decades of fire suppression. *Nature*, 603(7901), 445–449.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** Comparison between uncorrected  $\delta^{18}\text{O}$  data from the Picarro WS-CRDS isotopic water analyzer and corrected  $\delta^{18}\text{O}$  data.

**Figure S2.** Calibration curves used for correction of contaminated samples.

**Figure S3.** Measured  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for all soil depths (0, 10, 20 and >30 cm) sampled at four time points during the 2021 (A) and 2022 (B) growing seasons.

**Figure S4.** Soil isotope profiles at four time points during the 2021 (A) and 2022 (B) growing seasons.

**Figure S5.** Stable isotope profiles for deep soil cores (0–1 m) collected during each growing season.

**Figure S6.** (A) Net photosynthetic rates ( $A_{\text{net}}$ ), (B) Stomatal conductance ( $g_s$ ), (C) transpiration rates ( $E$ ), and (D) intrinsic water use efficiency (iWUE) for *A. gerardii* ( $C_4$  grass; green) and *C. drummondii* ( $C_3$  shrub; blue) throughout the 2021 and 2022 growing seasons.

**Figure S7.** Changes in leaf internal  $\text{CO}_2$  concentration ( $C_i$ ) in response to changes in midday leaf water potential ( $\Psi_{\text{leaf}}$ ) for *A. gerardii* ( $C_4$  grass; green) and *C. drummondii* ( $C_3$  shrub; blue) during the 2021 and 2022 growing seasons.

**Figure S8.** Total fine root biomass (A),  $C_3$  fine root biomass (B), and  $C_4$  fine root biomass (C) in surface soil (0–10 cm) collected at the end of the 2022 growing season.

**Figure S9.** Predawn leaf water potential (A), midday leaf water potential (B), diurnal change in leaf water potential (C; predawn  $\Psi_{\text{leaf}}$  – midday  $\Psi_{\text{leaf}}$ ) for *C. drummondii* and *A. gerardii* throughout the 2021 and 2022 growing seasons.

**Table S1.** Replication and sampling frequency for plant physiological variables and soil samples for isotopic analysis.

**Table S2.** Stable isotope mixing model (simmr) output for proportional use of each water source (0, 10, and >30 cm soil depth) at four time points during the 2021 and 2022 growing seasons for *A. gerardii* (A) and *C. drummondii* (B).

**Table S3.**  $p$ -Values from mixed effects models assessing differences in soil water  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values by soil depth and through time during the 2021 and 2022 growing seasons.

**Table S4.**  $p$ -Values for mixed effects models assessing changes in stomatal conductance ( $g_s$ ), transpiration rates ( $E$ ), water use efficiency (iWUE), and turgor loss point ( $\pi_{\text{TLp}}$ ) throughout the 2021 and 2022 growing seasons for *A. gerardii* (A) and *C. drummondii* (B).

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## APPENDIX 1

## SAVE OR SPEND? DIVERGING WATER-USE STRATEGIES OF GRASSES AND CLONAL SHRUBS IN TALLGRASS PRAIRIE

**ShRaMPs (Shrub Rainout Manipulation Plots) experimental design**

The ShRaMPs experimental site is located at Konza Prairie Biological Station (KPBS) in northeastern KS, USA. ShRaMPs is a drought × fire experiment located in adjacent watersheds K01B (1-year burn frequency, un-grazed) and K04A (4-year burn frequency, un-grazed) at KPBS (Figure A1). Watershed K01B has been annually burned for 12 years (2011–2022) but was previously burned every 4-years (1980–2008), which allowed for the limited establishment of *C. drummondii* shrubs before annual burning was implemented. K04A has been burned every 4 years since 1980. K01B was burned in the spring (March or April) each year of the experiment (2018–2022). K04A was burned in the spring of 2017 (prior to the start of this experiment) and again in the spring of 2021. In lowland positions on both watersheds, seven passive rainout shelters (6 × 6 m) were constructed over naturally-established, intact *C. drummondii* shrubs and their surrounding herbaceous communities. Shelters were built in 2017 and drought treatment was implemented in 2018. In each watershed, 4 shelters were built over randomly selected *C. drummondii* shrub islands for the drought treatment, and 3 shelters were built over randomly selected *C. drummondii* shrub islands for the control treatment.

Shelter design was modeled after the long-term RaMPs (Rainout Manipulation Plots) experiment, also at KPBS (Fay et al., 2002, 2003; <http://www.konza.ksu.edu/ramps/>). Drought shelters were designed to exclude ~50% of incoming precipitation using polyethylene roofing panels and gutter systems that route blocked precipitation away from the plots (Figure A2b). The sides and ends (north and south) of the shelters were left open to maximize airflow and minimize changes in relative humidity and temperature in the plots (Figure A1). Drought plots were trenched, and metal flashing was installed to a depth of 15 cm to reduce lateral water flow into the plots at the soil surface and through surficial soils. In 2021, we added (1) additional paneling to the north side of each drought shelter to reduce blow-in from rainstorms approaching from the northeast and (2) additional flashing on the north side of drought shelters

that were on a slope to reduce any overland flow that might occur during heavy rain events. Polyethylene roofs were also added to control shelters, but small circular holes cut in the roofing allowed ambient precipitation to reach the underlying plant communities. As such, control shelter roofs mimic any microclimatic effects without the rainfall exclusion (Figure A2a). Shelter roofs were removed for roughly 1 month each spring (typically mid-February to mid-March) when watersheds were burned, but otherwise remained intact year-round. Each watershed contained four drought shelters and three control shelters for a total of 14 shelters.

Each shelter was equipped with 30 cm time-domain reflectometry probes (Campbell Scientific) at soil depths of 10, 15, and 30 cm. Soil moisture data was recorded at 30-min intervals on Campbell Scientific data loggers (CR1000X). A weather station was constructed on-site to measure PAR (photosynthetically active radiation), relative humidity, and air temperature throughout the study period. Weather station data was recorded at 30-min intervals on a Campbell Scientific data logger (CR1000). Daily precipitation data was collected at KPBS headquarters, ~5 km away from the study site, using an Ott Pluvio2 rain gauge (Nippert, 2023).

## APPENDIX 2

## SAVE OR SPEND? DIVERGING WATER-USE STRATEGIES OF GRASSES AND CLONAL SHRUBS IN TALLGRASS PRAIRIE

**Stable isotope sample processing and analysis details****Cryogenic vacuum distillation line**

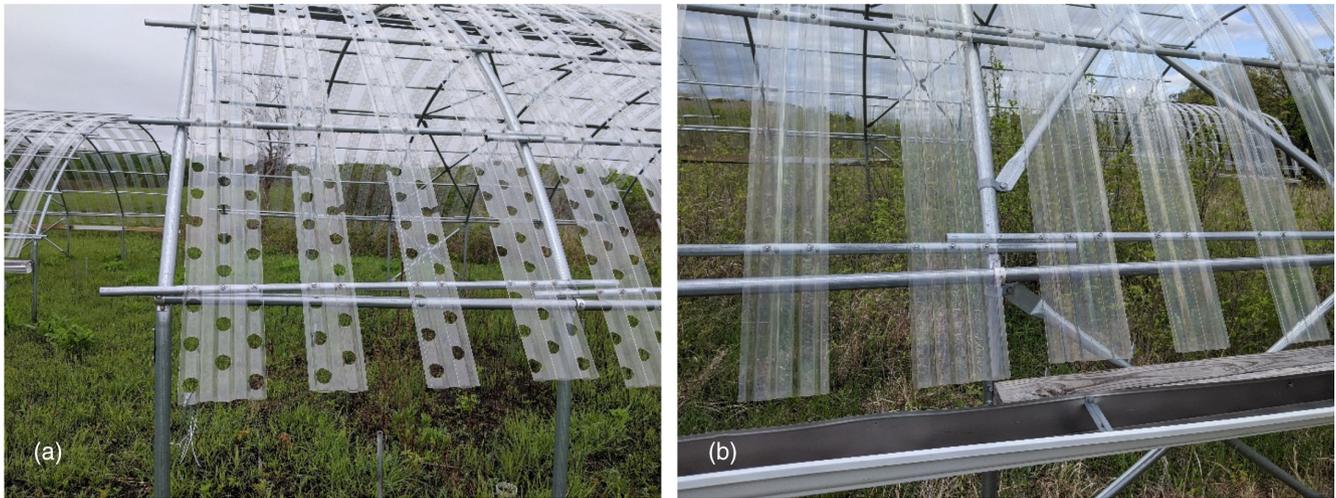
The water bath used during the extraction process was set at 100°C and samples were extracted for either 30 min (soils) or 45 min (plants). These extraction times have been previously assessed to result in complete extraction efficiency. For soils from KPBS, gravimetric content varies from ~13% to 28%. For the plant tissues collected for extraction, water content was ~25% to 40% by mass.

**Picarro analyses (K-State Stable Isotope Mass Spectrometry Lab)**

The Picarro collects six injections per sample with the first three discarded and the last three retained for averaging. The stable isotope lab at Kansas State University uses four internally created water lab standards, all calibrated against VSMOW every other

**FIGURE A1** ShRaMPs experimental design at KPBS. Drought and control shelters were built over intact *C. drummondii* shrub patches on neighboring 4-year burn (left) and 1-year burn (right) watersheds.





**FIGURE A2** Control (a) and drought (b) shelter roof and gutter design at ShRaMPs.

year. Three of the standards were used for the creation of a calibration curve and were run for every 16 samples processed. The fourth standard is a working lab standard used to assess potential drift over the time period of analysis. This working standard was analyzed after every four samples processed.

#### LGR analyses (University of Pennsylvania)

The LGR isotope analyzer collects ten injections per sample, with the first four discarded in high precision mode and the last six

retained for averaging. Three USGS standards were used (USGS 47, 48 and 50), but the same 4 lab standards from the Kansas State University stable isotope lab (see above) were also inserted throughout the run as a blind internal comparison. A standard was run after every four samples with all standards run in sequence at the beginning and end of each run.