ORIGINAL RESEARCH

Contrasting shrub and grass hydraulic responses to experimental drought

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Abstract

Whole-plant hydraulics provide important information about responses to water limitation and can be used to understand how plant communities may change in a drier climate when measured on multiple species. Here, we measured above- and belowground hydraulic traits in *Cornus drummondii*, an encroaching shrub within North American tallgrass prairies, and *Andropogon gerardii*, a dominant C_4 grass, to assess the potential hydraulic responses to future drought as this region undergoes woody expansion. Shelters that reduced precipitation by 50% and 0% were built over shrubs and grasses growing in sites that are burned at 1-year and 4-year frequencies. We then measured aboveground (K_{shoot}), belowground (K_{root}), and whole-plant maximum hydraulic conductance (K_{plant}) in *C. drummondii* and K_{root} in *A. gerardii*. We also measured vulnerability to embolism (P_{50}) in *C. drummondii* stems. Overall, we show that: (1) *A. gerardii* had substantially greater K_{root} than *C. drummondii*; (2) belowground hydraulic functioning was linked with aboveground processes; (3) above- and belowground *C. drummondii* hydraulics were not negatively impacted by the rainfall reductions imposed here. These results suggest that a multi-year drought will not ameliorate rates of woody expansion and highlight key differences in aboveground and belowground hydraulics for dominant species within the same ecosystem.

Keywords Grasslands · Woody encroachment · Drought · Roots · Plant hydraulics

Introduction

Woody plants have increased in abundance and distribution in herbaceous ecosystems globally over the past century (Ravi et al. 2009; Archer et al. 2017). This shift in land cover, known as woody encroachment, can have substantial impacts on the structure and functioning of grasslands and savannas. For instance, conversion from herbaceous to woody-dominated systems can alter aboveground net primary productivity (Knapp et al. 2008), reduce vegetation diversity (Ratajczak et al. 2012), alter soil microbial processes (Mureva and Ward 2017), reduce surface runoff and soil water recharge (Zou et al. 2018), and alter ecosystem-level water fluxes (Wang et al. 2010; Logan and Brunsell 2015; Keen et al. 2022). Woody encroachment has been attributed to a variety of drivers including reduced fire frequency (Briggs et al. 2005), overgrazing (Archer 2010), loss of native browsers (O'Connor et al. 2020), and rising atmospheric CO_2 concentrations (Archer et al. 1995). However, woody encroachment dynamics are often site-specific, which can complicate predictions of land cover change in different ecosystems. Predicting patterns and consequences of woody encroachment is also complicated by an incomplete understanding of how different woody species respond to ongoing climate changes. Improved predictions of ecosystem structure and function in historically herbaceous ecosystems will therefore require a detailed understanding of the mechanisms facilitating the expansion of individual woody species in specific ecosystems, under both current and future climate conditions.

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Several woody species are exhibiting encroachment throughout the Great Plains region of North America including *Cornus drummondii* C.A. Mey. (roughleaf dogwood), *Rhus glabra* L. (smooth sumac), and *Juniperus virginiana* L. (eastern red cedar) (Ratajczak et al. 2014a). *C. drummondii* is native to tallgrass prairie but has increased in abundance and distribution over the past several decades due to reduced fire frequency in the region (Ratajczak et al. 2014b). When burned infrequently, this clonal shrub grows large and densely enough to limit herbaceous growth in its understory and consequently reduces fuel loads such that subsequent fires are far less intense (Ratajczak et al. 2014a). Additionally, *C. drummondii* is deep-rooted and uses deep soil water throughout the growing season (Ratajczak et al. 2011). Consistent use of deep water allows this shrub species to maintain stable physiological rates despite seasonal fluctuations in temperature and precipitation (Muench et al. 2016), resulting in substantially greater water-use than co-occurring shrubs and herbaceous species (O'Keefe et al. 2020). Thus, the reliance of *C. drummondii* on a stable water source that is inaccessible to many neighboring species may also contribute to its ongoing expansion in the Great Plains.

That *C. drummondii* thrives on deep soil water raises the question: would encroachment by this species change without access to a reliable water supply? Water stored deep in the soil is typically recharged annually by winter precipitation (Ransom et al. 1998) and may decline over time as drought intensifies and/or woody utilization of deep water increases with expanding woody cover (Vero et al. 2018). Furthermore, the rate and extent of soil water depletion may be exacerbated by the fire regime experienced by the system. Fire frequency impacts soil water availability such that annually burned soils are drier and more susceptible to drought than infrequently burned soils (Knapp et al. 1993; Briggs and Knapp 1995). Drier, shallow soils can cause plants with dimorphic root systems to switch reliance from shallow to deep water sources (Nippert and Knapp 2007), which may further diminish deep supplies. If deep water stores are depleted, *C. drummondii* may experience water limitation that impacts its physiology, growth, and ability to shade out grasses. However, whether these responses also impact its expansion across the landscape depends in part on the response of grasses to drought. Grasses often dominate in herbaceous systems due to their superior abilities to use shallow soil water (Holdo and Nippert 2023). If water limitation impacts grass functioning such that they lose their competitive advantage for resource extraction, woody plants may increase in dominance. Consequently, an understanding of both woody and grass responses to drought, specifically under different fire regimes, is necessary to predict the extent of future woody expansion in tallgrass prairies.

In particular, hydraulic traits may provide key insights into responses of *C. drummondii* and co-occurring grasses to drought. Hydraulic traits (e.g., hydraulic conductivity, vulnerability to embolism, hydraulic capacitance) are those that influence water transport throughout the plant and consequently, plant performance when water is both abundant and limiting. Characteristics such as hydraulic conductivity and vulnerability to embolism mechanistically link water loss with carbon gain (Anderegg et al. 2018), reduce uncertainty in model estimates of water flux and community dynamics (Christoffersen et al. 2016; Mencuccini et al. 2019), and have even been used to explain widespread plant mortality across broad spatial scales (Choat et al. 2012; Anderegg et al. 2015). Furthermore, when measured on multiple species, these traits have the potential to predict community and ecosystem responses to drought (Griffin-Nolan et al. 2018). Hydraulic traits are tightly coordinated, and thus, are most informative when studied in multiple organs throughout entire plants (McCulloh et al. 2019). However, our understanding of whole-plant hydraulic functioning in herbaceous and woody grassland species is remarkably limited. Previous studies in grasslands have examined leaf hydraulics in grasses (Ocheltree et al. 2014, 2016), but almost no information exists regarding water movement through roots or stems (but see Martre et al. 2001; Meunier et al. 2018). Knowledge about hydraulic traits in *C. drummondii* roots and stems, as well as in the root systems of co-occurring grasses, will be crucial for understanding how tallgrass prairie communities respond to water limitation in an era of climatic and land-use changes.

Our objective was to assess how experimental changes in water availability and fire frequency impact hydraulic functioning in *C. drummondii* and *Andropogon gerardii* Vitman (big bluestem), a dominant C_4 grass. We were specifically interested in understanding the hydraulic responses that facilitate water movement aboveground (shoot maximum hydraulic conductance; K_{shoot}), belowground (root maximum hydraulic conductance; K_{root}), and throughout whole-plants (whole-plant maximum hydraulic conductance; K_{plant}), as well as aboveground hydraulic vulnerability to loss of function under water limitation (stem vulnerability to embolism as assessed by the pressure inducing 50% loss of hydraulic conductivity; P_{50}). Specifically, we assessed the following questions: (1) Does K_{root} differ between *A. gerardii* and *C. drummondii*? (2) How does *A. gerardii* K_{root} vary under experimental fire and drought? (3) How do *C. drummondii* hydraulics (K_{shoot}, K_{root}, K_{plant}, and stem P_{50}) vary under experimental fire and drought? Given that grasses have fibrous shallow root systems that are efficient at water uptake (Ma et al. 2018; Wargowsky et al. 2021), we hypothesized that *A. gerardii* would have greater K_{root} than *C. drummondii*. However, because the deep root system of *C. drummondii* relies on stable water while the shallow root system of *A. gerardii* routinely experiences fluctuations in water availability, we hypothesized that *C. drummondii* hydraulics would

be negatively impacted by a multi-year drought while *A. gerardii* would adjust its hydraulics less to drought. Because fire can further reduce soil moisture, we hypothesized that *C. drummondii* will experience greater reductions in maximum hydraulic conductance under drought when more frequently burned. Finally, we predicted that under more water stressed conditions, plants would increase the water supply to a given leaf area by reducing leaf area and/or increasing the pressure gradient from the roots to leaves.

Materials and methods

Site description

Research was conducted in 2019 and 2020 at the Konza Prairie Biological Station (KPBS), a Long-Term Ecological Research (LTER) site located in the Flint Hills region of northeastern Kansas, USA (39.1° N, 96.9° W). KPBS is a 3487 ha area of native tallgrass prairie that is divided into experimental watersheds, each of which receives various fire (burned every 1, 2, 4, or 20 years) and grazing (grazed by *Bison bison*, cattle, or ungrazed) treatment combinations. The landscape is generally dominated by few dominant C_4 grass species and many subdominant forb and shrub species (Smith and Knapp 2003).

KPBS experiences a midcontinental climate with cool, dry winters and warm, wet summers. Long-term mean annual precipitation is 842 mm (1982–2021), approximately 75% of which occurs during the growing season (April–September). Annual precipitation was 970.8 mm in 2019 and 832.8 mm in 2020. Long-term (1982–2021) mean annual temperature at this site is 12.7 ℃ and mean growing season (May–September) temperature is 22.6 ℃. Growing season temperatures averaged 22.5 and 22.3 ℃ in 2019 and 2020, respectively, and growing season maximum temperatures reached 36.8 and 35.6 ℃ in 2019 and 2020, respectively (Nippert 2024). During the growing season, mean relative humidity between 9:00 and 13:00 was 62.5% in 2019 and 61.9% in 2020; maximum relative humidity exceeded 99% during the same daytime hours in both growing seasons (Nippert 2024).

Experimental setup

This study was conducted in lowland locations of two ungrazed watersheds, one of which is burned every year (K1B) and one of which is burned every 4 years (K4B). Rainout shelters (6.3 m^2) that reduce precipitation by 50% (drought) or 0% (control) were built in 2018 over mature *C. drummondii* and co-occurring C_4 grasses. Four control shelters and three drought shelters were built in each watershed, resulting in 14 total shelters. Precipitation treatment differences were verified with volumetric soil moisture measurements recorded every 30 min using 30 cm Campbell Scientific CS605 time domain reflectometry (TDR) probes at 10, 15, and 30 cm depths in each shelter. These data showed that soil moisture at the 10, 15, and 30 cm depths was an average of 20.9%, 18.6%, and 10.4% lower, respectively, under the drought shelters compared to the control shelters (Table S1, Fig S1, Online Resource 1).

Maximum hydraulic conductance

We used a high-pressure flow meter (HPFM; Tyree et al. 1993) to measure maximum hydraulic conductance (K; g m^{-2} s^{-1} MPa^{-1}) in intact *C. drummondii* and *A. gerardii* roots (K_{root}) and in *C. drummondii* shoots (K_{shoot}). Maximum hydraulic conductance is a metric of ease of water flow through a tissue with native embolisms removed. Three clonal ramets were measured on the same individual shrub per shelter and three individual grasses were measured per shelter. *C. drummondii* maximum hydraulic conductance measurements were taken twice per year in 2019 and 2020 (June and August each year; DOY 142–149 and 217–227 in 2019; DOY 154–156 and 210–218 in 2020). Because *A. gerardii* stems were too small to maintain a tight seal with the HPFM in 2019 and early in the 2020 growing season, maximum hydraulic conductance measurements were only collected in August 2020.

For *C. drummondii*, each ramet was cut near the soil surface and the protruding root end was wrapped in a damp paper towel while the shoot end was attached to the HPFM. A 20 mmol L^{-1} KCl solution flowed from the HPFM into the shoot and the pressure differential of the solution entering the sample was monitored until stabilized (approximately 10–15 min). The pressure differential was then recorded along with the temperature of the solution. Immediately following the shoot measurement, the HPFM tubing was attached to the root end and maximum root hydraulic conductance was measured using the same method. For each ramet, stem diameter was recorded and each leaf was collected in a plastic bag. Leaves were then brought back to the laboratory and their area measured with a 3100C leaf area meter (Li-Cor, Inc., Lincoln, NE, USA). Maximum hydraulic conductance was measured on *A. gerardii* roots as described above for *C. drummondii* roots. We could not measure *A. gerardii* shoots due to extensive water leakage through the leaf sheath. For *A. gerardii*, a smaller gasket was used to attach the root crown tissue to the HPFM and the entire cut tiller was collected to measure leaf area.

Leaf area-specific maximum hydraulic conductance was calculated for all samples as:

$$K = \frac{\left(\left(\frac{F}{P}\right) \times \left(0.359 + 1.421^{(-0.04 * T)}\right)\right)}{\text{Leaf area}} \qquad (1)$$

where F is the flow rate calculated from the pressure differential and a calibration coefficient, P is the pressure of the water entering the sample, and T is the temperature of the solution (Yang and Tyree 1994). The calibration coefficient was calculated prior to measurements by measuring flow rate through the HPFM at set pressures using a gravity feed apparatus. The constants were used to correct the viscosity of the solution based on the solution temperature (Kestin et al. 1978), and the entire calculation was standardized by the leaf area of that sample. To understand the impacts of K_{root} and K_{shoot} on whole-plant water-use, we calculated whole-plant maximum conductance (K_{plant}) as:

$$K_{plant}^{-1} = K_{shoot}^{-1} + K_{root}^{-1} \qquad (2)$$

Vulnerability to embolism

We also assessed the vulnerability of *C. drummondii* stems to loss of hydraulic function by measuring hydraulic vulnerability curves in July 2019. One *C. drummondii* stem ($\sim$50 cm long and 2.1–7.5 mm diameter) was collected from each shelter, wrapped in damp paper towels, double-bagged, and shipped overnight to the University of Wisconsin-Madison. Samples were recut under water to 14.0–20.4 cm in length with a razor blade and rehydrated in a 20 mmol L^{-1} KCl solution under a partial vacuum overnight. The following day, hydraulic conductivity was measured on fully hydrated samples using a hydrostatic pressure head. Hydraulic conductivity was then repeatedly measured as each sample was subjected to increasingly negative xylem pressures using the centrifuge method (Alder et al. 1997). The pressure at which 50% of the maximum hydraulic conductivity was lost (P_{50}) was then calculated for all curves within each water treatment*fire treatment group. Vulnerability curves were analyzed with a Weibull model using the fitplc package (Duursma and Choat 2017). Differences in P_{50} values between treatments were determined as having non-overlapping 95% confidence intervals.

Leaf water potential

Hydraulic conductance is mediated in part by water potential gradients throughout the plant following:

$$K = \frac{Q}{\Delta\Psi} \qquad (3)$$

where Q is the flow rate of water and $\Delta\Psi$ is the pressure difference throughout the plant (e.g., $\Psi_{root} - \Psi_{leaf}$). To provide additional insight regarding patterns of whole-plant water movement, we estimated this pressure difference using predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potential measurements. Leaf water potential was measured on *C. drummondii* and *A. gerardii* using a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA). Measurements were made once in each month that maximum hydraulic conductance was measured (DOY 161 and 232 in 2019 and DOY 154 and 224 in 2020 for *C. drummondii*; DOY 224 in 2020 for *A. gerardii*), within approximately a week of conductance measurements. For each set of measurements, 3 leaves per species were collected in each plot and equilibrated in a plastic bag for approximately 60 min prior to measurement. Leaves for predawn measurements were collected approximately 1 h before sunrise and leaves for midday measurements were collected at 12:00 h. Assuming Ψ_{PD} is approximately equivalent to the water potential of the rhizosphere, we then calculated the pressure differential as $\Delta\Psi_{leaf} = -(\Psi_{PD} - \Psi_{MD})$.
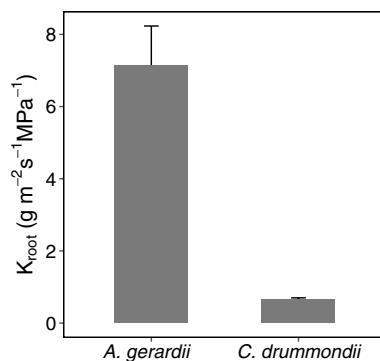
Statistics

All statistical analyses were performed in R version 4.1.1 (Team RC 2021). All data were checked for normality using normal qq-plots and homogeneity of variance was determined by assessing residuals versus fitted plots (Faraway 2016). Data were then log- or square-root transformed as necessary (see Online Resource 1). We used the following analyses to address each question:

(1) Does K_{root} differ between *A. gerardii* and *C. drummondii*? We addressed our first question using a fixed effects linear model to compare K_{root} between *A. gerardii and C. drummondii*, for all treatments combined. Because *A. gerardii* data were only collected in August 2020, we also only included *C. drummondii* data collected in August 2020 in these analyses.

(2) How does *A. gerardii* K_{root} vary under experimental fire and drought? To address our second question, we compared *A. gerardii* K_{root} across treatments using a fixed effects linear model, with precipitation treatment and fire treatment as fixed effects. We used the same model to compare *A. gerardii* leaf area, Ψ_{PD}, Ψ_{MD}, and $\Delta\Psi_{leaf}$ across precipitation and fire treatments. Finally, we used a linear regression model to compare the relationship between *A. gerardii* K_{root} (unstandardized by leaf area) and leaf area.

(3) How do *C. drummondii* hydraulics (K_{shoot}, K_{root}, K_{plant}, and stem P_{50}) vary under experimental fire and drought? We addressed our third question by comparing *C. drummondii* K_{shoot}, K_{root}, and K_{plant} across treatments with a fixed effects linear model, using precipitation treatment, fire treatment, and sampling date
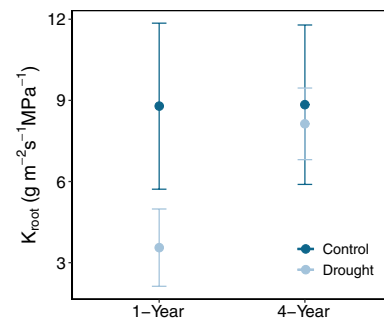
as fixed effects. The same model was used to evaluate *C. drummondii* leaf area, Ψ_{PD}, Ψ_{MD}, and $\Delta\Psi_{leaf}$ across precipitation treatments, fire treatments, and sampling date. The relationships of leaf area with K_{shoot}, K_{root}, and K_{plant} (all unstandardized by leaf area) were analyzed with linear regression models.

Results

(1) Does K_{root} differ between *A. gerardii* and *C. drummondii*? Maximum root hydraulic conductance (K_{root}) was significantly lower in *C. drummondii* than in *A. gerardii* ($p < 0.001$; Table S2, Online Resource 1). Specifically, K_{root} was 90% lower in *C. drummondii* compared to *A. gerardii* in August 2020 (Fig. 1).

(2) How does *A. gerardii* K_{root} vary under experimental fire and drought? We found that *A. gerardii* K_{root} was generally lower in the drought treatment when annually burned, although this trend was not significant ($p = 0.058$; Fig. 2; Table S3, Online Resource 1). Leaf area was significantly greater in the drought treatment for annually burned *A. gerardii* ($p = 0.042$; Fig. 3; Table S4, Online Resource 1), which would consequently lower leaf-area specific K_{root} (Eq. 1). However, when not standardized by leaf area, *A. gerardii* K_{root} and leaf area exhibited no relationship ($p = 0.480$; Fig. S2d; Table S5, Online Resource 1). Finally, we found evidence that the drop in K_{root} was associated with changing water potential gradients. Ψ_{MD} was lower in annually burned *A. gerardii* compared to those burned every four years ($p < 0.001$; Fig. S3; Table S6, Online Resource 1), resulting in a larger pressure differential ($\Delta\Psi_{leaf}$) ($p < 0.001$; Fig. 4; Table S7, Online Resource 1).

(3) How do *C. drummondii* hydraulics (K_{shoot}, K_{root}, K_{plant}, and stem P_{50}) vary under experimental fire

and drought? Aboveground shrub hydraulics differed between fire treatments but not precipitation treatments. K_{shoot} was significantly greater in annually burned shrubs compared to shrubs burned every four years, particularly in June and August 2020 ($p < 0.001$; Fig. 5b; Table S8, Online Resource 1). Annually burned shrubs had lower leaf area than shrubs burned every four years ($p < 0.001$; Fig. 6a, b; Table S4, Online Resource 1), which would increase leaf-area specific K_{shoot} in shrubs burned every four years (Eq. 1). When comparing pressure differentials, we found that $\Delta\Psi_{leaf}$ was significantly lower in annually burned shrubs compared to those burned every four years ($p < 0.040$) and during June compared to August ($p < 0.001$; Fig. 4a, b; Table S7, Online Resource 1).

Belowground shrub hydraulics differed between precipitation treatments but not fire treatments. K_{root} was generally greater in droughted *C. drummondi* shrubs compared to shrubs from the control treatment, particularly in August

Fig. 2 Maximum root hydraulic conductance (K_{root}) for *A. gerardii*, measured in control and drought precipitation treatments and in watersheds burned every 1 and 4 years. Shown are means $\pm$ 1SEM and $n = 3$–4 per treatment combination. See Table S3 for corresponding statistics
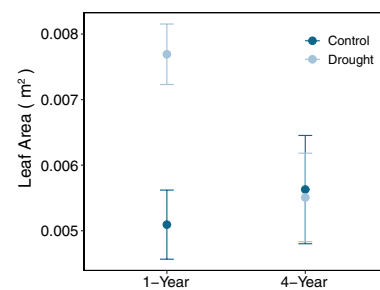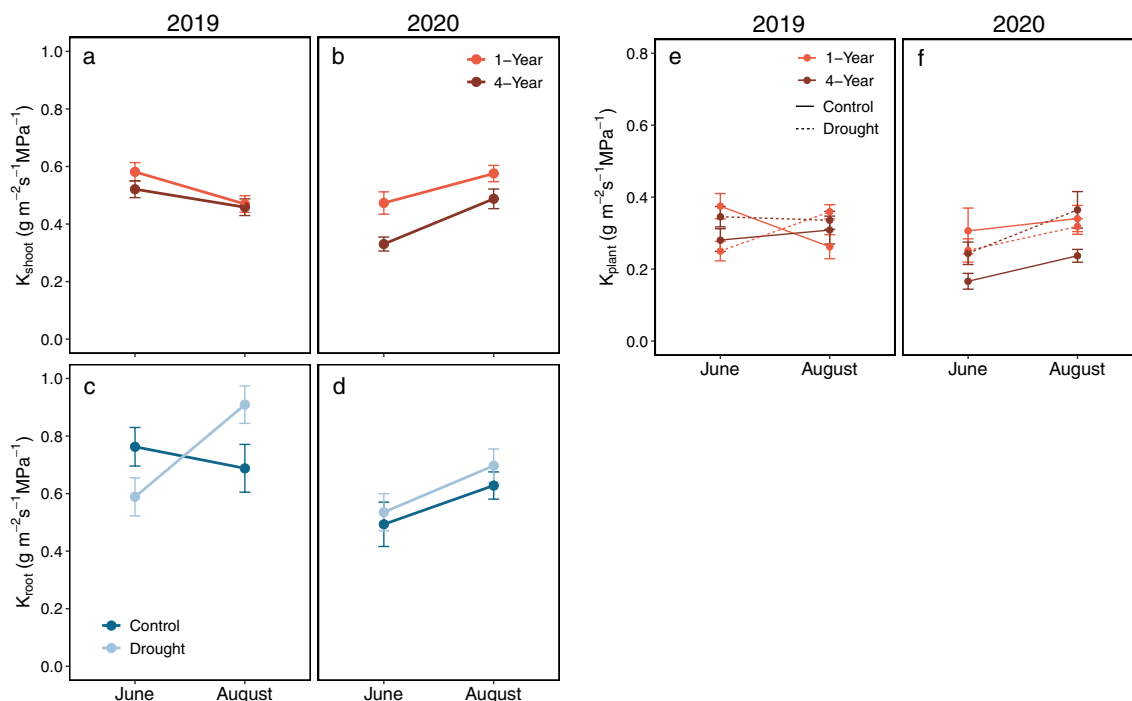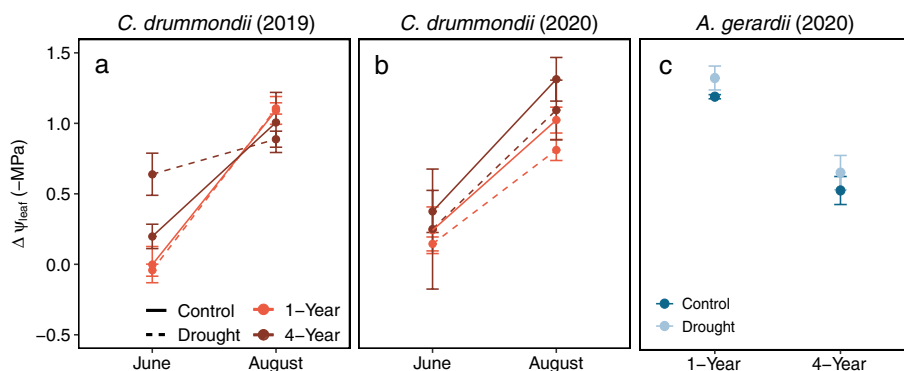

Fig. 1 Maximum root hydraulic conductance (K_{root}) for *A. gerardii* and *C. drummondii*. Shown are means $\pm$ 1SEM and $n = 3$–4 per treatment combination. See Table S2 for corresponding statistics

Fig. 3 Leaf area measured for *A. gerardii* in control and drought precipitation treatments and in watersheds burned every 1 and 4 years. Shown are means $\pm$ 1SEM and $n = 3$–4 per treatment combination. Corresponding statistics are shown in Table S4

Fig. 4 The pressure differential ($\Delta\Psi_{leaf}$) between $\Psi_{PD} - \Psi_{MD}$ calculated for *C. drummondii* in **a** 2019 and **b** 2020, as well as for **c** *A. gerardii* in 2020. Shown are means $\pm$ 1SEM and $n = 3$–4 per treatment combination. Corresponding statistics are shown in Table S7

Fig. 5 **a**, **b** Maximum shoot (K_{shoot}), **c**, **d** root (K_{root}), and **e**, **f** whole-plant (K_{plant}) hydraulic conductance for *C. drummondii* measured in **a**, **b**, **e**, **f** watersheds burned every 1 and 4 years and **c**, **d**, **e**, **f** for *C. drummondii* growin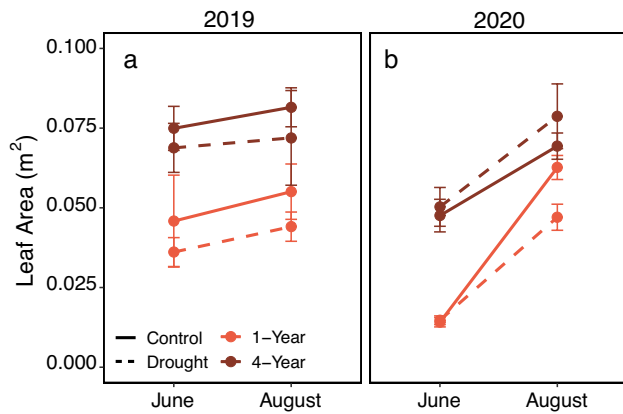g in control and drought precipitation treatments throughout **a**, **c**, **e** 2019 and **b**, **d**, **f** 2020. Shown are means $\pm$ 1SEM and $n = 3$–4 per treatment combination. See Table S8 for corresponding statistics

2019 ($p = 0.046$; Fig. 5c; Table S8, Online Resource 1). K_{root} was also greater in August compared to June for all treatments except the control shrubs in 2019 ($p = 0.002$; Fig. 5c, d). Leaf area was generally lower in drought treatment compared to the control ($p = 0.002$), noticeably so in August 2019, which likely increased K_{root} calculated on a leaf area basis (Eq. 1).

Whole plant hydraulics were generally more variable than above or belowground maximum conductance considered alone. We found that K_{plant} varied significantly across precipitation * fire treatment ($p = 0.033$), and fire * sampling date combinations ($p = 0.002$), with 4-year burned, droughted shrubs having higher K_{plant} values than control shrubs in

both years (Fig. 5e, f; Table S8, Online Resource 1). In 2020, K_{plant} was greater in annually burned shrubs under control water conditions compared to droughted shrubs and during August compared to June (Fig. 5f). Finally, when not calculated on a leaf area basis, K_{plant} ($p < 0.001$), as well as K_{shoot} ($p < 0.001$) and K_{root} ($p < 0.001$), all exhibited strong positive relationships with leaf area (Fig. S2a-b-c; Table S5, Online Resource 1).

Stem vulnerability to embolism (P_{50}) ranged between -4.22 and -3.18 MPa and was generally lower in stems from the 4-year burn treatment compared to stems from the 1-year burn treatment (Fig. S5, Online Resource 1; Table 1). Annually burned stems from the drought treatment

Fig. 6 Leaf area measured for *C. drummondii* in control and drought precipitation treatments and in watersheds burned every 1 and 4 years, across **a** 2019 and **b** 2020. Shown are means ± 1SEM and $n = 3$–4 per treatment combination. Corresponding statistics are shown in Table S4

Table 1 Stem P_{50} (the pressure at which 50% of the maximum hydraulic conductivity is lost) measured for *C. drummondii* in control and drought precipitation treatments and in watersheds burned every 1 and 4 years ($n = 3$–4 per treatment combination)

	1-year	4-year
Control	−3.71 (−3.51, −3.90)	−4.04 (−3.68, −4.51)
Drought	−3.18 (−2.90, −3.43)	−4.22 (−3.83, −4.65)

Shown are P_{50} values and their 95% confidence intervals. See Fig. S5 for corresponding vulnerability curves

had the greatest P_{50} (i.e., they were more vulnerable to embolism) and exhibited non-overlapping 95% confidence intervals with P_{50} values measured from all other treatment groups (Table 1).

Discussion

Whole-plant hydraulic functioning can provide crucial insight regarding how plants respond to water stress (McCulloh et al. 2019), yet we have limited information about aboveground hydraulic traits in grassland species and even less about the hydraulic traits of their roots. Here, we show how experimental changes in water availability and fire frequency can impact K_{shoot}, K_{root}, and K_{plant} in *C. drummondii*. We also provide a first assessment of K_{root} in *A. gerardii* which, to our knowledge, has never before been measured in grasses. Overall, we show several key findings: (1) *A. gerardii* has substantially greater K_{root} on a leaf area basis than *C. drummondii*; (2) belowground hydraulic functioning is tied to aboveground processes (e.g., *C. drummondii* leaf area exhibits a significantly positive relationship with K_{shoot}, K_{root}, and K_{plant}); (3) above- and belowground *C. drummondii*

hydraulics were not negatively impacted by drought. These results provide insight regarding woody encroachment dynamics under drought and highlight the importance of understanding both aboveground and belowground hydraulics for multiple dominant species within plant communities.

We found that *A. gerardii* had significantly greater K_{root} than *C. drummondii*. This result is not surprising given that C_4 grasses typically have dense, fibrous root systems that can maximize water uptake in resource-limited systems (Ma et al. 2018). In fact, C_4 grasses are often characterized as "aggressive" users of water because they exhibit root traits that allow them to sustain higher transpiration and growth rates than woody plants under wet conditions (Xu et al. 2015; Wargowsky et al. 2021; Belovitch et al. 2023). Consistent with this notion, previous work at this site has shown that grass root biomass and total root length are greatest within the top 20 cm of soil (Nippert et al. 2012), and that grass root biomass is significantly greater in shallower soil compared to woody root biomass (R. Keen, *unpublished data*). Additional work has shown that these grasses have thin, dense shallow roots (Tucker et al. 2011) that exhibit anatomical traits resulting in high theoretical conductance (Nippert et al. 2012; O'Keefe et al. 2022). Combined, these traits should produce root systems that have overall higher K_{root} than shrub root systems. Such a strategy likely benefits *A. gerardii* in the pulse-driven climate of the Great Plains, as this species can take advantage of periodic precipitation inputs to surface soils throughout the growing season more effectively than co-occurring forbs or shrubs that do not have an extensive fibrous root system. Given that *A. gerardii* experiences substantial fluctuations in water availability throughout a growing season, we hypothesized that the hydraulic response of this species would not differ under the contrasting precipitation treatments.

Contrary to our hypothesis, *A. gerardii* K_{root} was negatively impacted by drought—but only when annually burned. K_{root}, which indicates the root hydraulic conductance for a given leaf area (Eq. 1), could decline across treatments in this fashion if root hydraulic conductance declined and leaf area remained constant, if root hydraulic conductance remained the same and leaf area increased, or if root hydraulic conductance declined and leaf area increased. Each of these scenarios would decrease the hydraulic conductance per leaf area. Figure 3 indicates that there was a large increase in leaf area within the drought plots, which is consistent with a lower K_{root}. Interestingly, *A. gerardii* leaf area was not related to K_{root} when unstandardized by leaf area (Fig. S2d). Additional factors—such as root die-off under drought—may have also contributed to the decrease in K_{root} observed in annually burned, droughted *A. gerardii*. We unfortunately could not measure root biomass within these long-term sampling plots. However, recent work conducted in the same watersheds has shown that grass fine roots

have anatomical traits that might increase susceptibility to drought, and that these trait values differ with fire frequency. O'Keefe et al. (2022) showed that annually burned grass roots tend to have wider conduit diameters, fewer conduits, and lower conduit structural safety than roots from 4-year burned grasses. These traits are associated with greater hydraulic efficiency and reduced hydraulic safety (Sperry et al. 2002; Pratt et al. 2007), suggesting that annually burned grass roots may be more susceptible to drought than roots belonging to grasses burned every 4 years. Fire frequency drives differences in surface soil moisture availability, with less frequent fire resulting in greater soil moisture and grass productivity during drought (Knapp et al. 1993). With more frequent and pronounced dry soil conditions, grass roots in annually-burned locations are more likely to lose hydraulic connectivity with soil moisture, leading to a higher potential for root hydraulic dysfunction (Zeppel et al. 2015). Combined, these results indicate that annually burned grasses have a root system with a lower maximum hydraulic conductance that may also be more vulnerable to embolism than roots from 4-year burned grasses.

The reduction in K_{root} was also associated with a greater pressure differential between *A. gerardii* roots and leaves ($\Delta\Psi_{leaf}$). A larger $\Delta\Psi_{leaf}$ may have been a response to the reduction in K_{root} as a mechanism to maintain high transpiration rates (Eq. 3). Indeed, the increase in $\Delta\Psi_{leaf}$ resulted from a decrease in Ψ_{MD}, not from changes in Ψ_{PD}, suggesting that water availability in the rhizosphere did not influence midday leaf water status during this time period. This mechanism highlights that above- and belowground hydraulic functioning is tightly coordinated and that more studies should consider whole-plant responses to drought (McCulloh et al. 2019).

In *C. drummondii*, belowground hydraulic traits were more responsive to drought than aboveground traits, particularly later in the growing season. Furthermore, these responses were surprisingly positive; droughted *C. drummondii* roots had a greater capacity for moving water than control roots. We suggest the increase in K_{root} was likely a byproduct of aboveground responses to drought—specifically, leaf area dynamics. Leaf area was lower in droughted *C. drummondii*, which would increase K_{root} calculated on a leaf area basis. The reduction in leaf area is consistent with previous work showing that *C. drummondii* exhibits reduced growth rates under drought Wedel et al. (2021a) and suggests that this shrub is somewhat affected by water limitation. However, because hydraulic conductance can be positively correlated with growth rate (Zhang and Cao 2009; Fan et al. 2012), the higher K_{root} in droughted shrubs may promote rapid regrowth after wetter conditions return. This notion is supported by our observation that leaf area was positively related to K_{root}, K_{shoot}, and K_{plant} unstandardized by leaf area (Fig. S2a-c, Online Resource 1). Thus, a higher

maximum conductance supports greater *C. drummondii* leaf area which could, along with other factors such as leaf thickness and chlorophyll density (McClendon 1962; Pickett and Kenworthy 1939), facilitate the rapid growth of this species in tallgrass prairie.

K_{shoot} in *C. drummondii* did not differ in response to the precipitation treatments but was greater in annually burned shrubs compared to those burned every four years, especially in 2020. Similar to K_{root}, this pattern was likely due to the effect of lower leaf area on K_{shoot} (Eq. 1). Other factors, such as xylem diameter and number, could also be associated with differences in K_{shoot} across fire treatments. High K_{shoot} would theoretically be associated with wider xylem vessels (Tyree et al. 1994) and would promote rapid leaf regrowth after a fire in annually burned areas. Similarly, (Wedel et al. 2021b) found that resprouting *C. drummondii* stems produce leaves with lower leaf mass per area and higher leaf nitrogen, which promotes high photosynthetic rates during periods of regrowth. Thus, *C. drummondii* exhibits a variety of aboveground hydraulic, photosynthetic, and morphological traits that likely allow this species to persist in fire-dominated systems, even when frequently burned.

To investigate the impacts of K_{root} and K_{shoot} on whole-plant hydraulics, we calculated K_{plant} and compared these values to patterns of $\Delta\Psi_{leaf}$. K_{plant} was generally variable across treatments and through time, but was higher in the 4-year burn droughted treatment compared to 4-year burn control treatment. Throughout our experiment, $\Delta\Psi_{leaf}$ in these same shrubs was higher, the same, or lower than the 4-year control shrubs, suggesting that water potential gradients did not always compensate for differences in K_{plant} to maintain transpiration rates (Eq. 3). For instance, the greater K_{plant} and $\Delta\Psi_{leaf}$ in August of both years suggests that transpirational demand also increased during this time (Eq. 3). This result, supported by leaf gas exchange data (R. Keen, *in press*), is surprising given that previous work has shown that *C. drummondii* exhibits stable photosynthetic rates through time (Muench et al. 2016) and highlights the need to understand plant functioning when water is both plentiful and limiting.

Finally, *C. drummondii* stems grown under drought were more vulnerable to embolism than control stems, but only in the annually burned treatment (Table 1). This result could indicate that annually burned stems exhibit "cavitation fatigue" similar to others who have also observed increased hydraulic vulnerability in stems following drought (Hacke et al. 2001; Anderegg et al. 2013). Conversely, stems from the four-year burn treatment exhibited the lowest vulnerability to embolism (Table 1). These contrasting results may indicate that stems grown in different fire regimes vary in xylem traits (e.g., conduit diameter, wall thickness, or pit membrane thickness and frequency) that alter hydraulic responses to drought (Sperry et al. 2002; Pratt et al. 2007).

However, the link between fire regimes and plant hydraulics is generally not well known. Some previous work has shown that resprouted individuals exhibit greater hydraulic efficiency and lower hydraulic safety, compared to unburned plants, as a mechanism to increase growth following fire (Venturas et al. 2016). If occurring here, that same mechanism could explain our observed difference in P_{50} values across treatments. Overall, these observations highlight the need for further research into the relationships between fire and plant hydraulic function, particularly as they apply to understanding species responses to global change.

The extent of woody encroachment in the future will likely depend on the interplay between shrub and grass performance under drought i.e., reductions in shrub performance relative to grasses have the potential to slow rates of woody encroachment and vice versa. Here, we found little evidence suggesting that the hydraulic functioning of *C. drummondii* declines with water limitation. Although leaf area was significantly reduced, droughted *C. drummondii* exhibited greater K_{root} and lower root vulnerability to embolism than control shrubs. Lower root vulnerability to embolism would consequently allow *C. drummondii* to maintain hydraulic functioning under lower water potentials while greater K_{root} may facilitate the recovery of aboveground growth after drought ends. Fire frequency impacted some aboveground *C. drummondii* traits (e.g., K_{shoot}, leaf area, $\Delta\Psi_{leaf}$, and stem P_{50}), but these responses were not influenced by drought. These results highlight the remarkable capacity of this species to persist through periods of stress across different fire regimes. Based on these findings, we suggest that rates of shrub encroachment might not decline in a drier climate. Predicting encroachment trajectories in dynamic systems will remain challenging, as emphasized by our observations that *C. drummondii* hydraulic conductances often varied across the June and August sampling dates.

The hydraulic responses of *A. gerardii* to drought further support this notion. K_{root} in *A. gerardii* decreased during drought, but only when annually burned (i.e., under a fire regime that already limits woody encroachment). In the 4-year burn treatment, where woody encroachment is more prevalent, *A. gerardii* did not experience drought-induced changes K_{root}. These results suggest that water limitation does not likely impact the competitive ability of grasses such that they would change rates of woody encroachment in locations where this phenomenon typically occurs. Furthermore, the belowground response of *A. gerardii* to drought was associated with increased leaf area, suggesting that any reductions in hydraulic functioning did not translate to reductions in aboveground performance. Finally, *A. gerardii* K_{root} was substantially greater than that of *C. drummondii*, regardless of treatment, suggesting that *A. gerardii* should maintain competitive superiority for water uptake despite reductions in K_{root} under drought.

Finally, these results are reported after only two years of experimental drought. Longer or more intense periods of drought may reduce deep water stores to a greater extent and result in different dynamics within shrub-grass communities. Furthermore, whether these species will be able to adjust their hydraulic traits over longer periods is unclear, particularly given the contrasting literature supporting (Lemaire et al. 2021) and showing little evidence for (Bittencourt et al. 2020) hydraulic trait acclimation. Future studies should continue to investigate the responses of multiple species to long term drought in order to better understand the trajectories of ecosystem change in tallgrass prairies exposed to multiple interactive global change drivers. Aboveground and belowground hydraulic traits, in particular, will be key for understanding whole plant responses to water limitation and resolving uncertainty in predictions of grassland functioning under different climate change scenarios.

Author contribution statement All authors conceived the ideas and designed the methodology. KO and RK collected the data. KO analyzed the data and wrote the manuscript. All authors contributed critically to the drafts.

Availability of data and material Data used in this study are available upon request.

Code availability Not applicable.

Declarations

Conflict of interest Not applicable.

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