

STUDIES

Spatio-temporal differences in leaf physiology are associated with fire, not drought, in a clonally integrated shrub

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Associate Editor: Patrick Mitchell

Form & Function. Chief Editor: Kate McCulloh

Abstract

In highly disturbed environments, clonality facilitates plant survival via resprouting after disturbance, resource sharing among interconnected stems and vegetative reproduction. These traits likely contribute to the encroachment of deep-rooted clonal shrubs in tallgrass prairie. Clonal shrubs have access to deep soil water and are typically thought of as relatively insensitive to environmental variability. However, how leaf physiological traits differ among stems within individual clonal shrubs (hereafter 'intra-clonal') in response to extreme environmental variation (i.e. drought or fire) is unclear. Accounting for intra-clonal differences among stems in response to disturbance is needed to more accurately parameterize models that predict the effects of shrub encroachment on ecosystem processes. We assessed intra-clonal leaf-level physiology of the most dominant encroaching shrub in Kansas tallgrass prairie, *Cornus drummondii*, in response to precipitation and fire. We compared leaf gas exchange rates from the periphery to centre within shrub clones during a wet (2015) and extremely dry (2018) year. We also compared leaf physiology between recently burned shrubs (resprouts) with unburned shrubs in 2018. Resprouts had higher gas exchange rates and leaf nitrogen content than unburned shrubs, suggesting increased rates of carbon gain can contribute to recovery after fire. In areas recently burned, resprouts had higher gas exchange rates in the centre of the shrub than the periphery. In unburned areas, leaf physiology remained constant across the growing season within clonal shrubs (2015 and 2018). Results suggest single measurements within a shrub are likely sufficient to parameterize models to understand the effects of shrub encroachment on ecosystem carbon and water cycles, but model parameterization may require additional complexity in the context of fire.

Keywords: Clonal shrubs; *Cornus drummondii*; gas exchange; leaf physiology; shrub encroachment; tallgrass prairie.

Introduction

Shrubs are a widespread plant growth form which have evolved in response to multiple drivers of disturbance such as fire, drought and temperature stress (Stutz 1989; Rundel 1991).

Götmark *et al.* (2016) estimated that woody shrubs can grow in environments covering approximately 45 % of the terrestrial surface largely because they are successful in harsh climates and

Received: 22 December 2020; Editorial decision: 13 April 2021; Accepted: 8 June 2021

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frequently disturbed environments where trees do not thrive (e.g. grasslands, tundra, Mediterranean shrublands and semi-arid and arid ecosystems). Shrubs with a multi-stemmed growth form have a number of advantages in disturbed systems compared with single-stemmed shrubs or trees, including increased growth rates and protection from herbivory and wind damage (Peterson and Jones 1997; Rowe and Speck 2005; Götmark et al. 2016). In grasslands and savannas, established shrubs recover quickly after disturbances such as fire, drought and/or herbivory via resource sharing and resprouting from protected below-ground, basal or epicormic buds (Lamont et al. 2011).

Many shrubs are also clonal, where individuals reproduce asexually via the vegetative production of stems from below-ground buds and rhizomes, which can provide an additional competitive advantage over non-clonal shrubs in frequently disturbed environments. For instance, clonal shrubs are able to spread vegetatively and rapidly resprout after fire or herbivory due to their reserve population of below-ground buds (Ott et al. 2019). Clonal shrubs also exhibit physiological integration among connected stems ('ramets') which facilitates resource transfer across the entire individual shrub ('clone' or 'genet') and reduces competition with neighbouring plants. Clonal growth increases the survival and longevity of individuals by providing a stable source of resources to younger or damaged ramets, particularly in environments that experience considerable disturbance and climatic variability (Peterson and Jones 1997; Ratajczak et al. 2011; Killian 2012; Nippert et al. 2013; Pinno and Wilson 2014; Liu et al. 2016). Together, traits associated with clonal growth help mitigate competition for limiting resources, circumvent the challenges of seedling establishment (Hartnett and Bazzaz 1983), and increase the invasiveness of shrubs in disturbed landscapes (Song et al. 2013; You et al. 2014).

Clonal traits that increase shrub resistance to disturbance may also facilitate the spread of clonal shrubs across herbaceous ecosystems where they are native. Shrub encroachment—the increase in abundance and distribution of shrubs in grasslands and savannas—is occurring worldwide and has been associated with the spread of clonal shrubs within different ecosystems [e.g. *Cornus drummondii* in tallgrass prairie USA (Briggs et al. 2005; Ratajczak et al. 2011); *Dichrostachys cinerea* in lowveld savannas (Wakeling & Bond 2009); *Salix planifolia* and *S. glauca* in alpine tundra (Formica et al. 2014); *Larrea tridentata* in south western USA (MacMahon and Wagner 1985)]. Shrub encroachment is broadly driven by changes in climate including increased atmospheric CO₂ concentrations and altered precipitation regimes (Van Auken 2009; Devine et al. 2017), as well as reduced fire frequency and overgrazing associated with land management practices (Briggs et al. 2005; Archer et al. 2017). Many clonal species exhibit traits that enable their spread in response to these global, regional and local drivers. For example, in North American tallgrass prairie, clonal shrub establishment and growth is limited by frequent fire (burned every 1–3 years; Briggs et al. 2005; Ratajczak et al. 2014). During longer periods without fire, shrubs are able to establish and form dense canopies via clonal vegetative growth, which reduces light penetration for understory herbaceous species and consequently limits fine-fuel accumulation and the spread of fire through the shrub canopy (Ratajczak et al. 2011). Substantial non-structural carbohydrate storage below-ground also allows vigorous resprouting and improves competition with grasses in a post-fire environment (Janicke and Fick 1998; O'Connor et al. 2020). Combined, responses to infrequent fire that are associated with shrub clonality create positive feedbacks that can facilitate the conversion of grasslands to shrublands (Ratajczak et al. 2011, 2014).

Increased cover of deep-rooted clonal shrubs in grasslands is predicted to shift ecosystem carbon cycling and ecophysiology

through increased above-ground biomass and evapotranspiration (Knapp et al. 2008; Logan and Brunsell 2015; O'Keefe et al. 2020). Given that clonal shrubs are more likely to expand across grasslands and savannas than non-clonal shrubs (Ratajczak et al. 2011; Formica et al. 2014), understanding how this growth form uses resources and responds to environmental variation will be key for predicting how shrub encroachment may alter carbon and water cycling in the future. There is abundant evidence showing clonal shrubs can translocate resources throughout a clone, and these dynamics can impact the response of a clone to disturbance. For instance, Zhang et al. (2002) observed that carbon translocation within the rhizomatous shrub *Hedysarum laeve* can improve growth following defoliation, while Luo et al. (2015) showed that *Alhagi sparsifolia* increases the water status of younger ramets by transferring water within a clone. Early work on clonality suggested that resource translocation may be transient; Hartnett and Bazzaz (1983) showed that physiological integration within a common forb (*Solidago canadensis*) decreases with ramet age but that individuals can become 'reintegrated' when resources become limiting. Together, these studies indicate that physiological integration, and the resulting impacts on leaf physiological processes, may vary in response to climate and disturbance and may not always be uniform within an individual clone. If this is the case, land-surface models that predict woody plant dynamics may require detailed assessments of physiological differences across ramets within single clones (hereafter 'intra-clonal' differences in physiology) to produce more accurate estimates of shrub demography, competition dynamics between shrubs and grasses and resultant shifts in grassland carbon and water cycling.

In this study, we examined how leaf-level physiology differs among ramet locations within a major encroaching clonal shrub, *C. drummondii*, in North American tallgrass prairie. *Cornus drummondii* expands radially via rhizomes forming discrete clones where the centre of the shrub is older than the periphery (Fig. 1). Mature *C. drummondii* shrubs resprout rapidly after fire and have deep roots near the centre of the shrub that access deep soil water which can be transferred to juvenile ramets on the shrub periphery (Ratajczak et al. 2011). Access to deep and stable water sources and resource transfer among ramets mitigates competition for water with neighbouring grasses and can decouple shrub leaf physiology from typical environmental variability (Ratajczak et al. 2011; Nippert et al. 2013; Muench et al. 2016). While this physiological decoupling has been shown to buffer intraspecific variation among shrubs across the landscape (Nippert et al. 2013), how physiological integration impacts leaf-level physiology within a clone, or if the effects of integration on leaf physiology shift in response to more extreme environmental variation such as fire or drought, is unknown. To address this knowledge gap, we asked the following questions: (i) Does leaf-level physiology differ among ramet locations within *C. drummondii* clones? (ii) How does leaf-level physiology within clonal shrubs differ between a wet vs. extremely dry year? and (iii) How does fire affect clonal shrub leaf-level physiology? We compared the leaf-level physiology, including water potential, photosynthetic gas exchange rates, water use efficiency (WUE), nitrogen content and photosynthetic nitrogen use efficiency (PNUE) of *C. drummondii* ramets from the periphery to the centre within shrub clones during a wet (2015) and extremely dry (2018) year. In addition, we compared the leaf-level physiology of *C. drummondii* ramets resprouting after fire (burned in 2018) to shrubs with the same fire frequency (every 4 years) but burned the previous year (burned in 2017). We hypothesized that (i) clonal integration

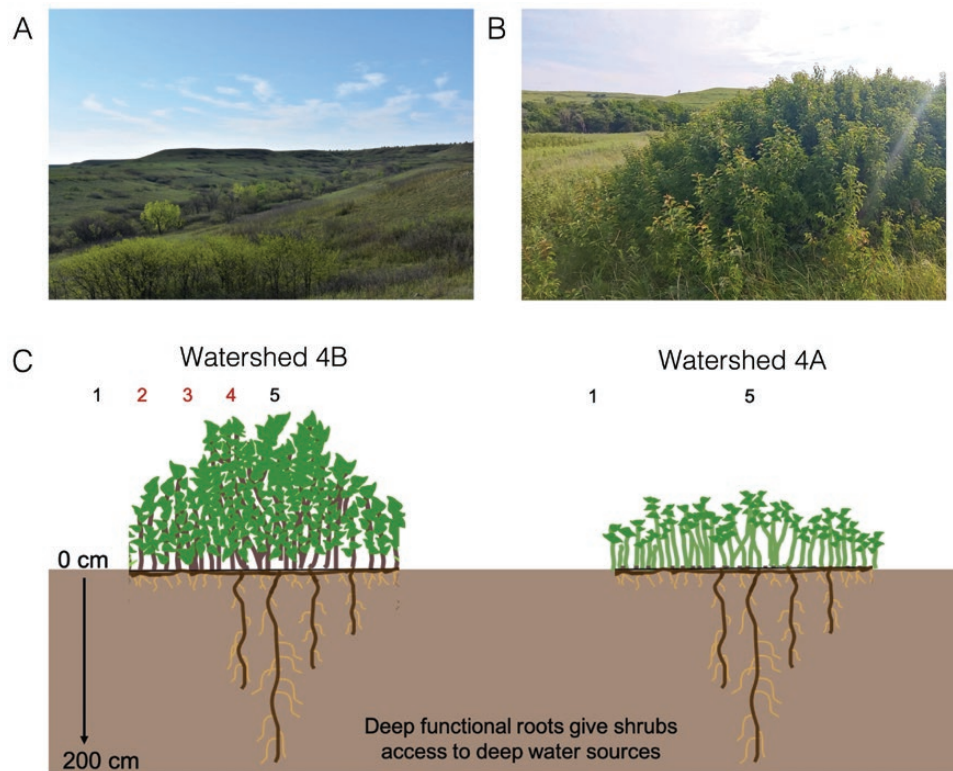


Figure 1. (A) Photo from Konza Prairie Biological Station (KPBS) showing *Cornus drummondii* shrubs scattered across the hilly landscape. KPBS is made up of rolling hills where woody cover is highest in the lowland areas. The brown and green patches are discrete *C. drummondii* shrub clones. (B) Discrete *C. drummondii* shrub clone. Clones grow radially to create discrete groups of stems with dense canopies. Photograph: E. Greg Tooley. (C) Diagram of *C. drummondii* growth form and sampling locations. We sampled shrubs within watershed 4B in 2015 and 2018. Red numbers indicate ramet locations that were only sampled in 2015. In 2018, we sampled resprouting shrubs in watershed 4A that had 100 % above-ground ramet mortality after fire. Shrubs in watershed 4A were burned in April of 2018.

will buffer physiological differences among ramets within a shrub clone resulting in no differences among ramet locations; (ii) deep functional roots provide *C. drummondii* with a stable water source and will reduce differences in photosynthetic gas exchange rates across wet and dry years (Nippert et al. 2013); (iii) shrubs resprouting post-fire (hereafter referred to as ‘resprouts’) will have higher gas exchange rates, lower WUE and increased foliar nitrogen (N) content than shrubs burned the previous year (hereafter referred to as ‘unburned’) because fire increases N availability and nutrient use efficiency in infrequently burned grasslands (Ojima et al. 1994; McCarron and Knapp 2003). Additionally, shrubs that experience complete mortality of above-ground biomass, or topkill, after fire have an increased root:shoot ratio (Clemente et al. 2005; Nolan et al. 2014), increasing water and nutrient availability for resprouting ramets and allowing shrubs to maximize carbon gain to recover lost biomass.

Methods

Study site

Data were collected at Konza Prairie Biological Station (KPBS; 39°05'N, 96°35'W) located south of Manhattan, KS, USA. KPBS is a 3387 ha native tallgrass prairie characterized by rocky limestone hillslopes. KPBS is divided into watersheds with various burn frequency treatments (1-, 2-, 4-, and 20-year burn intervals) and grazing treatments (bison, cattle or no large mammalian grazers). Frequently burned areas (burned every

1–2 years) are dominated by C_4 warm season grasses (*Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans* and *Schizachyrium scoparium*). Infrequently burned areas (burned every 4 or more years) are encroached by clonal shrubs (*C. drummondii* and *Rhus glabra*). Long-term mean annual precipitation (1982–2018) is 835 mm with 75 % of precipitation occurring over the growing season (April–September). In 2015 and 2018, growing season precipitation was 747 mm and 516 mm, respectively [see Supporting Information—Fig. S1]. In 2018, only 30 % of growing season precipitation fell during the sampling period (June–August) and herbaceous biomass was only 25 % of the long-term average (Blair and Nippert 2020). Mean daily temperatures (1982–2018) range from a low of -1.2°C in January to a high of 26.1°C in July. In 2018, maximum daily temperatures were 5.6°C and 3.6°C higher than the long-term average for May and June, respectively.

Study species

Cornus drummondii is a deciduous clonal shrub native to eastern Kansas that has undergone encroachment of tallgrass prairie during the past 40 years (Briggs et al. 2005). *Cornus drummondii* flowers May–June and sets fruit in July. Shrubs spread laterally via rhizomes, forming new ramets from below-ground buds, and each shrub appears as a discrete group of stems (Fig. 1). Discrete shrubs can grow extremely large and have been reported to cover up to 830 m^2 (Connell et al. 2021). Ramets can grow between 2 and 6 m tall and expand laterally with younger and shorter ramets on the periphery of the clone compared with those in the centre. Fire increases ramet densities and lateral expansion

(McCarron and Knapp 2003) as ramets resprout rapidly from basal and below-ground buds.

Sampling

We sampled mature *C. drummondii* shrubs of varying sizes (14–347 m²) in 2015 and 2018 at KPBS. We calculated shrub canopy area using an ellipse area equation by measuring the length of the longest axis and its perpendicular width through each discrete shrub clone. Shrubs were located in the lowlands of two adjacent watersheds (referred to at KPBS as 4A and 4B; 18.84 and 54.5 ha, respectively), each with a 4-year burn frequency. KPBS hosts a long-term ecological research station where prescribed burning occurs during the spring (March–April). Burn history and data legacies from watersheds 4A and 4B date back to 1981 and are publicly available (lter.konza.ksu.edu). Watershed 4B was last burned in 2017 and 4A was last burned in April 2018.

2015 sampling

In 2015, we collected leaf-level gas exchange and leaf water potential from six shrubs located in the lowlands of 4B. We sampled five ramets equidistant from the periphery of the clone to the centre (Location 1 = outermost ramet on the periphery and Location 5 = the innermost ramet in the centre; Fig. 1C) on six sampling dates throughout the growing season (June–September; see Supporting Information—Table S1). Instantaneous leaf-level gas exchange measurements of net photosynthetic rate (A_{net} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration (E , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and stomatal conductance to vapour (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured and intrinsic water use efficiency (iWUE; A_{net}/g_s) was estimated on one fully expanded, sun-exposed leaf at the five ramet locations within each shrub ($n = 30$) using the Li-6400XT open-system gas analyser (Li-Cor, Inc., Lincoln, NE, USA). Cuvette conditions were set to [reference CO_2] = 400 $\mu\text{mol CO}_2 \text{ mol}^{-1}$, relative humidity = 40–60 %, and photosynthetically active radiation (PAR) = 2000 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. Leaves were allowed to stabilize to chamber conditions before recording the measurement. Gas exchange measurements were collected between 10:00 h and 15:00 h. This is a similar time window used in other studies on this species (Muench et al. 2016; O’Keefe and Nippert 2018; O’Connor et al. 2020) and there is no evidence of midday decline in sap flux throughout the growing season (O’Keefe et al. 2020). Sampling order was random for each sampling date to minimize variability contributed by diurnal effects.

Predawn (ψ_{pd}) and midday (ψ_{md}) leaf water potentials were measured on clear, sunny days six times throughout the growing season [see Supporting Information—Table S1]. Leaves were collected for ψ_{pd} measurements approximately 1 h prior to dawn and leaves used for ψ_{md} measurements were collected at approximately 12:00 h. We collected the youngest, fully expanded leaf and equilibrated the leaf for 1 h in a dark, high [CO_2] moist plastic bag to ensure stomatal closure. Leaf water potential was measured using a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA).

2018 sampling

In 2018, we assessed leaf-level physiological responses to fire. We measured leaf physiological traits on 20 shrubs located in 4B (unburned) and 20 shrubs located in 4A (resprouts). For the resprouts, we selected individuals that experienced 100 % above-ground ramet mortality following fire. We measured leaf-level gas exchange and iWUE using the same methods as in 2015 on one ramet on the periphery and one ramet in the centre of each shrub clone during four sampling dates throughout the growing

season (June–August; see Supporting Information—Table S1). Samples from resprouting shrubs and unburned shrubs were collected on consecutive days due to time constraints. We collected four young, fully expanded leaves, including the leaf used for gas exchange measurements, from the periphery and centre of each shrub for stable isotope analysis. Leaves from the first sampling date were collected 2 weeks after gas exchange measurements and all other leaves were collected immediately following gas exchange measurements. Leaves were stored in moist plastic bags within a cooler until returned to the laboratory. We measured leaf area using LEAFSCAN smartphone application (Anderson and Rosas-Anderson 2017). We then dried the leaf tissue at 60 °C for 72 h, and subsequently weighed each leaf for dry mass. We calculated leaf mass per area (LMA) as leaf dry mass (g)/leaf area (m²).

For each shrub, the four dried leaves from the periphery and centre were each combined and ground. We measured leaf nitrogen content per unit dry mass (N_{mass} , mg g⁻¹) and the stable carbon isotopic composition ($\delta^{13}\text{C}$) of leaves at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. Total C and N of homogenized samples were determined following combustion using an Elementar vario Pyro cube coupled to an Elementar Vision mass spectrometer for isotope analysis. Isotopic abundance ratios were converted to δ notation using:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] * 1000 \quad (1)$$

where R is the ratio of heavy to light isotopes for the sample and standard (Vienna-Pee-Dee Belemnite), respectively. Within-run and across-run variability of the laboratory working standard was <0.05 ‰. $\delta^{13}\text{C}$ was used as a proxy for integrated water use efficiency (WUE). Larger, enriched leaf $\delta^{13}\text{C}$ values indicate a higher integrated WUE (Farquhar et al. 1989). We expected trends to be similar for integrated WUE and iWUE (A/g_s) with differences reflecting integrated WUE as a longer-term measurement incorporating daily WUE over the physiologically active life span of the leaf while iWUE is an instantaneous measurement.

N content per unit leaf area (N_{area} , g m⁻²) was calculated as the N content of the homogenized leaf samples (g g⁻¹) multiplied by the average leaf area (m²) for the centre and periphery of each shrub. Photosynthetic nitrogen use efficiency (PNUE) was calculated as:

$$\text{PNUE} = \frac{A (\mu\text{mol m}^{-2} \text{ s}^{-1})}{N_{\text{area}} (\text{g m}^{-2})} \quad (2)$$

Statistical analysis

Statistical analyses were performed in R V3.6.0 (R Core Team 2020). We used repeated measures type III ANOVA using the lmer function from the lme4 package (Bates 2015) and the ANOVA function from the car package (Fox and Weisburg 2019). For each analysis, pairwise comparisons were made using the emmeans package (Lenth 2020) with Tukey’s HSD adjustment. We ran three separate analyses to answer each of our three main questions:

- (1) To assess how leaf physiology differed across ramet locations within the shrub, we analysed data from 2015 and 2018 separately since year of measurement had a different number of sampling dates and sampling locations within each shrub clone. We used location within clone (periphery to centre) as a fixed factor and sampling date (Day of Year, [DOY]) as a continuous predictor. Shrub ID was included as a random effect to account for repeated measures. We scaled DOY and shrub area to have a mean of 0 and a standard

deviation of 1 so all numeric predictors had equal weight in the analyses (Greig-Smith 1983). We included shrub canopy area as a covariate, but it was not significant in any model ($P > 0.05$).

- (2) To assess how leaf physiology differed between wet and dry years, we compared gas exchange and iWUE between 2015 (wet) and 2018 (dry). We used the same model as above and included year as a main effect, only comparing data from the common watershed (4B or unburned shrubs) and common sampling locations within each shrub (periphery and centre).
- (3) We only used data from 2018 to assess differences in leaf physiology between resprouting shrubs (burned in 2018) and unburned shrubs. We used the same model as above and included fire as a main effect. For this model, we log transformed stomatal conductance (g_s) to meet the assumptions of normality and homogeneity of variance.

Results

Effect of ramet location on leaf physiology depended on fire not year

Leaf physiology did not differ across ramet locations within unburned shrubs (Fig. 2; see Supporting Information—Tables S2–S5). In 2015, photosynthetic rates (A_{net}) slightly but significantly increased and transpiration rates (E) significantly decreased across the growing season (Table 1; Fig. 2A and E), but gas exchange rates did not differ among ramet locations within the shrubs (Table 1). Differences in average A_{net} were largest between the periphery (location 1: 14.81 ± 0.46 SEM $\mu\text{mol m}^{-2} \text{s}^{-1}$) and centre (location 5: 13.01 ± 0.46 SEM $\mu\text{mol m}^{-2} \text{s}^{-1}$) but these were not significant. On average, intrinsic water use efficiency (iWUE) was significantly higher on the periphery (location 1: 52.00 ± 2.25 SEM $\mu\text{mol mol}^{-1}$) than near the centre

(location 4: 42.55 ± 2.22 SEM $\mu\text{mol mol}^{-1}$) of the shrub ($P = 0.013$; see Supporting Information—Table S2). Leaf water potentials significantly differed throughout the growing season ($P < 0.001$; see Supporting Information—Table S5), where ψ_{pd} was lowest during the middle of the season in July (DOY 192 and 211) and ψ_{md} decreased across the growing season (Fig. 3). Neither ψ_{pd} nor ψ_{md} differed among ramet locations within shrub clones.

In 2018, gas exchange rates were significantly different between the centre and periphery or resprouting shrubs [see Supporting Information—Table S2]. These differences were largest at the beginning of the growing season and diminished through time (Fig. 2B, D, F and H). On average, the centre of resprouting shrubs had 52 % greater E and almost 145 % greater g_s than the periphery during the first sampling period, but these differences decreased by the second sampling date (Fig. 2D and F). For unburned shrubs, there were no differences in gas exchange rates between the centre and periphery [see Supporting Information—Table S2]. In resprouting shrubs, leaves from the centre of the clone had significantly higher N_{mass} ($P < 0.001$) and lower LMA ($P < 0.001$) than leaves from the periphery of the clone (Fig. 4A and C; see Supporting Information—Table S6). Integrated WUE ($\delta^{13}\text{C}$) showed a significant 3-way interaction among burn treatment, location within shrub, and DOY (Fig. 4D; see Supporting Information—Table S6; $P = 0.004$). $\delta^{13}\text{C}$ values had low variability across the growing season for leaves from the periphery of resprouting and unburned shrubs (standard deviation: 0.610 and 0.653, respectively) while $\delta^{13}\text{C}$ slightly but significantly increased in leaves from the centre of the shrub clones over time (DOY and location interaction: $P < 0.001$).

Leaf physiology of unburned shrubs differed between wet and dry years

On average, the centre and periphery of unburned shrubs in 2015 had significantly higher gas exchange rates (A_{net} , E and g_s) and lower iWUE than unburned shrubs in 2018 (Fig. 2A–H; Table 2).

Table 1. Type III ANOVA table of results for gas exchange measurements among ramet locations within *C. drummondii* shrubs in 2015 and 2018, including net photosynthetic rate (A_{net} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$), and intrinsic water use efficiency (iWUE $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$). Shown are F- and P-values for the fixed effects of ramet location within shrub, day of year (DOY), fire treatment, and their interactions. Shrub area was used as a covariate and was not included in the interactions. Bold P-values are statistically significant ($\alpha = 0.05$).

Year	Predictor	A_{net}		g_s		E		iWUE	
		F	P	F	P	F	P	F	P
2015	Location	2.329	0.058	0.264	0.901	1.291	0.276	2.672	0.034
	DOY	6.873	0.009	1.399	0.239	49.337	<0.001	0.244	0.622
	Area	0.261	0.636	0.224	0.661	0.039	0.854	0.467	0.532
	Location $\times$ DOY	0.456	0.768	0.247	0.911	0.115	0.977	0.064	0.993
2018	Fire	39.600	<0.001	57.408	<0.001	94.979	<0.001	17.981	<0.001
	Location	1.109	0.293	5.973	0.015	13.099	<0.001	2.409	0.122
	DOY	0.560	0.455	7.577	0.006	83.988	<0.001	15.682	<0.001
	Area	0.335	0.566	0.506	0.481	0.217	0.644	0.021	0.886
	Fire $\times$ Location	2.628	0.106	7.791	0.006	10.852	0.001	1.089	0.298
	Fire $\times$ DOY	12.820	<0.001	59.781	<0.001	70.823	<0.001	22.117	<0.001
	Location $\times$ DOY	4.894	0.028	18.356	<0.001	24.258	<0.001	6.676	0.010
	Fire $\times$ Location $\times$ DOY	0.026	0.873	0.934	0.335	4.744	0.030	1.723	0.191

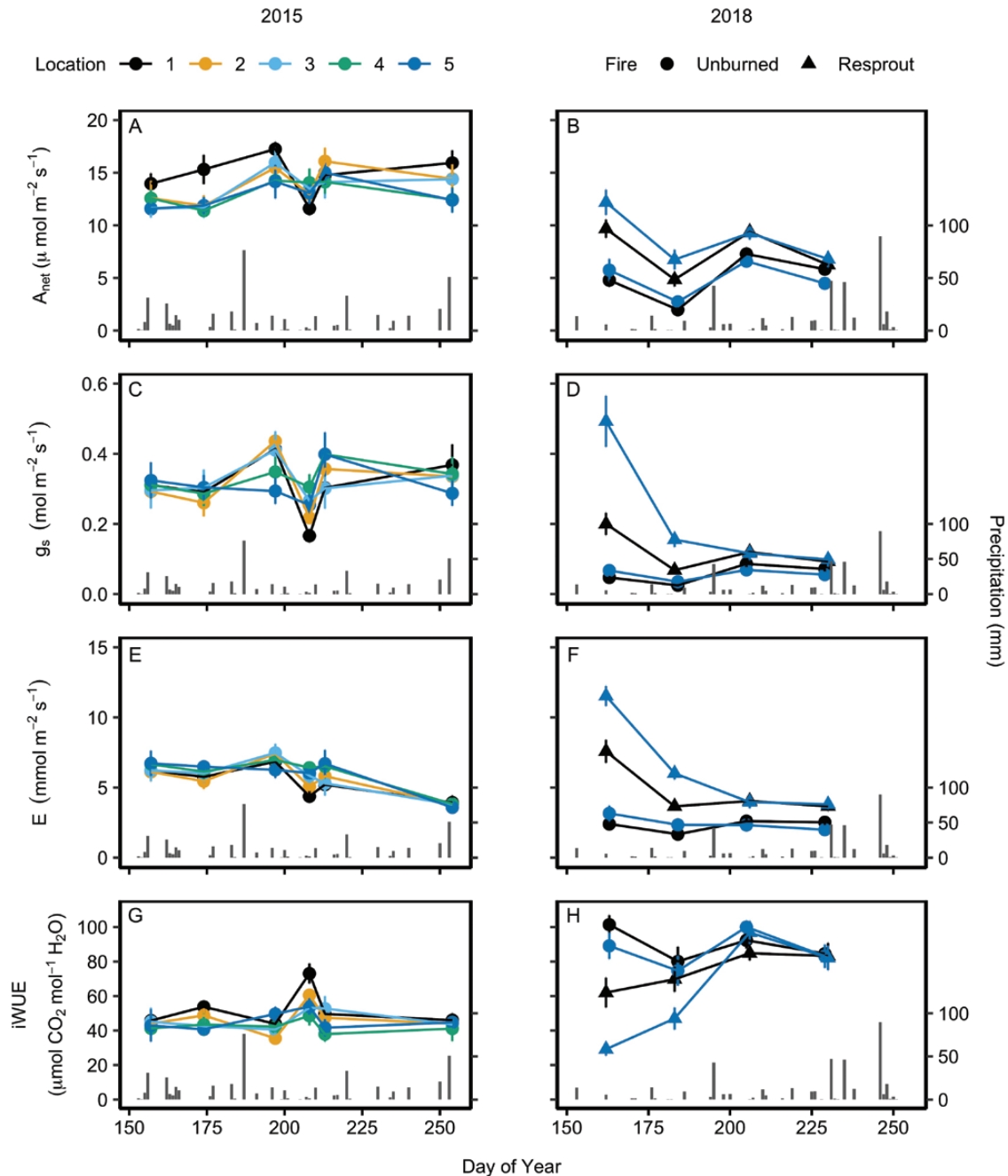


Figure 2. Mean (± 1 SE) leaf gas exchange rates across various locations within *C. drummondii* clones during the 2015 and 2018 growing seasons, including net photosynthetic rate, A_{net} (A and B), stomatal conductance to vapour, g_s (C and D), transpiration rate, E (E and F) and intrinsic water use efficiency, iWUE (G and H). Locations 1 through 5 represent the outermost ramet to the centremost ramet of a shrub clone, respectively. For 2018, resprouting shrubs were shrubs recovering from prescribed fire (burned in 2018) and unburned shrubs were burned the previous year (burned in 2017). See Table 1 for repeated measures ANOVA statistics.

Leaf physiology differed between resprouts and unburned shrubs in 2018

In 2018, resprouting shrubs had on average 60 % higher A_{net} , 165 % higher g_s and 122 % higher E than unburned shrubs (Fig. 2B, D and F). The interaction between DOY and fire was significant (Table 1) for all gas exchange variables due to resprouts having higher rates at the beginning of the season. Resprouting and unburned shrubs exhibited similar trends in A_{net} following precipitation events. Average A_{net} rates on the second sampling date were nearly half the rates measured on the first sampling

date and increased again after the site received multiple days with rainfall (Fig. 2B). Resprouting shrubs had significantly lower iWUE (Table 1) than the unburned shrubs, with greater differences between fire treatments at the beginning of the season than the end of the season (Fig. 2H). Leaves from resprouting shrubs had significantly higher N_{mass} and lower LMA than unburned shrubs ($P < 0.001$; Fig. 4A and C). Integrated WUE followed similar trends, as resprouting shrubs had lower $\delta^{13}\text{C}$ in the centre of the clones at the beginning of the season than unburned shrubs and this difference decreased over time (Fig. 4D).

PNUE was significantly higher in resprouts than unburned shrubs ($P < 0.001$; Fig. 4B).

Discussion

Clonal growth is the dominant growth strategy in grasslands (Benson and Hartnett 2006) and is associated with increased establishment, growth and survival. Although clonal shrubs are increasing in abundance throughout grasslands and savannas globally, we currently lack an understanding of how physiological processes differ among ramet locations within shrub clones or shift in response to disturbance. In this study, we investigated if leaf-level physiology differs among ramet locations within a

common clonal shrub known to exhibit water transfer among ramets and if these patterns are altered by fire or drought. Gas exchange rates did not differ among ramet locations within unburned *C. drummondii* during both wet and dry years. However, leaf physiology differed among ramets within resprouting shrubs shortly after a fire, and gas exchange rates of recently burned shrubs were higher than that of unburned shrubs. These results suggest that physiological integration within this clonal shrub maintains similar intra-clonal gas exchange rates in both wet and dry years, and that increased gas exchange rates after fire can contribute to recovery of above-ground biomass, particularly during an extremely dry growing season.

Effect of ramet location on leaf physiology depended on fire not year

Resource transfer among ramets can increase the physiological uniformity, productivity and competitive ability of clonal shrubs (reviewed in Song et al. 2013). Previous work has shown that *C. drummondii* transfers resources within individuals, where younger ramets on the clone periphery are reliant on water derived from older ramets in the centre (Ratajczak et al. 2011; Killian 2012). Here, we found that leaf-level physiology did not differ across different ramet locations within unburned *C. drummondii* shrubs, suggesting that clonal integration among ramets likely buffers differences in gas exchange rates among ramet locations throughout a clone (Fig. 2). Maintaining high carbon assimilation rates throughout a clone may be beneficial for survival of the entire individual in a variable environment and may also increase shrub size by promoting clonal lateral growth (Götmarm et al. 2016). While it is unknown how long *C. drummondii* ramets remain connected, we found no differences in leaf-level physiology across different sized clones (Table 1), suggesting that this clonal shrub retains physiological integration at large sizes (at least up to 347 m²).

Lower LMA and higher N_{mass} of ramets in the centre compared with the periphery of resprouting shrubs likely enabled the high photosynthetic rates observed early in the growing season, which could potentially facilitate carbon movement within the shrub and allow for both rapid regrowth in peripheral leaves and increased lateral spread of the clone (McCarron and Knapp 2003). LMA indicates the carbon invested per light-capturing area and can represent a trade-off between leaf longevity and carbon

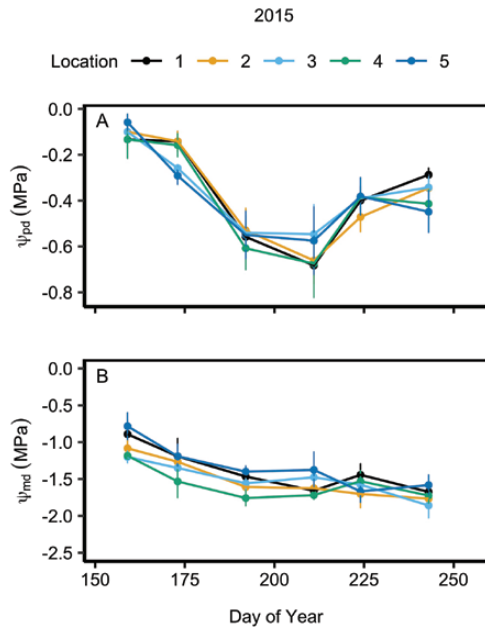


Figure 3. Mean (± 1 SE) predawn, ψ_{pd} (A) and midday, ψ_{md} (B) water potentials across various locations within *C. drummondii* clones during 2015. Locations 1 through 5 represent the outermost ramet to the centremost ramet of a shrub clone, respectively. See [Supporting Information—Table S5](#) for repeated measures ANOVA statistics.

Table 2. Type III ANOVA table of results comparing gas exchange measurements between center and periphery ramet locations within *C. drummondii* shrubs in 2015 and 2018, including net photosynthetic rate (A_{net} $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s $\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E $\text{mmol m}^{-2} \text{s}^{-1}$), and intrinsic water use efficiency (iWUE $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$). Shown are F- and P-values for the fixed effects of ramet location within shrub, day of year (DOY), fire treatment, and their interactions. Shrub area was used as a covariate and was not included in the interactions. Bold P-values are statistically significant ($\alpha = 0.05$).

Comparison	Predictor	A_{net}		g_s		E		iWUE	
		F	P	F	P	F	P	F	P
2015 vs. 2018	Year	295.694	<0.001	439.689	<0.001	189.625	<0.001	75.599	<0.001
	Location	6.064	0.015	0.003	0.953	3.867	0.051	1.464	0.228
	DOY	5.607	0.019	1.490	0.223	24.656	<0.001	0.063	0.801
	Area	0.163	0.692	1.356	0.267	0.008	0.929	0.0002	0.989
	Year $\times$ Location	3.668	0.057	0.078	0.780	2.460	0.118	0.493	0.483
	Year $\times$ DOY	0.670	0.414	0.466	0.495	15.056	<0.001	0.389	0.533
	Location $\times$ DOY	1.286	0.258	2.299	0.131	5.122	0.025	0.686	0.408
	Year $\times$ Location $\times$ DOY	2.221	0.138	0.010	0.919	2.135	0.145	0.398	0.528

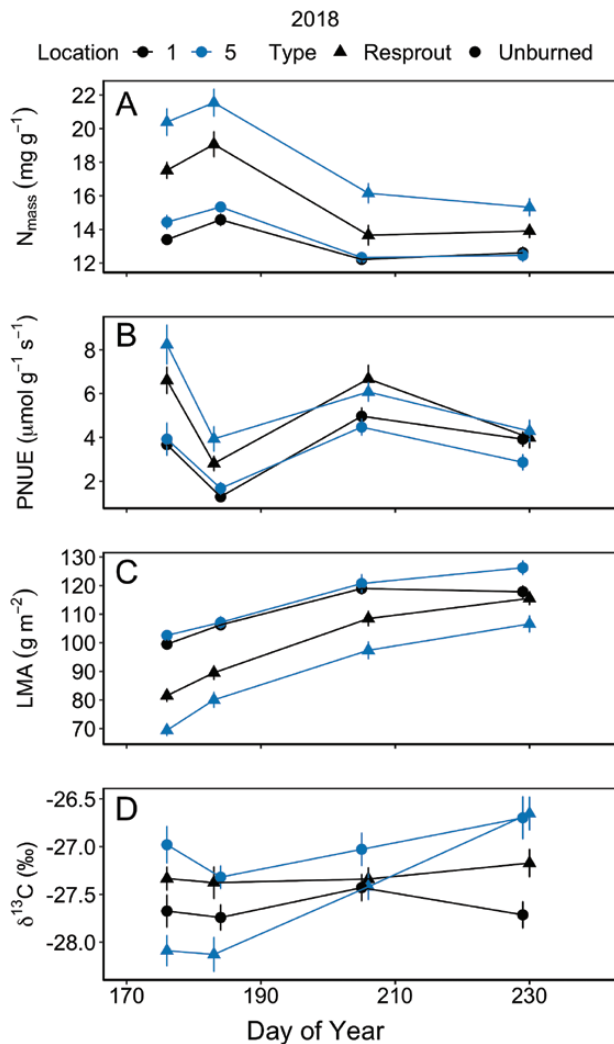


Figure 4. Mean (± 1 SE) leaf traits between the periphery (location 1) and centre (location 5) of *C. drummondii* clones during 2018, including leaf nitrogen content, N_{mass} (A), photosynthetic nitrogen use efficiency, PNUE (B), leaf mass per area, LMA (C) and integrated water use efficiency, $\delta^{13}\text{C}$ (D). See [Supporting Information—Table S6](#) for repeated measures ANOVA statistics.

gain, where species with lower LMA have thinner, ‘cheaper’ leaves that are more efficient at acquiring carbon (Wright et al. 2004). Species with low LMA also tend to have higher foliar N content, which facilitates the higher photosynthetic rates observed in the leaves of resprouts (Evans 1989; Poorter et al. 2009). Additionally, greater leaf area in the centre of the shrub would support higher area-based transpiration where the root:shoot ratio is the highest, which may enable more efficient water transfer throughout the clone.

Leaf physiology of unburned shrubs differed between wet and dry years

The summer of 2018 was one of the driest and hottest on record at KPBS leading to lower gas exchange rates in 2018 than 2015. Although gas exchange rates were lower overall during the dry year, g_s did not differ significantly in unburned shrubs within growing seasons, suggesting that *C. drummondii* may exert loose stomatal control over leaf water status (i.e. exhibit ‘anisohydric’ stomatal behaviour; Tardieu and Simonneau 1998). For instance, in 2015, average g_s between the initial and final sampling periods differed by $\sim 8\%$, despite intra-annual variation in precipitation,

while ψ_{md} declined by $\sim 68\%$ across the growing season (Figs 2C–3B). Although leaf water potential was not measured in 2018, stomata were similarly insensitive to intra-annual variation in microclimate during the drought. *Cornus drummondii* is deep-rooted and has access to a consistent water source (Ratajczak et al. 2011; Nippert et al. 2013), which likely allows leaves to maintain similar g_s throughout the growing season despite intra-annual fluctuations in precipitation, atmospheric temperature or vapour pressure deficit (O’Keefe et al. 2020). However, if deep water stores are depleted during extreme or prolonged future drought, *C. drummondii* may experience a leaf water potential threshold that induces stomatal closure (Klein 2014; Meinzer et al. 2016) and overall gas exchange rates may decline. Thus, the maintenance of similar g_s and A_{net} throughout a growing season may benefit *C. drummondii* during years of average or above average precipitation but might negatively impact the functioning of this species in a warmer, drier climate.

Leaf physiology differed between resprouts and unburned shrubs in 2018

Increased carbon gain could facilitate more rapid plant recovery from fire to compete with vigorous grass growth, recover lost above-ground biomass and replenish below-ground reserves used for resprouting (Landhausser and Lieffer 2002; Schutz et al. 2009). The high gas exchange rates we observed in resprouting shrubs is consistent with other studies (DeSouza et al. 1986; McCarron and Knapp 2003; Goorman et al. 2011) and likely due to a combination of factors, including increased leaf N content and root:shoot ratio. Increased leaf N after fire supports the transient maxima hypothesis, which posits the accumulation of N in infrequently burned grasslands is readily taken up by resprouting plants after fire due to a transient release from N and light limitation (Seastedt and Knapp 1993; Blair 1997; McCarron and Knapp 2003). High leaf N content is positively correlated with photosynthetic capacity as the majority of leaf N is used to build photosynthetic machinery (Field and Mooney 1986; Evans 1989), resulting in resprouts that have higher photosynthetic and growth rates than shrubs that were not recently burned (DeSouza et al. 1986; Castell et al. 1994; Fleck et al. 1998; Goorman et al. 2011).

In addition to increased leaf N_{mass} , the higher gas exchange rates observed in the resprouts may also be due to a greater root:shoot ratio resulting in increased soil water and nutrient availability for individual leaves due to reduced canopy size (Peña-Rojas et al. 2004; Goorman et al. 2011). Deep roots provide a consistent water source allowing new above-ground growth to exert high transpiration rates at the beginning of the season. Increased transpiration rates in resprouting shrubs can buffer leaf microclimate by increasing latent heat and decreasing leaf temperature after fire (Gates 1968; Michaletz 2015). For instance, O’Connor (2019) showed that leaf temperature was lower than the ambient air temperature for recently burned, but not unburned *C. drummondii*, and was likely due to the increased stomatal conductance and transpiration rates observed during resprouting. This suggests the deep roots of *C. drummondii* likely have multiple impacts on leaf carbon, water and thermal energy budgets following disturbance by favouring latent over sensible heat exchange and creating a more favourable microclimate for photosynthesis during regrowth. Increased latent heat may be particularly important for regrowth during drought, like 2018, when air temperatures near the soil surface are high due to decreased albedo after fire in conjunction with warmer than average air temperatures (Bremer and Ham 1999).

Increased gas exchange rates and lower iWUE observed in recently burned shrubs correspond with changes in leaf

structure associated with higher growth rates (i.e. LMA). As described previously, resprouts had lower LMA, higher N_{mass} , and higher PNUE than unburned shrubs, suggesting that resprouting shrubs invest in structurally ‘cheap’ leaves with high N to maximize carbon gain and increase growth rates after fire. These post-fire physiological traits are attributes that help clonal shrubs respond to disturbances, increase competitive success with grasses, and ultimately promote long-term persistence and expansion in highly disturbed environments.

Implications

Clonal shrub encroachment can have significant ecosystem consequences for tallgrass prairie. In mesic grasslands, shrub encroachment often increases above-ground net primary productivity (Lett et al. 2004; Knapp et al. 2008) and soil organic carbon (Li et al. 2016). Deep-rooted shrubs may also shift grassland ecohydrology by using deep soil water relative to herbaceous species (Nippert and Knapp 2007) and by transpiring more water than other co-occurring species (O’Keefe et al. 2020). Considering grasslands will likely become warmer and drier as climate change progresses, a shift in vegetation cover from herbaceous species to deep-rooted woody shrubs may deplete deep water stores and increase evapotranspiration, altering ecosystem water flux (Huxman 2005; Scott et al. 2014; Logan and Brunsell 2015; Honda and Durigan 2016; Acharya et al. 2017; O’Keefe et al. 2020).

Given the global increase in shrub encroachment, as well as the numerous ecosystem consequences associated with this phenomenon, there is current interest in modelling changes in grass-shrub cover, as well as the resulting shifts in ecosystem carbon and water budgets, within herbaceous ecosystems (O’Donnell and Caylor 2012; Qiao et al. 2015; Scheiner-McGraw et al. 2020). Traditionally, ecosystem models that predict the distribution of vegetation types (i.e. dynamic global vegetation models), as well as land-surface models that estimate the exchange of mass and energy within the Earth-atmosphere system, group plant species into coarse plant functional type groups based on life history traits (e.g. deciduous or evergreen) and morphological plant traits (e.g. leaf life span, leaf mass per area, leaf nitrogen content; Fisher et al. 2015). More recent efforts to improve model predictions of vegetation dynamics have incorporated additional biological complexity to models (e.g. by parameterizing plant hydraulic traits; Christoffersen et al. 2016), but still do not account for potential heterogeneity in physiological functioning within clones. Consequently, if clonal shrubs do exhibit heterogeneity in functioning, models that represent these species as a single functional type based on coarsely defined plant traits may produce inaccurate assessments of shrub encroachment dynamics.

Our data show that leaf physiological traits do not differ substantially among ramet locations within *C. drummondii* clones at a given point during the summer, nor do these patterns shift in response to typical intra-annual variability in microclimate or in response to drought. These results are important because they suggest that single trait values of shrub clones measured within the growing season may be sufficient for parameterizing *C. drummondii* in vegetation models that aim to understand shrub encroachment under different climate scenarios. However, we did find intra-clonal physiological differences shortly after fire, as well as substantial differences in physiology between recently burned and unburned shrubs. These results indicate that fire dynamics should be considered when modelling shrub encroachment dynamics (e.g. using the SPITFIRE model; Thonicke et al. 2010), and that trait parameters may require additional complexity in the context of fire. Future work should conduct detailed investigations of intra-clonal

physiology for other shrubs that are expanding across grasslands and savannas in order to improve predictions of vegetation cover and ecosystem functioning in a changing climate.

Supporting Information

The following additional information is available in the online version of this article—

Table S1. Sampling dates for photosynthetic gas exchange and water potential in 2015 and 2018.

Table S2. Tukey’s pairwise comparisons for gas exchange rates among ramet locations within clonal shrubs in 2015 and 2018.

Table S3. Tukey’s pairwise comparisons for gas exchange rates between unburned shrubs in 2015 and 2018.

Table S4. 95% confidence interval of gas exchange rates for each ramet location in 2015 and 2018.

Table S5. Type III ANOVA table of results for predawn and midday water potential among ramet locations with *C. drummondii* shrubs in 2015.

Table S6. Type III ANOVA table of results for leaf traits among ramet locations within *C. drummondii* shrubs in 2018.

Figure S1. Cumulative precipitation, mean daily temperature, and vapor pressure deficit from April 1–September 30 in 2015 and 2018.

File S1. R code for gas exchange data.

File S2. R code for water potential data.

File S3. R code for leaf trait data.

Sources of Funding

Funding was provided by the Konza Prairie Long Term Ecological Research program (NSF DEB-1440484) and the Department of Energy (DE-SC0019037).

Contributions by the Authors

E.R.W., K.O., J.B.N., and R.C.O. designed the study. E.R.W., K.O., B.H., and R.C.O. conducted the field work. E.R.W., K.O., J.B.N., B.H., and R.C.O. wrote the manuscript.

Conflict of Interest

None declared.

Acknowledgements

We thank the Konza Prairie Biological Station for access to the site and maintenance of the long-term experimental fire treatments. We thank Samuel Long, MaKenna Miller and Lindsey Swartz for their help with field and laboratory work.

Data Availability

Data and metadata are available on the Long Term Ecological Research Network Data Portal <https://portal.edirepository.org>.

Literature Cited

- Acharya BS, Hao Y, Ochsner TE, Zou CB. 2017. Woody plant encroachment alters soil hydrological properties and reduces downward flux of water in tallgrass prairie. *Plant and Soil* 414:379–391.
- Anderson CJR, Rosas-Anderson PJ. 2017. Leafscan (Version 1.3.21). [Mobile application software]. www.leafscanapp.com (1 May 2018).
- Archer SR, Anderson EM, Predick KL, Schwinning S, Steidl RJ, Woods SR. 2017. Woody plant encroachment: causes and consequences. In:

- Briske DD, ed. *Rangeland systems: processes, management and challenges*. New York: Springer, 25–84.
- Bates D, Maechler B, Bolker B, Walker S. 2015. Fitting linear mixed effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Benson EJ, Hartnett DC. 2006. The role of seed and vegetative reproduction in plant recruitment and demography in tallgrass prairie. *Plant Ecology* 187:163–178.
- Blair JM. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. *Ecology* 78:2359–2368.
- Blair JM, Nippert J. 2020. PAB01 Aboveground net primary productivity of tallgrass prairie based on accumulated plant biomass on core LTER watersheds (001d, 004b, 020b). Environmental Data Initiative. <http://dx.doi.org/10.6073/pasta/9aa53de93c12b115a0402986b50ee0cf> (1 November 2020).
- Bremer DJ, Ham JM. 1999. Effect of spring burning on the surface energy balance in a tallgrass prairie. *Agricultural and Forest Meteorology* 97:43–54.
- Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK. 2005. An ecosystem in transition: causes and consequences of the conversion of mesic grassland to shrubland. *BioScience* 55:243–254.
- Castell C, Terradas J, Tenhunen JD. 1994. Water relations, gas exchange, and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L. *Oecologia* 98:201–211.
- Christoffersen BO, Gloor M, Fauset S, Fyllas NM, Galbraith DR, Baker TR, Kruijt B, Rowland L, Fisher RA, Binks OJ, Sevanto S, Xu C, Jansen S, Choat B, Mencuccini M, McDowell NG, Meir P. 2016. Linking hydraulic traits to tropical forest function in a size-structured and trait-driven model (TFS v.1-Hydro). *Geoscientific Model Development* 9:4227–4255.
- Clement AS, Rego FC, Correia OA. 2005. Growth, water relations and photosynthesis of seedling and resprouts after fire. *Acta Oecologia* 27:233–243.
- Connell RK, O'Connor RC, Nippert JB, Blair JM. 2021. Spatial variation in soil microbial processes as a result of woody encroachment depends on shrub size in tallgrass prairie. *Plant and Soil* 460:1–15.
- DeSouza J, Silka PA, Davis SD. 1986. Comparative physiology of burned and unburned *Rhus laurina* after chaparral wildfire. *Oecologia* 71:63–68.
- Devine AP, McDonald RA, Quafe T, Maclean IM. 2017. Determinants of woody encroachment and cover in African savannas. *Oecologia* 183:939–951.
- Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* 78:9–19.
- Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–537.
- Field C, Mooney HA. 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish R, ed. *On the economy of plant form and function*. Cambridge: Cambridge University Press, 25–55.
- Fisher RA, Muszala S, Versteinstein M, Lawrence P, Xu C, McDowell NG, Knox RG, Koven C, Holm J, Rogers BM, Spessa A. 2015. Taking off the training wheels: the properties of a dynamic vegetation model without climate envelopes, CLM4. 5 (ED). *Geoscientific Model Development* 8:3593–3619.
- Fleck I, Hogan KP, Llorens L, Abadía A, Aranda X. 1998. Photosynthesis and photoprotection in *Quercus ilex* resprouts after fire. *Tree Physiology* 18:607–614.
- Formica A, Farrer EC, Ashton IW, Suding KN. 2014. Shrub expansion over the past 62 years in rocky mountain alpine tundra: possible causes and consequences. *Arctic, Antarctic, and Alpine Research* 46:616–631.
- Fox J, Weisberg S. 2019. *An [R] companion to applied regression*, 3rd edn. Thousand Oaks CA: Sage.
- Gates DM. 1968. Transpiration and leaf temperature. *Annual Review of Plant Physiology* 19:211–238.
- Goorman R, Bartual A, Paula S, Ojeda F. 2011. Enhancement of photosynthesis in post-disturbance resprouts of two co-occurring Mediterranean *Erica* species. *Plant Ecology* 212:2023–2033.
- Götmark F, Götmark E, Jensen AM. 2016. Why be a shrub? A basic model and hypotheses for the adaptive values of a common growth form. *Frontiers in Plant Science* 7:1–14.
- Grieg-Smith, P. 1983. *Quantitative plant ecology*, 3rd edn. Oxford: Blackwell Science Publications.
- Hartnett DC, Bazzaz FA. 1983. Physiological integration among intracolonial ramets in *Solidago canadensis*. *Ecology* 64:779–788.
- Huxman TE, Wilcox BP, Breshears DD, Scott RL, Snyder KA, Small EE, Hultine K, Pockman WT, Jackson RB. 2005. Ecohydrological implications of woody plant encroachment. *Ecology* 86:308–319.
- Honda EA, Durigan G. 2016. Woody encroachment and its consequences on hydrological processes in the savannah. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371:20150313.
- Janicke GL, Fick WH. 1998. Prescribed burning effects on total nonstructural carbohydrates of roughleaf dogwood. *Transactions of the Kansas Academy of Science* 101:39–48.
- Killian PD. 2012. *Mechanisms driving woody encroachment in the tallgrass prairie: an analysis of fire behavior and physiological integration*. Masters Thesis, Kansas State University.
- 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* 28:1313–1320.
- Knapp AK, Briggs JM, Collins SL, Archer SR, Bret-Harte MS, Ewers BE, Peters DP, Young DR, Shaver GR, Pendall E, Cleary MB. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. *Global Change Biology* 14:615–623.
- Landhäusser SM, Loeffers VJ. 2002. Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following above-ground disturbance. *Journal of Ecology* 90:658–665.
- Lamont BB, Enright NJ, He T. 2011. Fitness and evolution of resprouters in relation to fire. *Plant Ecology* 212:1945–1957.
- Lenth R. 2020. *emmeans: estimated marginal means, aka least-squares means*. R package version 1.5.4.
- Lett MS, Knapp AK, Briggs JM, Blair JM. 2004. Influence of shrub encroachment on aboveground net primary productivity and carbon and nitrogen pools in a mesic grassland. *Canadian Journal of Botany* 82:1363–1370.
- Li H, Shen H, Chen L, Liu T, Hu H, Zhao X, Zhou L, Zhang P, Fang J. 2016. Effects of shrub encroachment on soil organic carbon in global grasslands. *Scientific Reports* 6:28974.
- Liu F, Liu J, Doug M. 2016. Ecological consequences of clonal integration in plants. *Frontiers in Plant Science* 7:770.
- Logan KE, Brunsell NA. 2015. Influence of drought on growing season carbon and water cycling with changing land cover. *Agricultural and Forest Meteorology* 213:217–225.
- Luo W, Zhao W, Zeng F, Liu B. 2015. Water but not photosynthates integration exists between mother and daughter ramets of a root-derived clonal shrub. *Plant Ecology* 216:331–342.
- MacMahon JA, Wagner FH. 1985. The Mojave, Sonoran, and Chihuahuan deserts of North America. In: Evenari M, Noy-Meir I, Goodall DW, eds. *Ecosystems of the world*. Amsterdam: Elsevier, 105–202.
- McCarron JK, Knapp AK. 2003. C3 shrub expansion in a C4 grassland: positive post-fire responses in resources and shoot growth. *American Journal of Botany* 90:1496–1501.
- Meinzer FC, Woodruff DR, Marias DE, Smith DD, McCulloh KA, Howard AR, Magedman AL. 2016. Mapping 'hydroscales' along the iso- to anisohydric continuum of stomatal regulation of plant water status. *Ecology Letters* 19:1343–1352.
- Michaletz ST, Weiser MD, Kaspari M, Helliker BR, Enquist BJ. 2015. Plant thermoregulation: energetics, trait-environment interactions, and carbon economics. *Trends in Ecology & Evolution* 30:714–724.
- Muench AT, O'Keefe K, Nippert JB. 2016. Comparative ecohydrology between *Cornus drummondii* and *Solidago canadensis* in upland tallgrass prairie. *Plant Ecology* 217:267–276.
- Nippert JB, Knapp AK. 2007. Linking water uptake with rooting patterns in grassland species. *Oecologia* 153:261–272.
- Nippert JB, Ocheltree TW, Orozco GL, Ratajczak Z, Ling B, Skibbe AM. 2013. Evidence of physiological decoupling from grassland ecosystem drivers by an encroaching woody shrub. *PLoS ONE* 8:e81630.
- Nolan RH, Mitchell PJ, Bradstock RA, Lane PN. 2014. Structural adjustments in resprouting trees drive differences in post-fire transpiration. *Tree Physiology* 34:123–136.
- O'Connor RC. 2019. *Drivers, mechanisms, and thresholds of woody encroachment in mesic grasslands*. PhD Dissertation, Kansas State University.
- O'Connor RC, Taylor JH, Nippert JB. 2020. Browsing and fire decreases dominance of a resprouting shrub in woody encroached grassland. *Ecology* 101:e02935.

- O'Donnell FC, Caylor KK. 2012. A model-based evaluation of woody plant encroachment effects on coupled carbon and water cycles. *Journal of Geophysical Research: Biogeosciences* 117:G202012.
- Ojima DS, Schimel DS, Parton WJ, Owensby CE. 1994. Long- and short-term effects of fire on nitrogen cycling in tallgrass prairie. *Biogeochemistry* 24:67–84.
- O'Keefe K, Bell M, McCulloh KA, Nippert JB. 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:e2019JG005446.
- O'Keefe K, Nippert JB. 2018. Drivers of nocturnal water flux in a tallgrass prairie. *Functional Ecology* 32:1155–1167.
- Ott JP, Klimešová J, Hartnett DC. 2019. The ecology and significance of below-ground bud banks in plants. *Annals of Botany* 123:1099–1118.
- Peña-Rojas K, Aranda X, Fleck I. 2004. Stomatal limitation to CO₂ assimilation and down-regulation of photosynthesis in *Quercus ilex* sprouts in response to slowly imposed drought. *Tree Physiology* 24:813–822.
- Peterson CJ, Jones RH. 1997. Clonality in woody plants: a review and comparison with clonal herbs. In: De Kroon H, van Groenendaal J, eds. *The ecology and evolution of clonal plants*. Leiden, The Netherlands: Backhuys Publishers, 263–289.
- Pinno BD, Wilson SD. 2014. Nitrogen translocation between clonal mother and daughter trees at a grassland–forest boundary. *Plant Ecology* 215:347–354.
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *The New Phytologist* 182:565–588.
- Qiao L, Zou CB, Will RE, Stebler E. 2015. Calibration of SWAT model for woody plant encroachment using paired experimental watershed data. *Journal of Hydrology* 523:231–239.
- R Core Team. 2020. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ratajczak Z, Nippert JB, Hartman JC, Ocheltree TW. 2011. Positive feedbacks amplify rates of woody encroachment in mesic tallgrass prairie. *Ecosphere* 2:art121.
- Ratajczak Z, Nippert JB, Briggs JM, Blair JM. 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.
- Rowe N, Speck T. 2005. Plant growth forms: an ecological and evolutionary perspective: Research review. *New Phytologist* 166:61–72.
- Rundel, PW. 1991. Shrub life-forms. In: Mooney HA, Winner WE, Pell EJ, eds. *Response of plants to multiple stresses*. San Diego, CA: Academic Press, 345–370.
- Schutz AE, Bond WJ, Cramer MD. 2009. Juggling carbon: allocation patterns of a dominant tree in a fire-prone savanna. *Oecologia* 160:235–246.
- Schreiner-McGraw AP, Ajami H. 2020. Impact of uncertainty in precipitation forcing datasets on the hydrologic budget of an integrated hydrologic model in mountainous terrain. *Water Resources Research* 56:e2020WR027639.
- Scott RL, Huxman TE, Barron-Gafford GA, Jenerette GD, Young JM, Hamerlynck EP. 2014. When vegetation change alters ecosystem water availability. *Global Change Biology* 20:2198–2210.
- Seastedt TR, Knapp AK. 1993. Consequences of nonequilibrium resource availability across multiple time scales: the transient maxima hypothesis. *The American Naturalist* 141:621–633.
- Song YB, Yu FH, Keser LH, Dawson W, Fischer M, Dong M, van Kleunen M. 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171:317–327.
- Stutz, HC. 1989. Evolution of shrubs. In: McKell CM, ed. *The biology and utilization of shrubs*. San Diego, CA: Academic Press, 323–340.
- Tardieu F, Simonneau T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *Journal of Experimental Botany* 49:419–432.
- Thonicke K, Spessa A, Prentice IC, Harrison SP, Dong L, Carmona-Moreno C. 2010. The influence of vegetation, fire spread and fire behaviour on biomass burning and trace gas emissions: results from a process-based model. *Biogeosciences* 7:1991–2011.
- Van Auken OW. 2009. Causes and consequences of woody plant encroachment into western North American grasslands. *Journal of Environmental Management* 90:2931–2942.
- Wakeling JL, Bond WJ. 2009. Disturbance and the frequency of root suckering in an invasive savanna shrub, *Dicrostachys cinerea*. *African Journal of Range & Forage Science* 24:73–76.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas ML, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004. The worldwide leaf economics spectrum. *Nature* 428:821–827.
- You W, Fan S, Yu D, Xie D, Liu C. 2014. An invasive clonal plant benefits from clonal integration more than a co-occurring native plant in nutrient-patchy and competitive environments. *PLoS One* 9:e97246.
- Zhang C, Yang C, Dong M. 2002. Clonal integration and its ecological significance in *Hedysarum laeve*, a rhizomatous shrub in Mu Us Sandland. *Journal of Plant Research* 115:113–118.