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RESEARCH ARTICLE

Divergent resource-use strategies of encroaching shrubs: Can traits predict encroachment success in tallgrass prairie?

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Abstract

1. Changes in climate and land management over the last half-century have favoured woody plants native to grasslands and led to the rapid expansion of woody species. Despite this being a global phenomenon, it is unclear why some woody species have rapidly expanded while others have not. We assessed whether the most abundant woody encroaching species in tallgrass prairie have common growth forms and physiology or unique traits that differentiate their resource-use strategies.
2. We characterized the abundance, above-ground carbon allocation, and leaf-level physiological and structural traits of seven woody encroaching species in tallgrass prairie that span an order of magnitude in abundance. To identify species-specific increases in abundance, we used a 34-year species composition dataset at Konza Prairie Biological Station (Central Great Plains, USA). We then compared biomass allocation and leaf-level traits to determine differences in carbon and water use strategies among species.
3. While all focal species increased in abundance over time, encroachment in this system is primarily driven by three species: *Cornus drummondii*, *Prunus americana* and *Rhus glabra*. The most dominant species, *Cornus drummondii*, had the most extreme values for several traits, including the lowest leaf:stem mass ratios, lowest photosynthetic capacity and highest turgor loss point.
4. Two of the most abundant species, *Cornus drummondii* and *Rhus glabra*, had opposing growth forms and resource-use strategies. These species had significantly different above-ground carbon allocation, leaf-level drought tolerance and photosynthetic capacity. There were surprisingly few interspecific differences in specific leaf area and leaf dry matter content, suggesting these traits were poor predictors of species-level encroachment.
5. *Synthesis*. Woody encroaching species in tallgrass prairie encompass a spectrum of growth forms and leaf physiology. Two of the most abundant woody species fell at opposite ends of this spectrum. Our results suggest niche differences among a community of woody species facilitate the rapid encroachment by a few species. This study shows that woody encroaching species do not conform to a 'one-size-fits-all' strategy, and a diversity of growth forms and physiological strategies may

make it more challenging to reach management goals that aim to conserve or restore grassland communities.

KEYWORDS
allometry, clonal plants, ecophysiology, functional ecology, functional traits, grasslands, niche partitioning, plant strategies, resource-use strategies, woody encroachment

1 | INTRODUCTION

Grasslands contain diverse assemblages of woody and non-grassy herbaceous species that coexist with the highly abundant grass species. These non-grassy species contribute to plant diversity and ecosystem heterogeneity, support a diversity of pollinators and provide supplemental food for livestock (Bråthen et al., 2021). Over the past half-century, woody species native to grasslands have increased in abundance and replaced herbaceous species (Wieczorkowski & Lehmann, 2022). The encroachment of native woody plants has resulted in reduced grassland biodiversity, altered ecosystem water and carbon cycling, and billions of dollars in lost revenue for the cattle ranching industry (Archer & Predick, 2014; Morford et al., 2022; Ratajczak et al., 2012). Multiple interacting global change factors are contributing to woody encroachment, including fewer disturbances (e.g. suppression of fire and browsing) and increased atmospheric CO_2 concentrations, which facilitate the growth of C_3 woody plants over the C_4 grasses that dominate warm temperate to tropical grasslands (Venter et al., 2018). Despite common drivers that benefit woody plants over grasses, only a select few native woody species tend to become dominant encroachers (Ratajczak et al., 2014; Zhou et al., 2021). This study took place in the Central Great Plains (eastern Kansas, USA), where we characterized the growth forms and resource-use strategies of encroaching woody species in a tallgrass prairie that span an order of magnitude in abundance. We measured above-ground carbon allocation and a suite of leaf-level traits to determine whether the most abundant encroaching species have similar traits that promote their success or unique traits that differentiate their carbon and water use strategies.

Like many mesic grasslands and savannas, fire suppression is the primary driver of woody encroachment in the tallgrass prairie of the Central Great Plains (USA), and clonal shrubs are the primary species driving encroachment (Case et al., 2020; Case & Staver, 2017; Charles-Dominique, Beckett, et al., 2015; Charles-Dominique, Staver, et al., 2015; Ratajczak et al., 2014). Some clonal shrubs have dense canopies that shade out herbaceous species, decreasing fire spread and intensity, which further promotes shrub growth (e.g. aggregated stems in Figure 1a; Ratajczak et al., 2011; Tooley et al., 2022). However, encroaching species in this system span a broad range of growth forms that can coexist within a single site (Figure 1a). And in grasslands in general, little work has been done to identify interspecific variation in woody plant physiology and biomass allocation (e.g. leaf:stem ratios) or to link traits with the expansion of encroaching species.

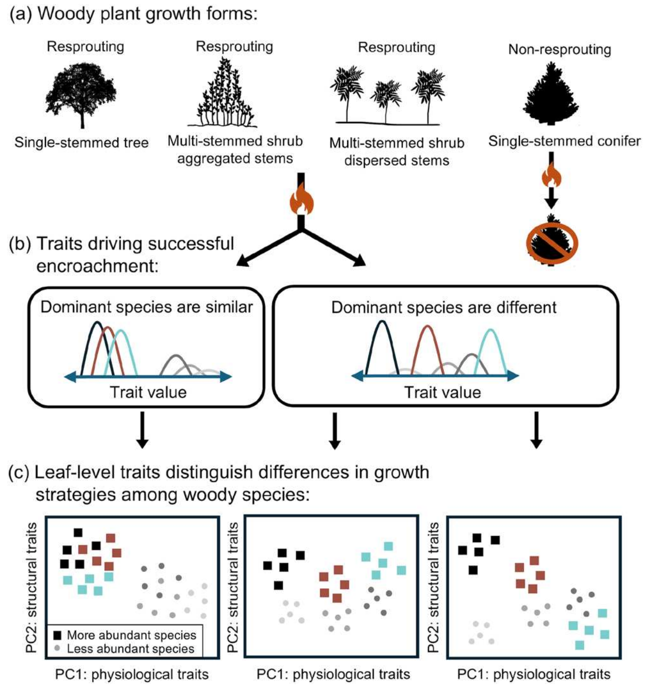

FIGURE 1 Conceptual figure depicting the growth forms and traits that distinguish growth strategies among encroaching woody species in tallgrass prairie. (a) Four growth forms of encroaching woody species at Konza Prairie Biological Station. Fire filters out woody species that cannot resprout in areas with a fire return interval of ≤4 years. (b) The most abundant encroaching species may have similar traits that increase their competitive ability or stress tolerance. This scenario (left) suggests the environment and/or competition with herbaceous species drives the community assembly of encroaching species with common traits that confer dominance. Alternatively, the most abundant encroaching species may have different traits. This scenario (right) suggests that niche differences among species s multiple species to coexist and become highly abundant at a single site. (c) Analyses that integrate leaf-level traits that reflect interspecific carbon and water use strategies can be used to infer whether the most abundant encroaching species are similar (left panel) or have unique growth strategies (middle and right panels). Species may vary along Axis 1 (middle panel), Axis 2 (not shown) or both (right panel).

Expansion by certain woody species within a community over others may be driven by community assembly processes, which are dependent on the physiological and morphological traits of coexisting woody species (Chesson, 2000; Fukami, 2015). Periodic fire, drought and competition with herbaceous species may select for encroaching species with a similar growth forms or physiological strategies. For example, frequent fire filters for encroaching species capable of resprouting after loss of their above-ground tissue (Figure 1a; Bond & Midgley, 2003) and/or thick bark to resist fire (Hoffmann et al., 2012). If abiotic factors or competition with herbaceous plants selects for certain traits, then the most abundant encroaching species should have similar growth forms and resource-use strategies that provide the species a fitness advantage (Grime, 2006; Mason et al., 2011; Scheffer & Van Nes, 2006). In this scenario, low trait diversity among species would suggest that strong environmental filters select for functionally similar species. Alternatively, environmental heterogeneity and competition among coexisting woody species may increase niche partitioning among the most abundant encroaching species (Chesson, 2000; MacArthur & Levins, 1967). In this scenario, high trait diversity among encroaching species would suggest that niche differences allow several encroaching species to become highly abundant within a single site (Figure 1b,c).

The biotic and abiotic filters governing whether the most abundant encroaching species have similar or different growth strategies are not mutually exclusive (Cadotte & Tucker, 2017; Kraft et al., 2015). We might expect that a community shows filtering on one niche axis (e.g. disturbance traits) and partitioning on another axis (e.g. resource acquisition traits; Hallett et al., 2019; Mason et al., 2011). For example, fast-growing woody species can quickly grow above the grass canopy reaching size classes less susceptible to fire and browsing (Higgins et al., 2000; Staver et al., 2009). However, these species are often shallow-rooted or have 'risky' water use strategies (i.e. anisohydric species) and are more susceptible to drought-induced mortality than slower-growing species (Case et al., 2020). Identifying the resource-use strategies of the most abundant encroaching species can help us understand community assembly processes when woody species expand in grasslands and ultimately predict whether species with certain growth strategies are more likely to encroach under varying environmental conditions. To identify successful growth strategies, we can measure traits that reflect interspecific carbon and water use strategies of woody species that vary in their abundance.

Here, we measured a suite of physiological and structural traits related to carbon capture and drought tolerance (Table 1) from six encroaching clonal shrub species (*Cornus drummondii*, *Prunus americana*, *Rhus aromatica*, *Rhus glabra*, *Rubus pensilvanicus* and *Zanthoxylum americanum*) and one encroaching tree species (*Gleditsia triacanthos*) at Konza Prairie Biological Station (KPBS, Manhattan, KS, USA). These species were chosen because they span the three major woody growth forms encroaching in frequently burned grasslands (Figure 1a), grow above the grass canopy and, as a group, comprise over 63% of woody cover across the entire site (excluding sub-shrubs and riparian areas; Figure S1). All the measured woody species have increased in abundance at KPBS over the past four decades but are encroaching at vastly different rates, and only three species have become dominant on the landscape. This study has three parts: First, we identified the trends in relative cover, frequency and dominance of the focal woody species using a 34-year species composition dataset (1988–2022). Second, we used allometry data to summarize leaf:stem mass ratios of the focal woody species to understand above-ground carbon allocation. Third, we compared leaf-level traits related to gas exchange, drought tolerance and leaf structure to determine whether species increasing the most across the landscape have unique carbon and water use strategies compared with shrub species with lower rates of increase over time.

2 | METHODS

2.1 | Study site and study design

Konza Prairie Biological Station (KPBS) is a 3487-ha native tallgrass prairie site located in the Flint Hills of eastern Kansas (39°05′ N, 96°35′ W). The site is an experimental landscape divided into watersheds with differing fire frequencies (1, 2, 3 to 4 or 20-year fire return intervals) and grazing regimes (year-long bison, seasonal cattle or no large mammalian grazers) prescribed to each landscape unit. The topography is diverse, with rocky uplands with shallow

TABLE 1 Traits and number of replicates per species measured in this study.

	Abbreviation	Trait	Unit	Replicates
Structural	SLA	Specific leaf area	$cm^2 g^{-1}$	10
	LDMC	Leaf dry matter content	$mg g^{-1}$	10
	Foliar C:N	Leaf carbon:nitrogen ratio	Unitless	10
	wd	Wood density	$g cm^{-3}$	10
Physiological	$\delta^{13}C$	Leaf water use efficiency	‰	10
	π_{tlp}	Leaf turgor loss point at the end of the growing season	MPa	8
	A_{sat}	Light-saturated photosynthetic rate	$\mu mol\ m^{-2} s^{-1}$	8
	LCP	Light compensation point	$\mu mol\ m^{-2} s^{-1}$	8
	φ	Apparent quantum yield	mol CO_2 mol incident photon^{-1}	8
	J_{max}	Maximum rate of electron transport	$\mu mol\ m^{-2} s^{-1}$	8
	Vc_{max}	Maximum rate of carboxylation	$\mu mol\ m^{-2} s^{-1}$	8

soils, benches that resemble shallow upland soils, lowlands with deeper soils (~2 m) and steep slopes that connect these soil types. Frequently burned areas with fire return intervals of 1–2 years are dominated by C_4 grasses, primarily *Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans* and *Schizachyrium scoparium*. The site has high herbaceous diversity with 99 grass species (C_3 and C_4) and 340 forb species (Donnelly et al., 2023; Nippert et al., 2019). Areas with fire return intervals >2 years are encroached by native clonal shrubs. Encroachment is highest in the lowlands that have deeper soils and higher water availability, but shrubs have also expanded onto the slopes and some uplands. Average historical fire return intervals in the region were every 3–4 years, which is no longer frequent enough to limit shrub establishment and expansion (Briggs et al., 2005; Ratajczak et al., 2014). Mean annual precipitation is 846 mm (from 1982 to 2022), 75% of which falls from April to September.

We focused on six species of encroaching clonal shrubs (*Cornus drummondii* C.A. Mey, *Prunus americana* Marshall, *Rhus aromatica* Aiton, *Rhus glabra* L., *Rubus pensilvanicus* Poir. and *Zanthoxylum americanum* Mill.) and one nonclonal resprouting tree (*Gleditsia triacanthos* L.). Three species of the clonal shrubs have aggregated stems that grow close together, two shrub species have dispersed stems that grow further apart, and one shrub species grows with either the aggregated or dispersed stem growth form (Table 2; Figure 1a). We focused on two watersheds with a 4-year burn frequency, one grazed by bison and one ungrazed. These watersheds were historically dominated by C_4 grasses but have undergone significant encroachment by clonal woody shrubs in the past three decades (Ratajczak et al., 2014). We chose these two watersheds because they both burned in March 2021, the year before our field sampling. In this study, our goal was to capture variation in the traits and growth forms among the most abundant woody encroaching species, all of which are present in both grazed and ungrazed areas. Bison grazing is not expected to impact shrub growth and physiology directly but can impact competition with herbaceous species and soil compaction and chemistry, which may indirectly affect shrub water use dynamics (Greenwood & McKenzie, 2001; O'Keefe & Nippert, 2017).

2.2 | Long-term species cover

We assessed long-term species cover for the seven focal woody species using permanent community composition plots within the two focal watersheds at KPBS (Collins & Calabrese, 2012; Hartnett et al., 2023). Plots were established in 1983, and bison was introduced into the grazed area between 1987 and 1991. Each watershed included four 50 m long transects in the lowlands with five 10 m^2 plots per transect ($n = 20$ plots per watershed). Species cover was estimated in each plot using a modified Daubenmire scale (0%–1% cover, 2%–5% cover, 6%–25% cover, 26%–50% cover, 51%–75% cover, 76%–95% cover and 96%–100% cover). For this study, all Daubenmire scale values were converted to the midpoint values for analysis (0%, 0.5%, 3.5%, 15%, 32.5%, 62.5%, 85% or 97.5% cover), which is the standard approach to using this type of dataset (Collins & Calabrese, 2012). We only used cover in the lowlands because woody cover is limited in the uplands. We calculated relative cover for the focal woody species as the cover of each species divided by the total cover of all species in the plots, including shrubs, trees and herbaceous species. We calculated species frequency as the percentage of plots where each species occurred. We then calculated a species dominance index as:

$$\frac{\text{average relative cover} + \text{relative frequency}}{2},$$

where values close to 1 indicate a species has a high relative cover and is distributed across the sampling plots (Avolio et al., 2019). We used the average species dominance index over the last 10 years (2012–2022) to rank the shrub species from the most to least abundant in all the figures.

2.3 | Shrub above-ground biomass allocation

Leaf area, stem mass and leaf mass for the focal woody species were opportunistically sampled over 3 weeks in late June to early July in 2020. The nonclonal tree, *G. triacanthos*, was not included in

TABLE 2 Mean ± 1 SE and median values for leaf:stem mass, leaf area:above-ground biomass and height:diameter ratios for each focal species. Letters indicate significant differences ($p < 0.05$) among species using the Dunn's test with a Bonferroni correction. Species are ordered from most abundant to least abundant. *Gleditsia triacanthos*, a single-stemmed nonclonal tree, was not measured for above-ground carbon storage and is not included in the table.

		Leaf:stem mass		Leaf area:above-ground biomass		Height:diameter	
Species	Growth form	Mean	Median	Mean	Median	Mean	Median
Cornus drummondii	Aggregated stems	0.32 ± 0.01	0.31^{a}	3.73 ± 0.14	3.42^{a}	2.31 ± 0.07	2.23^{a}
Prunus americana	Aggregated stems	0.44 ± 0.04	0.44^{abc}	3.59 ± 0.26	3.91^{ab}	1.66 ± 0.12	1.56^{b}
Rhus glabra	Dispersed stems	1.16 ± 0.13	1.08^{c}	4.99 ± 0.30	4.79^{bc}	1.32 ± 0.14	1.15^{b}
Rubus pensilvanicus	Dispersed stems	0.97 ± 0.07	1.02^{cd}	8.49 ± 0.67	7.89^{c}	1.84 ± 0.10	1.77^{ab}
Rhus aromatica	Aggregated stems	0.62 ± 0.07	0.53^{bd}	3.88 ± 0.23	4.03^{ab}	1.49 ± 0.10	1.42^{b}
Zanthoxylum americana	Aggregated or dispersed stems	0.65 ± 0.05	0.63^{bcde}	4.53 ± 0.18	4.62^{abc}	1.65 ± 0.19	1.28^{b}

these surveys because of its larger size and single-stemmed growth form. The measurements were sampled across eight watersheds at KPBS, including 2- and 4-year burn frequencies in bison-grazed and ungrazed areas (see Table S1 for sample sizes and locations). Stems of each species were harvested, placed in a bucket of water and immediately transported to an air-conditioned laboratory to minimize water loss. Stem height, stem diameter at 0.50 m height and leaf area were measured for each stem within 6 h of harvest. The cumulative leaf area for each stem was measured using a Li-3100C area metre (Li-COR Biosciences, Lincoln, NE). Fewer stems were measured for *Z. americanum* and *R. pensilvanicus* because these species have prickles on their leaves, which could damage the Li-3100C. However, the stems that were measured for these species spanned the full range of size classes encountered in field surveys. Leaves and stems were dried at 60°C for at least 4 days and weighed for stem and leaf dry mass. We used these measurements to calculate leaf:stem mass ratios, height:diameter ratios and the ratio of leaf area to the above-ground biomass.

2.4 | Physiological and structural traits

In 2022, we sampled detailed leaf-level physiological traits and commonly measured structural traits related to carbon gain and drought tolerance for the seven focal woody species (Table 1). We refer to traits directly measuring leaf-level photosynthetic efficiency and drought tolerance as physiological traits and traits describing leaf and stem construction as structural traits (e.g. traits common in the leaf economic spectrum; Wright et al., 2004). Precipitation in 2022 was average, with 601 mm occurring between April and September. We sampled individual shrubs in the lowlands of bison-grazed and ungrazed areas with a 4-year fire return interval near the long-term species composition plots described above. Shrubs of the same species were a minimum of 10 m apart to ensure the independence of replicates. Measurements included leaf-level physiological traits derived from light response curves, photosynthetic CO_2 response curves, pressure–volume curves, leaf chemistry and structural traits (specific leaf area [SLA], leaf dry matter content [LDMC] and wood density). We measured eight replicates per species for response curve parameters ($n = 4$ individuals per watershed) and 10 replicates per species for structural trait measurements and leaf chemistry ($n = 5$ individuals per watershed).

2.4.1 | Rapid light response curves

We sampled light response curves in July of 2022 using the Li-6400XT photosynthesis system (Li-Cor, Inc., Lincoln, NE). We sampled four replicates of each species in grazed and ungrazed areas ($n = 8$ per species). Measurements were taken between 930 and 1500 h. Light intensities were set to 2000, 1600, 1200, 800, 400, 200, 100, 50, 30, 15 and 0 $\mu mol\, m^{-2} s^{-1}$. CO_2 inside the chamber was set to 400 ppm and relative humidity was maintained between 40 and 50%. Each leaf was allowed 90–200 s to stabilize between light intensities. We derived apparent quantum yield (ϕ), light compensation point (LCP) and light-saturated photosynthetic rate (A_{sat}) using the *photosynthesis* package in R (Stinziano et al., 2023).

2.4.2 | A-Ci response curves

In July 2022, we sampled A-C_i response curves on the same shrubs as light response curves using the Li-6800 photosynthesis system (Li-Cor, Inc., Lincoln, NE). We used the dynamic assimilation response curve method that uses dynamic equations to continuously change CO_2 concentrations from 1600 to 40 ppm (Saathoff & Welles, 2021). Leaves were allowed to stabilize to chamber conditions before starting each curve. Initial chamber conditions were set to $CO_2 = 400$ ppm, temperature = 30°C, relative humidity = 50%, light = 2000 $\mu mol\, m^{-2} s^{-1}$. We derived the maximum electron transport rate (J_{max}) and maximum carboxylation capacity (Vc_{max}) using the *plantecophys* package in R (Duursma, 2015).

2.4.3 | Pressure–volume curves

Pressure–volume curves were sampled in August of 2022 on the same shrubs used for photosynthetic response curves using the bench dehydration method ($n = 8$ curves per species; Bartlett et al., 2012; Tyree & Hammel, 1972). Terminal sections of branches were collected before sunrise between 400 and 530 h, placed in a moist plastic bag and stored in a refrigerator until measuring. All branches were collected on the same morning as they were measured. To start each curve, a portion of the stem with the youngest developed leaves was cut underwater with a razor blade and weighed on a microbalance (0.1 mg, Ohaus Pioneer, Ohaus Corporation, Parsippany, NJ, USA). Leaf water potential was measured using a Scholander Pressure Chamber (PMS Instrument Company, Albany, OR, USA). The leaf was weighed again immediately after the water potential measurement, allowed to dry down on the bench top for 2 min and placed in the plastic bag to equilibrate for 20 min, and then, water potential was measured again. This process was repeated with dry-down periods of 2 min to 2 h until a pressure of 3.0 to 3.5 MPa was reached. Each leaf was dried at 60°C for 48 h and weighed. We derived turgor loss point (π_{tlp}) for each sample using the pressure–volume analysis spreadsheet tool (Sack et al., 2010).

2.4.4 | Leaf structural traits and leaf chemistry

SLA and LDMC were measured in late July of 2022 (sensu Pérez-Harguindeguy et al., 2013). Briefly, five leaves from a total of 10 shrubs per species were used for leaf trait measurements ($n = 350$ leaves). We included all parts of each leaf including the rachis and petiole. Leaves were collected, placed in a moist plastic bag and stored in a cooler until processing. We measured fresh leaf area using imageJ. Leaves were then rehydrated overnight and measured for water-saturated mass. We dried the leaves for 48 h at 60°C and weighed them for leaf-dried mass. SLA and LDMC were calculated for each individual leaf and then averaged for each individual shrub. Leaves for each replicate shrub were combined, ground and measured for foliar C:N and δ^{13}C. Foliar %C, %N and δ^{13}C were measured at the Stable Isotope Mass Spectrometry Laboratory at Kansas State University using an Elementar vario Pyro cube coupled to an Elementar Vision mass spectrometer. Isotopic abundance was converted to δ notation using:

$$\delta = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right] \times 1000,$$

where R is the ratio of heavy to light isotopes (^{13}C:^{12}C) for the sample and standard. Standards were calibrated to the international standard, Vienna Pee-Dee Belemnite for δ^{13}C. Within and across run variability of the working standard was <0.05 ‰.

2.4.5 | Woody density

Wood density, a metric of drought tolerance, was measured as described in Pérez-Harguindeguy et al. (2013). Briefly, a 10 cm long section of stem was cut 10 cm above the ground and measured for fresh volume. Stem sections were then dried at 60°C for 72 h and weighed to calculate stem density (mg cm^{-3}; $n = 10$ per species).

2.5 | Statistical analysis

All statistical analyses were performed in R V4.2.1 (R Core Team, 2022). We tested for differences in leaf:stem mass, height:diameter and leaf area:above-ground biomass ratios among species using a Kruskal–Wallis test. We chose a non-parametric test because of the largely unequal sample sizes among species, particularly *C. drummondii*, for which we had three times as many stems as the other species (Table S1). We then used a Dunn's test with a Bonferroni correction to assess which species had significantly different biomass ratios.

We used principal component analysis (PCA) to summarize trait correlations among species using the *prcomp* function in the stats package (R Core Team, 2022). Trait data were normalized and log transformed to linearize relationships among variables and to ensure each trait had equal weight in the analysis. One replicate of turgor loss point was missing for *Cornus drummondii*, so we used the average of all other replicates to fill in the missing value. We used a Type II one-way ANOVA to assess whether average PC1 and PC2 coordinates differed among species using the car package (Fox & Weisberg, 2019).

To test for individual trait differences among species, we used linear mixed models using the *lmer* function in the lme4 package (Bates et al., 2015). We included species as a fixed effect and replicate nested within watershed as a random effect. Light response and CO$_2$ response curves were taken over multiple days due to time restraints, and we included replicate nested within date as a random effect. Light-saturated photosynthetic rate (A_{sat}) and SLA were log transformed to meet the assumptions of homogeneity of variance and normality. We used Tukey's HSD to test for pairwise differences among species using the *emmeans* package (Lenth et al., 2019).

3 | RESULTS

3.1 | Long-term species cover

Over 34 years (1988–2022), the relative cover of woody species increased from approximately 0% to 45% in the ungrazed watershed and 0% to 20% in the bison-grazed watershed (Figure 2a,b). This increase was primarily driven by three species: *C. drummondii*, *P. americana* and *R. glabra*. The primary encroacher, *C. drummondii*, was present in over 80% of sampled plots in the ungrazed area and over 60% of plots in the grazed area in 2022. *P. americana* was the second most dominant encroacher and was present in 40% of the plots in grazed and ungrazed areas. The species *R. glabra* has decreased in cover since 2014 in the watersheds measured here but has an average of 20% cover across all sampling plots at KPBS (Figure S1; $n = 450$ plots). Other species, *G. triacanthos*, *R. aromatica* and *R. pensilvanicus* had lower cover than the other species but are present in up to 20% of the plots (Figure 2c,d). A less abundant species, *Z. americanum*, did not appear in any plots until 2020, but this species is present at comparable densities to *R. pensilvanicus* and *R. aromatica* across watersheds burned every 3 to 4 years (*unpublished data*; Figures S1 and S2).

3.2 | Species biomass allocation

The most abundant species, *C. drummondii*, had significantly lower leaf:stem mass ratios than all other species, except for *P. americana*, the second most abundant species with a very similar growth form (Table 2). Low leaf:stem mass ratios indicate a lower investment in leaf mass for a given stem mass than the other focal species. *C. drummondii* also had the highest height:diameter ratio compared with other species. *R. pensilvanicus* and *R. glabra* showed increased leaf investment with the higher leaf:stem mass and leaf area:above-ground biomass ratios than the other species, but these were only significantly higher than *C. drummondii* and *P. americana* ($p < 0.05$; Table 2).

3.3 | Physiological and structural traits

3.3.1 | PCA

PC1 and PC2 accounted for 30.40% and 18.88% of the variation in traits, respectively (Figure 3). The three most dominant woody species (*C. drummondii*, *P. americana* and *R. glabra*) were all significantly

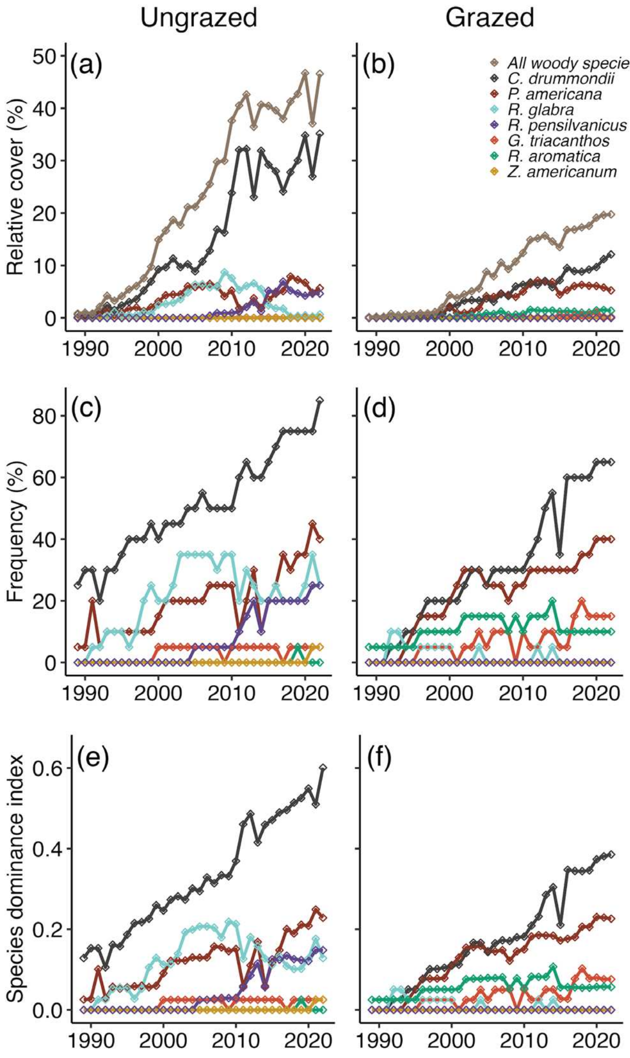

FIGURE 2 Relative cover (a, b), frequency (c, d) and species dominance index (e, f) of for the focal woody species from 1989 to 2022. Relative cover of each species was calculated using all species, woody and herbaceous, present in the plots ($n=20$ plots per ungrazed and grazed areas). The tan line in panels (a, b) is the total relative cover of all woody species, including all trees and shrubs that grow above the grass canopy. Frequency was calculated as the percentage of plots where each species occurred. Species dominance index is calculated as the average relative cover and frequency, where an index closer to 1 means the species has high cover and is found in a high number of plots (Avolio et al., 2019).

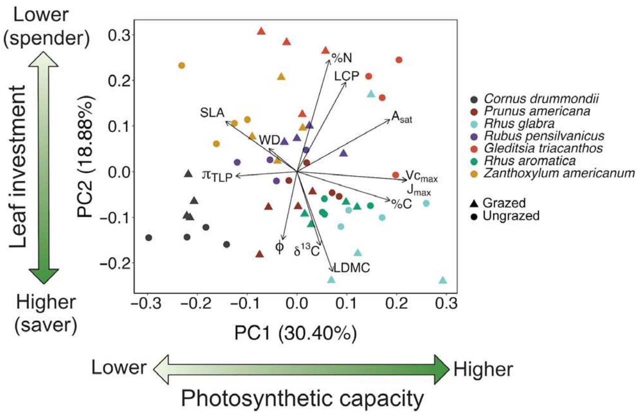

FIGURE 3 Principal components analysis of the seven focal species and measured physiological and structural traits ($n=8$ per species). Colours represent species and shapes represent the two locations sampled, one grazed (triangles) and one ungrazed (circles). Species names in the legend are ordered from most to least abundant. Physiological traits: A_{sat}, light-saturated photosynthetic rate; J_{max}, maximum electron transport rate; LCP, light compensation point; φ, apparent quantum yield; Vc_{max}, maximum carboxylation rate; $\delta^{13}C$, integrated leaf water use efficiency; π_{tlp}, turgor loss point. Structural traits: %C, leaf carbon content; %N, leaf nitrogen content; LDMC, leaf dry matter content; SLA, specific leaf area; wd, woody density.

different from one another along PC1 ($p<0.05$) but did not vary along PC2. *C. drummondii* and *R. glabra* fell at opposite ends of PC1 and their mean PC1 coordinate value was significantly different from almost all other species ($p<0.05$; Table S2). Traits related to leaf photosynthetic capacity (A_{sat}, J_{max} and Vc_{max}) divided species along PC1, where maximum electron transport (J_{max}) and carboxylation rates (Vc_{max}) contributed the most to PC1 (Table S3). Traits related to leaf structure and chemistry (LDMC, foliar %N, $\delta^{13}C$) divided species along PC2, with foliar %N and LDMC contributing the most to PC2. Light compensation point also contributed highly to PC2, which was most likely driven by the nonclonal tree, *G. triacanthos*, that had a higher compensation point than the other species (Figure 4B). Traits related to drought tolerance (i.e. turgor loss point and wood density) contributed less to the first two PC axes than traits related to carbon capture. Turgor loss point was associated with *C. drummondii*, *R. pensilvanicus* and *Z. americanum*, which have higher turgor loss points than other species (Figure 5A). The only nonclonal species, *G. triacanthos*, was isolated on PC2 and had higher variance along both PC1 and PC2 than the other species. Two less abundant species, *R. pensilvanicus* and *Z. americanum*, had different PC2 scores than most other species (*C. drummondii*, *R. aromatica* and *R. glabra*), likely driven by greater SLA and foliar %N (Figure 6A).

3.3.2 | Light response and A-Ci response curves

Parameters from light and CO_2 response curves varied significantly among species (Figure 4). Parameters related to photosynthetic capacity (A_{sat}, Vc_{max} and J_{max}) were highly correlated and showed similar trends among species, where the most abundant shrub, *C. drummondii*, had significantly lower values than most other species (Figure 4). The third most abundant shrub, *R. glabra*, had the highest maximum carboxylation and electron transport rates (Vc_{max}: 72.47 ± 3.87 and J_{max}: 156.8 ± 9.10, respectively) that were nearly twofold higher than *C. drummondii* (Vc_{max}: 36.79 ± 2.03 and J_{max}: 76.88 ± 2.46, respectively). Light compensation point and apparent quantum yield did not differ among species, except *G. triacanthos* had significantly higher compensation points, on average, than most other species (Figure 4B,C).

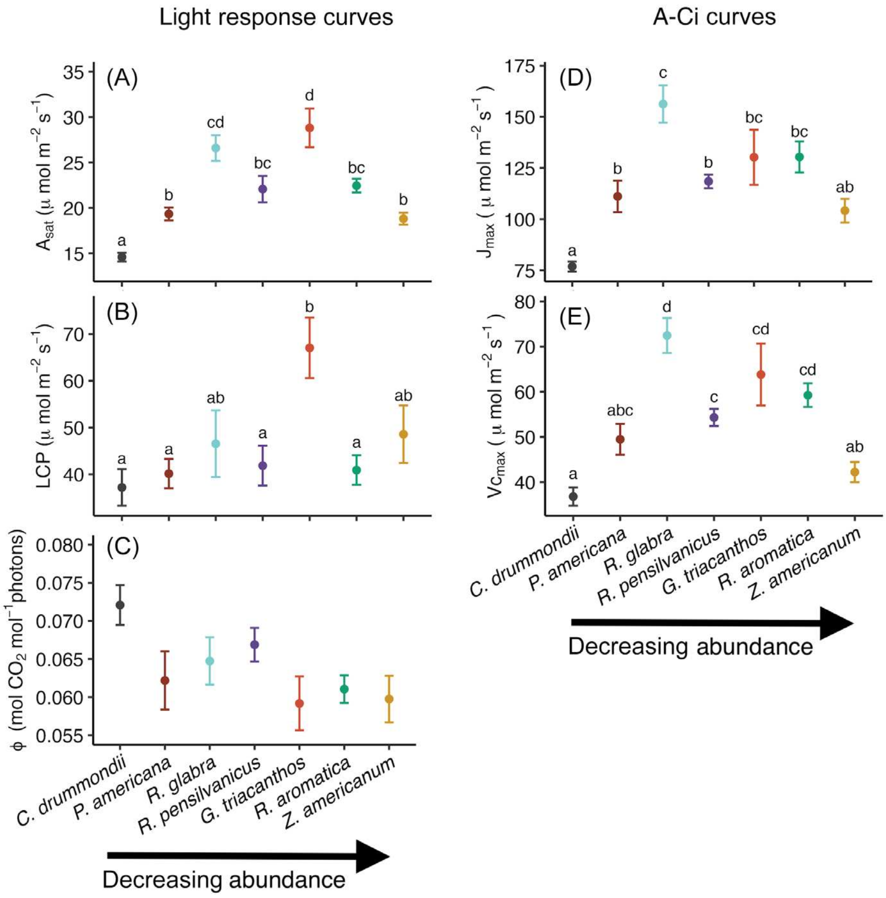

FIGURE 4 Parameters from light response (A–C) and CO_2 response curves (D, E; mean ± 1 SE) of the seven focal shrub species ($n = 8$ curves per species). (a) Light-saturated photosynthetic rates, (b) light compensation point, (c) apparent quantum yield, (d) maximum electron transport rate and (e) maximum rate of carboxylation. Species are ordered from most abundant to least abundant. Letters represent significant differences among species ($p < 0.05$).

3.3.3 | Water use/drought tolerance traits

Turgor loss point, δ^{13}C, and woody density varied significantly among species (Figure 5). *C. drummondii* and *R. pensilvanicus* had higher turgor loss points than most other species (Figure 5A). Intraspecific variability for wood density was very low, and all species differed significantly from one another except for the species with the lowest wood densities (Figure 5B; *R. glabra* and *R. pensilvanicus*). *P. americana*, *R. aromatica* and *R. glabra* had higher δ^{13}C than most other species, but only by ~0.5‰–1‰. The tree, *G. triacanthos*, had the lowest δ^{13}C, indicating lower leaf-level water use efficiency.

3.3.4 | Leaf structural traits

Foliar C:N ratios varied more among species (on average 19 to 34) than SLA or LDMC, which were similar for most species (80 to 118 cm^2 g^{-1} and 303 to 388 mg g^{-1}, respectively). *G. triacanthos*, *R. pensilvanicus* and *Z. americanum* had lower C:N than all other shrub species. The lower C:N ratio in these species was primarily driven by higher leaf nitrogen content, which is typically associated with high photosynthetic and leaf turnover rates. Lower C:N ratios are reflected in *R. pensilvanicus*, *Z. americanum* and *G. triacanthos* positions along PC2 (Figure 3). *C. drummondii* and *R. glabra*, the two shrub species with the largest differences in photosynthetic capacity, had similar C:N ratios, but *C. drummondii* had ~7% lower leaf carbon content than other species.

4 | DISCUSSION

Encroachment by trees and shrubs is one of the greatest threats to remaining grasslands world-wide by pushing grasslands into woody-dominated states that are difficult to reverse (Collins et al., 2021; Staver et al., 2011; Wieczorkowski & Lehmann, 2022). Despite the widespread threat of woody encroachment, it is unclear why some native woody species have increased in abundance while others have not. We provide detailed physiological and structural trait data for the most common encroaching shrub species in a tallgrass prairie that span an order of magnitude in their abundance. Our results show that coexisting encroaching species encompass a spectrum of growth forms and leaf physiology that reflect interspecific differences in resource-use strategies. Surprisingly, some of the most commonly measured functional traits, such as SLA and LDMC, showed little variation among species. However, for almost all traits related to carbon allocation and leaf-level physiology, the

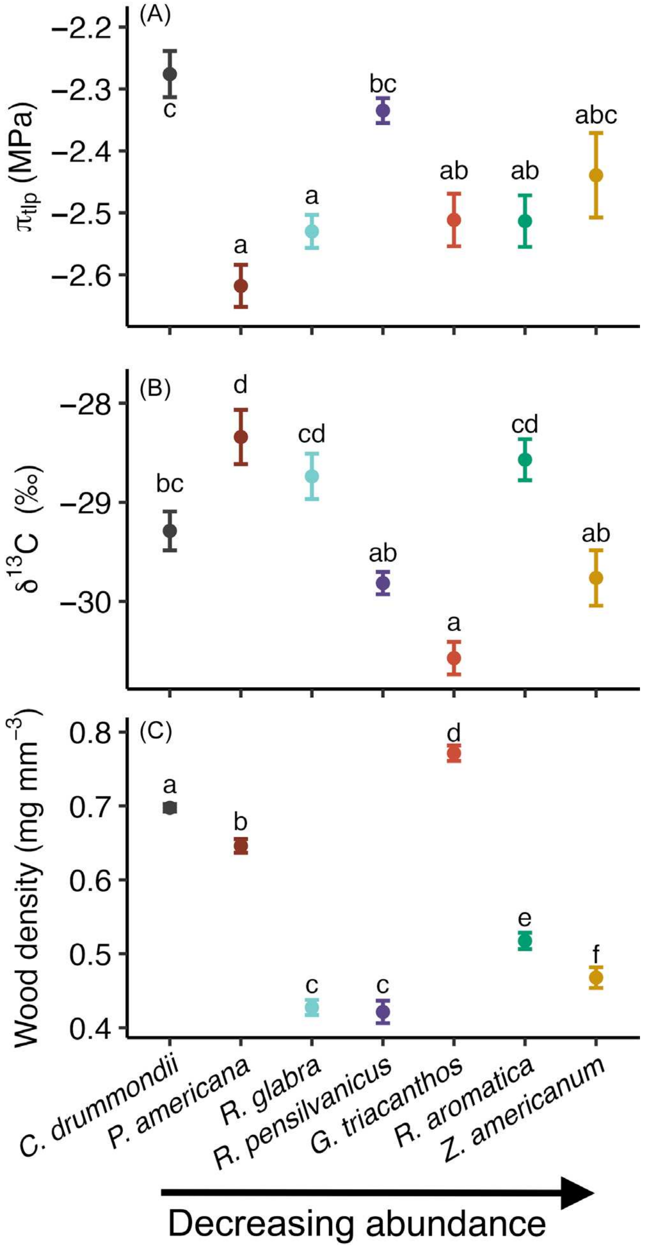

FIGURE 5 Traits related to water use and drought tolerance of the seven focal woody species. (a) Turgor loss point, (b) integrated leaf water use efficiency and (c) wood density. Species are ordered from most abundant to least abundant. Letters represent significant differences among species ($p < 0.05$).

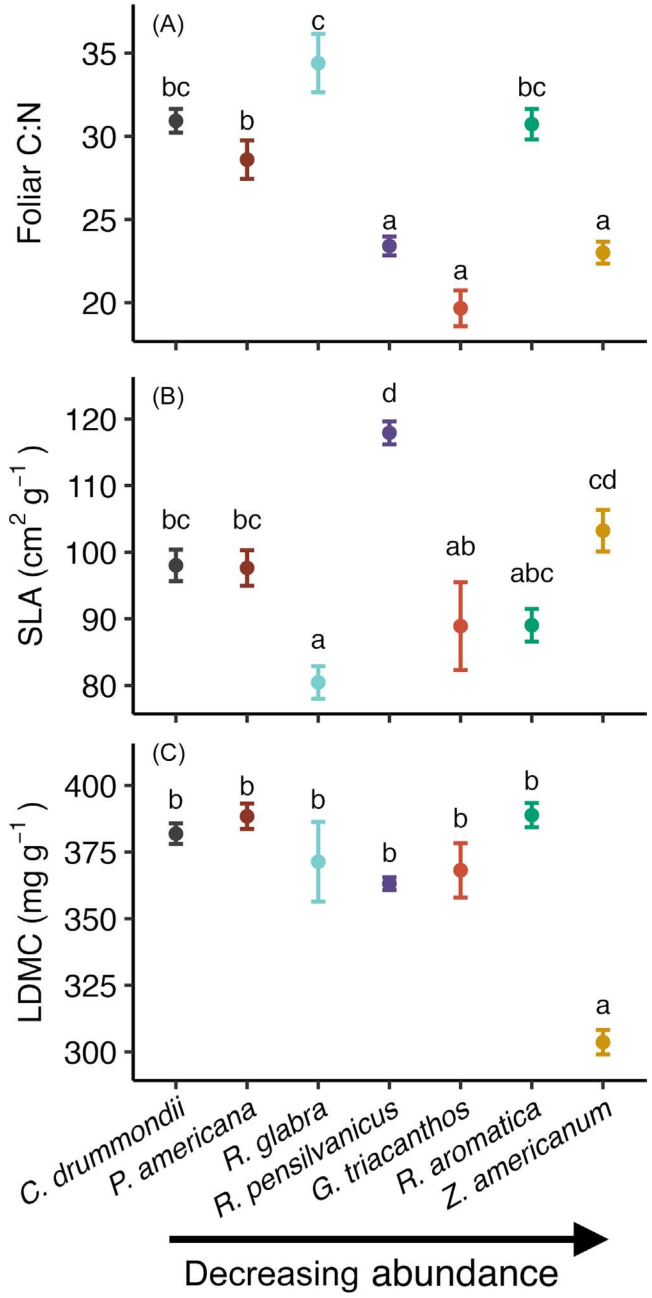

FIGURE 6 Leaf functional traits of the seven focal woody species. (a) Foliar C:N, (b) specific leaf area and (c) leaf dry matter content. Species are ordered from most abundant to least abundant. Letters represent significant differences among species ($p < 0.05$).

most dominant encroacher, *C. drummondii*, had the most extreme values, such as very high height:diameter ratios and very low leaf-level photosynthetic capacity. These interspecific differences differentiate the most abundant encroaching species from one another and suggest that niche differences allow several species to become highly abundant within a grassland ecosystem.

We posited that we might see evidence of filtering for some traits (resulting in small trait differences among species) versus niche partitioning for others (resulting in larger trait variation among species; Mason et al., 2011). Based on classic plant functional traits, we would probably conclude that niche partitioning plays a limited role in the encroachment of multiple species because SLA and LDMC values showed marginal variation across species. For instance, the two most abundant species did not have significantly different SLA values than the two least abundant species. Other traits showed more evidence of niche partitioning, specifically for the most extreme trait values, with *C. drummondii* and *P. americana* on one end of the spectrum, and *R. glabra* on the other. Two of the most abundant species (*C. drummondii* and *R. glabra*) had the most extreme values for leaf:stem mass ratio, height:diameter ratio, maximum photosynthetic rate and wood density. But interestingly, these two species varied in opposite directions, with *C. drummondii* investing more in vertical growth and *R. glabra* investing in larger leaves with high photosynthetic capacity, lower vertical growth and less allocation to woody stems. While we cannot explicitly quantify the relative importance of filtering versus niche partitioning in driving woody plant communities in this study, these data suggest that both mechanisms interact to determine which species become abundant (Angert et al., 2009; Hallett et al., 2019). Our results illustrate the diversity of growth strategies that can occur among the most successful encroaching species within a single site.

The extreme differences between *C. drummondii*, *P. americana* and *R. glabra* suggest there is a spectrum of woody growth forms: at one end are species that invest in stems to grow tall above the grass canopy (*C. drummondii* and *P. americana*), and at the other end are shorter species that allocate more above-ground biomass to leaves (*R. glabra* and *R. pensilvanicus*). The two most abundant species, *C. drummondii* and *P. americana*, have an aggregated growth form, where stems grow close together. These species invest in height and then form dense canopies (LAI = 8), which has been shown to further promote shrub growth by reducing fuel loads and suppressing fire intensity (Ratajczak et al., 2011; Tooley et al., 2022). This mirrors the thicket-forming species in the coastal United States (Brantley & Young, 2007) and South Africa (Charles-Dominique, Beckett, et al., 2015; Charles-Dominique, Staver, et al., 2015), which also form dense canopies and typically do not coexist with herbaceous species in their understorey. At the other end of the spectrum are species that have high leaf:stem mass ratios and exhibit a dispersed growth strategy, where stems grow further apart and are less effective at shading out herbaceous species (LAI = 2–3; Knapp, 1986). Shrubs in this category have lower stem densities and relative cover than the aggregated growth forms but have a similar frequency (Figure 2c). The relative advantage of these different growth strategies likely shifts across disturbance regimes and resource gradients. For example, *R. glabra* has an advantage in more frequently burned grasslands because it resprouts quickly following most fires and can persist at heights similar to the dominant grasses and forbs (Hajny et al., 2011). This is comparable to resprouting woody species in frequently burned savannas (fire every 1–2 years) of the Cerrado and lowveld savanna, where short growth forms with high below-ground storage and bud protection are common (Charles-Dominique, Beckett, et al., 2015; Charles-Dominique, Staver, et al., 2015; Chiminazzo et al., 2023).

Even within similar growth strategies, species vary in their resource-use and drought tolerance. Difference in turgor loss point (π_{tlp}) between the two most abundant species, *C. drummondii* and *P. americana*, likely reflects differences in drought tolerance (low π_{tlp}) versus avoidance (access to deep soil water). *C. drummondii* is known to rely on deep soil water (>30 cm), while *P. americana* relies more on surface soils (McCarron & Knapp, 2001) and is likely more sensitive to extended droughts. These two species provide an excellent example of how filtering and niche complementarity may act on different niche axes, where disturbance and herbaceous competition have filtered for resprouting species with dense canopies, but these species have contrasting drought tolerance strategies.

No single characteristic differentiated the most abundant encroaching species from the less abundant species, as the ability to encroach reflects an amalgamation of physiological tolerances, dispersal, phenotypic plasticity, biomass allocation, growth rates and characteristics of the environment. The same has been seen in several other open ecosystems, where single traits rarely explain species abundances (Charles-Dominique, Beckett, et al., 2015; Charles-Dominique, Staver, et al., 2015; Cornwell & Ackerly, 2010; García Criado et al., 2023; Higgins et al., 2012). We found that few species overlapped in multivariate space, suggesting each species has a unique suite of traits. Most notably, the most abundant encroaching species, *C. drummondii*, was isolated from all other species along PC1 (Figure 3). PC1 was primarily driven by differences in photosynthetic response curve parameters, where *C. drummondii* had the lowest average carboxylation rate (Vc_{max}), electron transport rate (J_{max}) and photosynthetic rate (A_{sat}). Low leaf-level photosynthetic capacity is consistent with previous studies showing this species has low instantaneous photosynthetic rates (McCarron & Knapp, 2001; Wedel et al., 2021). This seemingly low leaf-level assimilation capacity is compensated for by dense canopies (Tooley et al., 2022), high apparent quantum yield (Figure 4C) and deep root systems that decrease the likelihood of stems and leaves facing drought (Keen et al., 2024; Ratajczak et al., 2011). Access to more perennial deep soil water is especially critical, because deeper soil water is often more stable, which allows the species to maintain growth under variable growing season weather (Keen et al., 2024; Nippert et al., 2013) and results in slow but steady carbon capture throughout the growing season. For instance, a study of *C. drummondii* water use efficiency using carbon isotopes found no difference in years with average growing season weather versus a year with one of the most extreme droughts in 40 years (Nippert et al., 2013; see Ratajczak et al., 2022 for climate analyses).

Less dominant species, such as *R. pensilvanicus* and *Z. americanum*, tended to cluster together in the PCA and had similar carbon and water use traits that differentiated them from other species. These two species had traits associated with favouring growth over drought tolerance, including higher SLA and turgor loss point and lower wood density and leaf water use efficiency ($\delta^{13}C$; Figures 4 and 5). Frequent drought may keep these species at lower relative abundances, particularly if they have more shallow root systems than other woody species. Other demographic components not measured in this study (seed dispersal, germination success, resprout and clonal growth rates, etc.) may also keep these species at lower abundances.

There has long been hope that easily measured leaf structural traits could predict species abundance or dominance, but this has proven difficult, leading some authors to refer to this idea as a 'holy grail' in ecology (Funk et al., 2017; Lavorel et al., 2007). We found surprisingly little variation in the common plant functional traits SLA and LDMC across species (Figure 6C,D). While some interspecific differences in these traits were significant, variation across species was low (SLA means ranging from 80 to 117 $cm^2 g^{-1}$) compared with coexisting woody species in other open systems (Case et al., 2020; SLA means ranging from 60 to 143 $cm^2 g^{-1}$). These results suggest that these commonly measured leaf traits are either (1) strongly filtered or (2) do not capture meaningful differences among species growth strategies for the shrubs in this system. Other studies have associated successful encroachment with acquisitive growth strategies characterized by high photosynthetic rates, fast leaf turnover and rapid growth (De Jonge et al., 2024), all of which are variables often correlated with SLA (Reich, 2014; Wright et al., 2004). We did not find expected correlations among SLA, LDMC and other measured traits. For example, LDMC is a commonly measured trait that is often linked to turgor loss point (Blumenthal et al., 2020; Laughlin et al., 2020). However, in this study, species showed similar LDMC despite having very different turgor loss points, a metric driven by cell wall rigidity and osmotic adjustment (Bartlett et al., 2012). Similarly, SLA is typically assumed to be positively correlated with assimilation and growth rates (Reich, 2014). Here, SLA and light-saturated photosynthetic rates were orthogonal in multivariate space, illustrating that the ability to forecast key interspecific differences in photosynthetic capacity or leaf-level drought tolerance would not be possible by examining SLA or LDMC alone. These commonly measured traits may be most useful across large environmental gradients and may not show the expected relationships when determining differences among coexisting species at local scales (Wigley et al., 2016).

5 | CONCLUSION

We suggest that niche differences among a community of woody species facilitate rapid encroachment by a few species, which become highly abundant in mesic grasslands. Using a collection of physiological and structural traits, we illustrate the diversity of growth strategies that occur among the most successful encroaching species at a single site. These data illustrate that multiple strategies can result in successful encroachment, and that recognizing this diversity will shape our theoretical and practical understandings of community assembly in encroached grasslands. Niche partitioning is a well-cited mechanism driving grassland diversity–stability relationships by increasing ecosystem productivity and resistance to perturbations (Isbell et al., 2015). These mechanisms may be relevant in encroached grasslands, where a high diversity of encroaching species may reinforce the stability of an undesirable state and make finding management regimes that suppress an entire community of woody species more challenging. A wider range of functional types in the encroaching woody plant community likely means there will be higher 'response diversity' if conditions change in a way that would harm the currently dominant woody species (i.e. Elmqvist et al., 2003; Loreau et al., 2021). Ultimately, the diversity of encroaching woody species in mesic grasslands may make it more challenging to reach management goals that aim to combat encroachment and restore encroached grasslands.

AUTHOR CONTRIBUTIONS

Emily R. Wedel designed the study, collected the trait data, and led the data analysis and writing the manuscript. Zak Ratajczak and E. Greg Tooley collected the allometry data and assisted in writing the manuscript. Jesse B. Nippert aided in the study design and writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14456.

DATA AVAILABILITY STATEMENT

Shrub leaf physiology and structural trait data are available through the Konza Prairie Biological Station Long-Term Ecological Research (LTER) and the Environmental Data Initiative (EDI) data portal: https://doi.org/10.6073/pasta/fe327cebe79aab9ecf3dd10b45cbd485 (Wedel & Nippert, 2024).

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SUPPORTING INFORMATION

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

Figure S1. Cover of all woody species in the long-term species cover transect across the entire site.

Figure S2. Basal area ($m^2 ha^{-1}$) estimated across watersheds burned every 4 years on Konza Prairie.

Table S1. Total number of stems and locations for each species sampled for leaf area, stem mass, and leaf mass.

Table S2. 95% confidence intervals of PC1 and PC2 coordinates for each species estimated from a type-II ANOVA using the emmeans R package.

Table S3. Variance explained by the first three principal components and the variable loadings for each axis.

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