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RESEARCH ARTICLE

Root traits reveal safety and efficiency differences in grasses and shrubs exposed to different fire regimes

Kimberly O'Keefe[1,2] | Seton Bachle[3] | Rachel Keen[3] | E. Greg Tooley[3] | Jesse B. Nippert[3]

[1]Division of Biological Sciences, Saint Edward's University, Austin, TX, USA

[2]Department of Botany, University of Wisconsin, Madison, WI, USA

[3]Division of Biology, Kansas State University, Manhattan, KS, USA

Correspondence
Kimberly O'Keefe
Email: kokeefe@stedwards.edu

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Abstract

1. Roots are key components of terrestrial ecosystems, yet little is known about how root structure and function vary across a broad range of species, functional groups and ecological gradients in situ.
2. We assessed how woody and grass root anatomical traits vary among soil depths and different fire frequencies to better understand the water-use strategies exhibited by these two functional groups in tallgrass prairie experiencing woody encroachment. Specifically, we asked: (a) Do root anatomical traits differ with fire frequency or soil depth?; (b) Do relationships between anatomical traits that confer hydraulic safety versus efficiency vary by fire frequency or soil depth?; (c) Is root anatomy associated with integrative root traits (e.g. root diameter, specific root length (SRL) and root biomass)?; and (d) When scaled by root biomass, do root water-use traits impact the capacity for water uptake?
3. We collected grass and woody roots from 10, 30 and 50 cm deep soil in areas burned every 1, 4 and 20 years. We then measured xylem conduit diameter, conduit cell wall thickness, conduit number, conduit mechanical safety (t/b), stele area, endoderm thickness, hydraulic diameter, theoretical hydraulic conductivity and root-system theoretical hydraulic conductance.
4. We observed: (a) Woody roots had high hydraulic conductance in shallow soils and greater mechanical strength in deeper soils, which may provide a competitive advantage in less frequently burned, more diverse plant communities; (b) shallow grass roots had unique trait combinations at the anatomical and root-system levels (thinner, more numerous conduits and higher root-system hydraulic conductance compared to deeper roots) that likely allow these plants to rapidly use water but tolerate dry soils under multiple fire regimes; and (c) hydraulic safety versus efficiency trade-offs translate between different hierarchical scales (i.e. from anatomical to integrative root traits).
5. These results provide anatomical evidence to explain water-use dynamics in tallgrass prairie and also provide novel insight regarding functional strategies that may facilitate the conversion from grassland to shrubland in less frequently burned tallgrass prairie. Future work should investigate these dynamics in situ,

as they may explain current and future patterns of woody-grass coexistence in tallgrass prairies.

KEYWORDS
grasslands, hydraulic conductivity, hydraulic efficiency, hydraulic safety, roots

1 | INTRODUCTION

Plant roots are critical components of terrestrial ecosystems that play important roles in plant and ecosystem functioning (Freschet et al., 2021). Roots provide structural support for plants, absorb and store soil resources and facilitate interactions between plants and symbiotic fungi, which can have cascading impacts on a variety of ecosystem processes such as terrestrial carbon storage, biogeochemical fluxes and soil microbial dynamics (Bardgett et al., 2014; Grigulis et al., 2013; Jones et al., 2004). Given that roots directly determine plant access to and uptake of soil resources, root functioning will have tremendous consequences for terrestrial ecosystems as they experience ongoing ecological change (Bardgett et al., 2013).

Root uptake of water is particularly important to consider given that many ecological processes are directly tied to precipitation and soil water availability (Knapp et al., 2017; Reichstein et al., 2013). Water is a key limiting resource for plants in many ecosystems (Gherardi & Sala, 2020; Nemani et al., 2003; Sankaran et al., 2005) and its spatio-temporal availability controls the functioning of individual plants, competition between co-occurring species, primary productivity and the abundance/distribution of species (Anderegg et al., 2013; D'Onofrio et al., 2015; Maurer et al., 2020). Roots provide the greatest resistance to water movement through the soil–plant–atmosphere continuum, and consequently, determine the amount/rate of water used by plants through time (Sperry et al., 2002). Consequently, understanding how root traits influence plant water use will provide greater mechanistic insight regarding plant, community and ecosystem functioning.

Root water-use traits can be broadly categorized into two groups: those that facilitate rapid water use versus those that confer safety against stress associated with water limitation. At the anatomical level, traits such as xylem conduit diameter and number influence root hydraulic conductivity, or the flow rate at which water moves through the xylem for a given pressure gradient (Tyree & Ewers, 1991). Roots with wider and/or more numerous conduits have greater conductivity and are 'hydraulically efficient' (Sperry et al., 2006). Thinner root conduits have lower conductivities but are less likely to experience drought-induced embolism and often have thick cell walls that prevent conduit collapse under high xylem tensions (i.e. are 'hydraulically safe'; Pratt et al., 2007). At greater scales, larger specific root length (SRL; total root length per dry biomass) increases water uptake efficiency (Roumet et al., 2006), while maximum rooting depth, the vertical profile of root density throughout the soil and horizontal root range influence the volume of soil that a root system can potentially exploit. Combined, these traits determine the response of plants to varying soil water availability through time.

Despite the recognized importance of plant roots within terrestrial ecosystems, as well as the link between root traits and water use, surprisingly little is known about how root structure and function vary across a broad range of species, functional groups and ecological gradients. Recent efforts have been made to generate large root trait databases (e.g. the Fine-Root Ecology Database; Iversen et al., 2017), produce global root trait meta-analyses (Ma et al., 2018) and develop hypotheses relating root traits to resource-use strategies (e.g. the multidimensional 'Root Economics Space'; Bergmann et al., 2020). However, much of these data originated from studies conducted under controlled conditions limiting the inferences that can be made about root trait–plant functional relationships in situ (Bardgett et al., 2014; Kirfel et al., 2017). Controlled experiments provide vastly different growing conditions than those experienced in the field, which can dramatically alter root growth (Poorter et al., 2016). Additionally, root trait–plant functioning relationships are often assessed using integrative traits (e.g. SRL) that are attributed to multiple underlying components and do not provide mechanistic understanding of ecological phenomena (Freschet et al., 2021). While some studies have linked root anatomical traits with whole-plant functioning (e.g. Hummel et al., 2007; Rieger & Litvin, 1999; Wahl & Ryser, 2000), more work is needed to understand the relationships between root traits within and across hierarchical scales (i.e. anatomical, morphological and whole-root system levels), as well as their combined impact on plant water use in situ.

Here, we used native tallgrass prairie in the North American Central Great Plains to investigate the relationships of herbaceous and woody root traits in situ. This system provides a unique opportunity to observe changes in root traits across a dynamic landscape shaped by multiple, interacting ecological drivers (i.e. fire, grazing and climatic variability). We were specifically interested in understanding the effects of fire frequency on root traits because fire strongly impacts vegetation structure and composition across the tallgrass prairie landscape. Frequently burned tallgrass prairie is dominated by a few C_4 grasses and numerous subdominant C_3 forbs (Smith & Knapp, 2003). However, woody plants increase in abundance and distribution when burned less frequently than every 3 years, substantially reducing the dominance of C_4 grasses (Ratajczak et al., 2014). This change in land cover is known as woody encroachment and can alter numerous grassland processes—for example plant diversity (Ratajczak et al., 2012) and soil carbon and nitrogen (Connell et al., 2020). A common woody encroaching species in the eastern North American tallgrass prairie, *Cornus drummondii* (Family: Cornaceae), exhibits unique water-use traits that may also

contribute to its spread across these landscapes. *Cornus drummondii* utilizes soil water from deeper soil depths than grasses to support consistently high leaf gas exchange rates, which may provide this species a competitive advantage over herbaceous species in a variable environment (O'Keefe et al., 2020). However, how woody and grass root anatomical traits vary through the soil profile, particularly in response to fire, is unknown. This system provides a unique framework to investigate root trait relationships among different plant functional types to develop greater insight regarding the mechanisms of land cover change in North American tallgrass prairie.

Our overall goal was to assess differences in anatomical traits across woody and grass roots as a function of depth in the soil profile to better understand the water-use strategies exhibited by these two functional groups in tallgrass prairie. Specifically, we addressed the following questions: (1) Do grass and woody root anatomical traits differ with fire frequency or soil depth?; (2) Do relationships between anatomical traits that confer hydraulic safety versus efficiency vary by fire frequency or soil depth?; (3) Is root anatomy associated with integrative root traits (e.g. root diameter, SRL and root biomass)?; and (4) When scaled by root biomass, do root water-use traits impact the capacity for water uptake across treatment contrasts? We hypothesized that: (1) grass roots will exhibit traits associated with efficient water use at shallow soil depths (10 cm deep) while woody roots will exhibit efficient water-use traits at deeper soil depths (50 cm deep) because grass roots typically use shallower water than woody roots in temperate North American prairies (Nippert & Knapp, 2007); (2) roots with efficient water-use traits at the anatomical level will also exhibit efficient water-use traits at the integrative trait level; (3) efficient water-use anatomical traits will be associated with greater water uptake capacity at the whole-system level; and (4) grass and woody roots will exhibit more efficient water-use traits in less frequently burned locations. Grasses likely experience greater competition for shallow soil water with co-occurring plants in less frequently burned, more diverse communities (Ratajczak et al., 2012; Smith & Knapp, 2003), and may consequently have more efficient *shallow* roots. Woody plants, however, may have more efficient *deep* roots to support their greater aboveground growth in less frequently burned areas. Overall, our results should provide novel perspective for how root traits might influence the dynamics of grasses and woody plants in tallgrass prairie.

2 | MATERIALS AND METHODS

2.1 | Site description

Fieldwork was conducted in June–July 2019 at the Konza Prairie Biological Station (KPBS), a Long-Term Ecological Research (LTER) site located in eastern Kansas, USA (39°05′N, 96°35′W). Permission was not needed for fieldwork. KPBS experiences a mid-continental climate, with warm, wet summers and cold, dry winters. Mean annual precipitation at the site is 835 mm (1985–2018), with 75% occurring during the growing season (April–September). In 2019, precipitation was 1336.6 mm.

Konza Prairie Biological Station is a 3,487-ha native tallgrass prairie and is divided into watersheds that receive varying combinations of fire frequency (burned every 1, 2, 4, 20 years) and grazing treatments (grazed by bison, cattle or ungrazed treatments; http://lter.konza.ksu.edu/sites/default/files/konzamap2017.jpg). These treatment combinations produce a heterogeneous landscape that is dominated by a few C_4 grass species, with many co-occurring subdominant C_3 forbs and shrubs (Smith & Knapp, 2003). Clonal shrubs (primarily *C. drummondii* and *Rhus glabra*) have increased in abundance and distribution within watersheds that are not frequently burned (Ratajczak et al., 2014). Additionally, long-term weathering at the site has produced a topographically variable landscape, with thin (<0.05 m), rocky upland soils, steep slopes and deep (>2 m), silty-clay lowland soils (Ransom et al., 1998).

2.2 | Sampling protocol

Soil cores were collected from lowland locations within six ungrazed watersheds (1D, K1B, K4A, 4F, 20B and 20C) of three different burn frequencies (burned every 1, 4 and 20 years). Vegetation cover type varied by burn frequency, with annually burned watersheds (1D and K1B) primarily covered by C_4 grasses (e.g. *Andropogon gerardii*, *Sorghastrum nutans* and *Panicum virgatum*), watersheds burned every 20 years (20B and 20C) primarily covered by clonal shrubs (e.g. *C. drummondii* and *R. glabra*) and watersheds burned every 4 years (K4A and 4F) covered by both C_4 grasses and shrubs. A 540MT Geoprobe Systems hydraulic push corer with a core tip that minimized soil compaction was used to collect 1 m deep cores of 5 cm diameter in each cover type (Salina, KS, USA). Three cores were collected from grass cover in each annually burned watershed, three cores were collected from shrub cover in each 20-year burned watershed and three cores were collected from separate areas of both grass and woody cover in each 4-year burned watershed (24 cores total; $n = 6$ per species × depth × fire treatment). While we could not definitively sample cores from specific plant species, we sampled grass roots from directly under C_4 grasses and woody roots from the centre of large mature *C. drummondii* individuals. The C_4 grass and mature *C. drummondii* were selected at random and were all located at least 50 m apart.

Cores were immediately returned to the laboratory and split into 10 cm increment subsamples. Roots were sieved and cleaned from each subsample, but here we focused only on 0–10, 20–30 and 40–50 cm increment sections (representative of 'shallow', 'intermediate' and 'deep' soil depths; hereafter '10', '30' and '50' cm depths for simplicity). A 5-cm long section of fine roots (approximately ≤0.5 mm diameter) was separated from each section, submerged in FAA (10% formalin, 5% glacial acetic acid and 50% ethanol) under a partial vacuum overnight, and stored at room temperature until further processing.

2.3 | Root anatomy

The preserved root samples were embedded in paraffin and cross-sectioned using a microtome at the Kansas State College of Veterinary Medicine Histopathology laboratory. Each cross section was then stained with Safranin-O and Fast Green (Ruzin, 1999), and images of stained sections were captured at 4×, 10×, 20× and 40× using an Infinity 2 digital camera attached to an Olympus BH-2 microscope (Olympus, Center Valley). Root anatomical traits were then measured using the open-source image processing software ImageJ2 Fiji (Schindelin et al., 2012). For each cross section, xylem conduit area, conduit cell wall thickness, conduit number, conduit mechanical safety (t/b; xylem cell wall thickness/conduit diameter), stele area, endoderm thickness and root diameter were measured. Assuming each xylem conduit was circular, we then calculated median conduit diameters (see Table 1 for a list of traits and their definitions).

Finally, we assessed the capacity of each root cross section to transport water by calculating the theoretical hydraulic conductivity and hydraulic diameter of each individual root cross section. Theoretical hydraulic conductivity (K_t), which indicates the ability for axial water transport through the xylem (Tyree & Ewers, 1991), was calculated as:

$$K_t = \sum \left(\frac{\pi r^4}{\eta} \right), \quad (1)$$

where r is the radius of each conduit within a cross section and η is the viscosity of water at 20°C. The hydraulic diameter (d_h) indicates the diameter of the conduit with the average hydraulic conductivity (Tyree & Zimmermann, 2002) and was calculated as:

$$d_h = \left(\sum \frac{d^4}{\eta} \right)^{1/4}, \quad (2)$$

where d is the diameter of each conduit and n is the number of conduits per cross section.

2.4 | Integrative root traits

The remaining root sample from each soil subsection was reserved to measure root biomass traits. Samples were stored in sealed plastic bags at 4°C for approximately 3–4 days before processing. Roots were scanned on a flatbed scanner (Epson Perfection 4870) and total root length per soil subsection was measured using WinRhizo Pro software (Regent Instruments). Roots were then dried at 60°C for 72 hr and weighed, and SRL was calculated as the total root length divided by the total dry biomass per soil subsection. The roots included in SRL calculations were primarily fine roots, as 80% had diameters less than 0.5 mm and 99.6% had diameters less than 2 mm.

To estimate the capacity of the root system to transport water, we calculated theoretical root conductance ($k_{t\text{-biomass}}$) at each soil depth. Theoretical hydraulic conductivity (K_t) was multiplied by the dry biomass for each corresponding root sample and was then averaged to estimate the mean $k_{t\text{-biomass}}$ for each depth.

2.5 | Statistics

All statistical analyses were conducted in R V. 4.0.3 (R Core Team, 2020). We checked all data for normality using normal q–q plots and assessed the homogeneity of variances by examining residuals versus fitted plots in base R (Faraway, 2005). All data were log-transformed to meet model assumptions. The following analyses were conducted to assess each question:

TABLE 1 Measured root anatomical and integrative traits, with a brief description and units associated with each variable

Root anatomical traits	Units	Description
Conduit diameter	μm	Median conduit diameter per root
Conduit cell wall thickness	μm	Xylem conduit cell wall thickness
Conduit number		Number of xylem conduits per root
Conduit mechanical safety (t/b)		Xylem conduit cell wall thickness/conduit diameter
Stele area	μm^2	Area of root stele
Endoderm thickness	μm	Thickness of root endoderm
Root diameter	mm	Root diameter
Theoretical hydraulic conductivity (K_t)	mmol mm $MPa^{-1}s^{-1}$	The ability for axial water transport through the xylem per root
Hydraulic diameter (d_h)	μm	The diameter of the conduit with the average hydraulic conductivity
Root integrative traits		
Specific root length (SRL)	m/g	The ratio of total roots length to total root dry biomass
Theoretical root conductance ($k_{t\text{-biomass}}$)	$kg^2m\ MPa^{-1}s^{-1}$	The capacity for individual root water transport multiplied by root dry mass
Root tissue density (RTD)	g/m^3	The ratio of root dry mass to root volume

2.5.1 | Do grass and woody root anatomical traits differ with fire frequency or soil depth?

We compared individual anatomical traits across burn treatments and soil depths using mixed effects models with the LME4 package V. 1.1-26 (Bates et al., 2015). Separate models were developed for woody and grass root traits because both root types were not present in all watersheds. Additionally, we did not directly compare woody and grass roots because we expected these two functional groups to differ substantially and preferred to maximize our statistical power in models that focused on responses to burn treatments and soil depths. Models comparing woody roots included burn treatment (4-year and 20-year burns) and soil depth (10, 30 and 50 cm) as fixed effects and watershed as a random effect. Models comparing grass roots included burn treatment (1-year and 4-year burns) and soil depth (10, 30 and 50 cm) as fixed effects and watershed as a random effect.

2.5.2 | Do relationships between anatomical traits that confer hydraulic safety versus efficiency vary by fire frequency or soil depth?

We created log–log regressions between conduit t/b (a metric of hydraulic safety) and hydraulic efficiency traits (median conduit diameter, conduit number, d_h and K_t) using the LME4 package. Individual models were constructed for each conduit t/b * efficiency trait pairwise comparison and for each functional group separately, including watershed as a random effect. Additionally, separate models were conducted for (a) each burn treatment (including traits for all soil depths) and (b) each soil depth (including traits for each burn treatment). Finally, we compared conduit wall thickness with conduit t/b using log–log regressions for each treatment combination to understand the relative control of conduit diameter versus conduit wall thickness over variation in conduit t/b.

2.5.3 | Is root anatomy associated with integrative root traits?

We conducted Information Theoretic (IT) Model Averaging (Burnham & Anderson, 2004) following O'Keefe and Nippert (2018) to evaluate which root anatomical traits contribute most strongly to variation in root biomass, SRL and root diameter. IT Model Averaging uses information criteria to compare multiple competing models, ranks and weights each model, and then produces a final model by averaging a top model set. Model averaging was chosen over traditional null hypothesis testing and model selection procedures because this approach better accounts for model uncertainty, allows for the evaluation of multiple competing hypotheses, avoids over-parameterization and provides more robust parameter estimates (Burnham & Anderson, 2004; Grueber et al., 2011). Each competing model initially included all anatomical traits except stele area, which was excluded due to low sample sizes. Burn treatment and root depth were included as random effects, and the top model set was chosen from a 4 AICc cut-off. Relative importance, a unitless metric which ranges from 0 to 1, was determined for each parameter included in the averaged model (0 = the parameter has no explanatory weight and 1 = the parameter is included in each top model). Finally, we created log–log regressions between anatomical traits and higher functional level root traits for the top three anatomical traits that exhibited relative importance values greater than 0.70.

2.5.4 | When scaled by root biomass, do root water-use traits impact the capacity for water uptake across treatment contrasts?

We compared root conductivity scaled by root biomass ($k_{t\text{-biomass}}$) across burn treatments and soil depths using mixed effects models with the LME4 package. Separate models were developed for woody and grass root traits, where burn treatment and soil depth were fixed effects and watershed was a random effect.

3 | RESULTS

3.1 | Do grass and woody root anatomical traits differ with fire frequency or soil depth?

Root anatomical traits associated with grass cover generally did not differ between burn treatments (Tables S1 and S2; Figure 1). Conduit number was the only grass trait that differed significantly across soil depths ($p = 0.01$; Table S1), where grass roots collected from shallower soil (i.e. 10 cm) had more xylem conduits than roots collected from deeper soil (Figure 1c). In annually burned grasses, conduits were also generally thinner in shallow roots than in deeper roots, although this trend was not significant ($p = 0.14$; Table S1). Conversely, woody anatomical traits exhibited greater differences between burn treatments and soil depths (Table S1; Figure 1). We found a significant interaction between burn treatment and soil depth for conduit diameter ($p = 0.04$) and t/b ($p = 0.02$), as well as a significant main effect of burn treatment for stele area ($p = 0.04$; Table S1). Shallow woody roots had wider conduits and stele area when burned every 4 years and wider conduits at deeper depths when burned every 20 years (Figure 1b,h). However, wider conduits did not correspond with greater theoretical water conducting capacity of individual roots, as woody K_t did not differ significantly between burn treatments or soil depth ($p > 0.05$; Tables S1 and S2). Finally, conduit t/b was greater in deep roots than shallow roots when burned every 4 years (i.e. conduits were 'safer'), but lower than conduit t/b in shallow roots when burned every 20 years (Figure 1f).

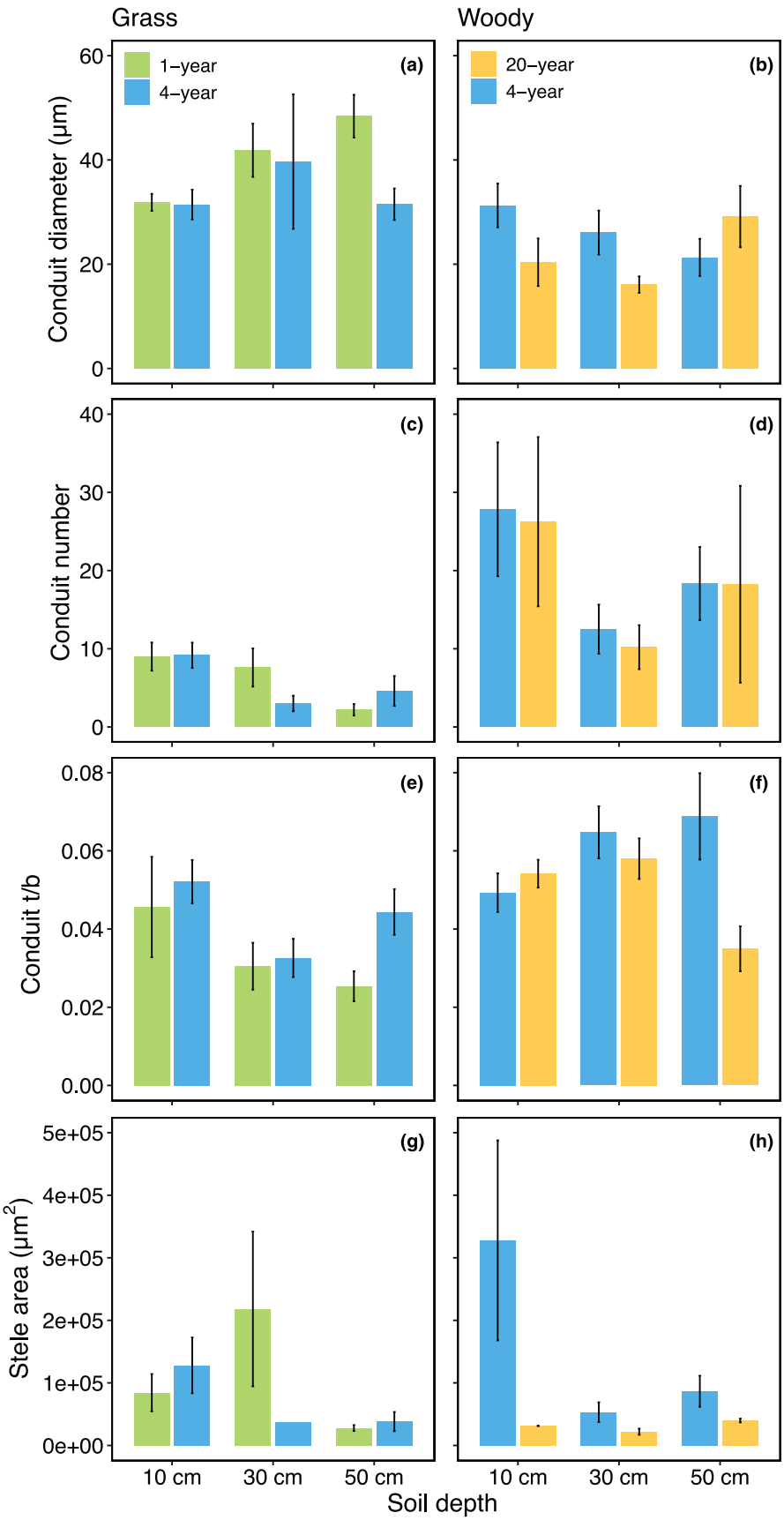

FIGURE 1 Anatomical traits measured in grass and woody roots across burn treatments (1-year, 4-year and 20-year burn intervals) and soil depths (10, 30 and 50 cm deep). Shown are mean (±1 *SE*) median conduit diameter (a, b), conduit number (c, d), conduit t/b (e, f) and stele area (g, h). Corresponding statistics are shown in Table S1

3.2 | Do relationships between anatomical traits that confer hydraulic safety versus efficiency vary by fire frequency or soil depth?

Woody hydraulic efficiency traits such as conduit diameter, d_h and K_t all exhibited strong negative relationships with conduit t/b in the 20-year burn treatment but not in the 4-year burn treatment (Figure 2d–f; Table S3). Conversely, grass d_h and K_t exhibited positive relationships with conduit t/b in the 4-year burn treatment (Figure 2b,c; Table S3). Most relationships between conduit t/b and hydraulic efficiency traits were not significant when compared across soil depths (Table S4). Finally, while conduit wall thickness is inherently related to conduit t/b, this relationship was only significant in 4-year burned woody roots (Table S3).

3.3 | Is root anatomy associated with integrative root traits?

Root anatomical traits did not contribute to variation in root biomass or root diameter for either grass or woody roots (Tables S5 and S6). Conduit t/b, diameter and cell wall thickness all contributed strongly to variation in SRL, having importance values above 0.8 and contributing to ≥11 models in the top model set for both woody and grass roots (Tables S5 and S6). Despite this, no anatomical trait was significant in the final averaged models (Tables S5 and S6), and only woody conduit t/b showed a significant negative relationship with SRL ($p = 0.04$; Figure 3; Table S7).

3.4 | When scaled by root biomass, do root water-use traits impact the capacity for water uptake across treatment contrasts?

Theoretical root hydraulic conductivity scaled by root biomass ($k_{t\text{-biomass}}$) measured at each soil depth differed significantly across depths for both grass and woody roots ($p < 0.01$ for both cover types; Table S8). Roots at shallower depths had greater $k_{t\text{-biomass}}$ than roots at deeper depths for both cover types, and this difference was most dramatic in woody roots (Figure 4). Woody roots in the 4-year burn treatment generally had greater $k_{t\text{-biomass}}$ than the 20-year burn treatment, particularly at 10 cm deep, while grass roots in the 1-year burn treatment had greater $k_{t\text{-biomass}}$ than the 4-year burn treatment at 30 cm deep (Figure 4). However, there were no significant effects

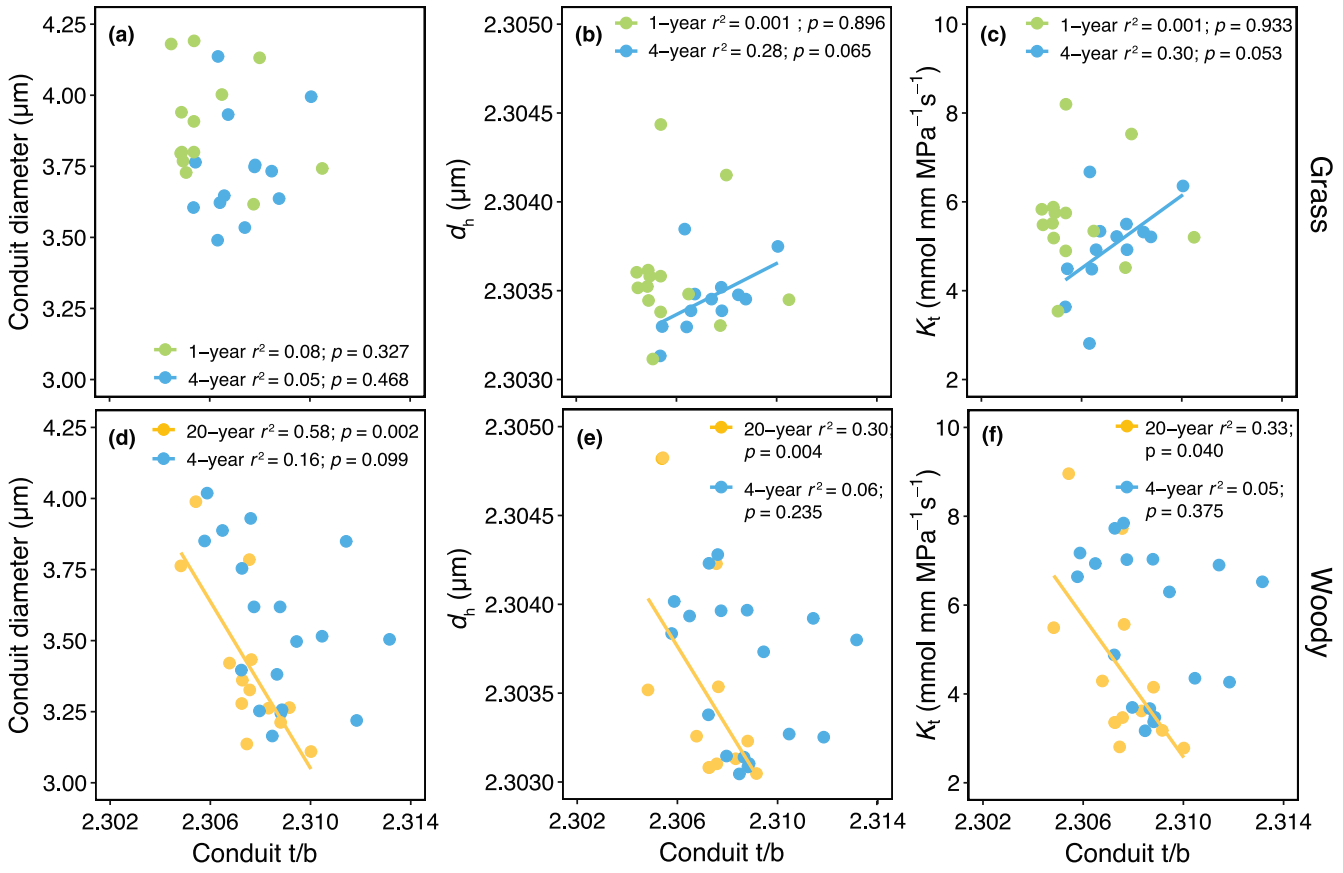

FIGURE 2 Relationships of grass and woody conduit t/b with hydraulic efficiency traits (median conduit diameter [a, d]; hydraulic diameter, d_h [b, e]; and theoretical hydraulic conductivity, K_t [c, f]) for different burn treatments (1-year, 4-year and 20-year burn intervals). Shown are log-transformed data and r^2 and p-values for all log–log models, but regression lines are only indicated for relationships with $r^2 > 0.20$ or $p < 0.05$. Corresponding statistics are shown in Table S3

of burn treatment or an interaction between burn treatment and depth for $k_{t\text{-biomass}}$ in either cover type (Table S8).

4 | DISCUSSION

4.1 | Do grass and woody root anatomical traits differ with fire frequency or soil depth?

Shrubs generally have deeper roots than grasses and their reliance on deep water has long been hypothesized to facilitate shrub–grass coexistence in grasslands and savannas (Jackson et al., 1996; Schenk & Jackson, 2002). In North American tallgrass prairie, shrubs such as *C. drummondii* use deeper soil water than co-occurring grasses, especially when shallow soils become dry (Nippert & Knapp, 2007; Ratajczak et al., 2011). As such, we hypothesized that woody roots would exhibit anatomical traits that allow for efficient deep water-use traits while grass roots would exhibit efficient water-use traits at shallower depths, and that these trends would vary by fire treatment. Our results show that woody conduit diameters vary by soil depth and fire frequency (i.e. deep conduits were narrower than shallow conduits when above-ground biomass burned every 4 years and wider than shallow conduits when burned every 20 years). This somewhat supports our hypothesis that woody plants have more efficient deep roots to support their greater above-ground growth in less frequently burned areas. However, K_t did not vary across fire treatments or depths (Tables S1 and S2), indicating that these differences in conduit diameter do not impact the capacity for individual roots to move water.

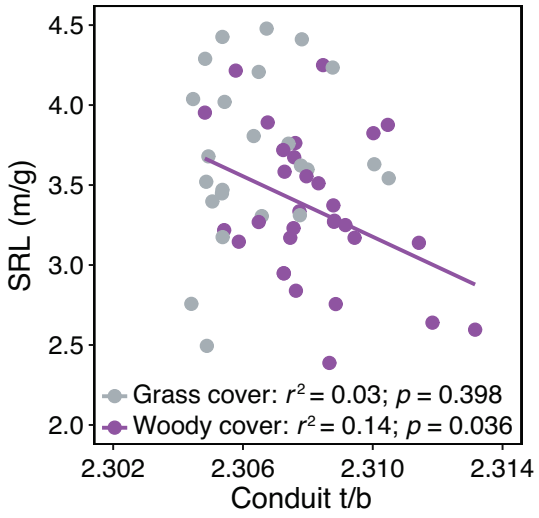

FIGURE 3 Relationship of specific root length (SRL) with conduit t/b for grass and woody roots. Shown are log-transformed data and r^2 and p-values for both log-log models, but regression lines are only indicated for relationships with $r^2 > 0.20$ or $p < 0.05$. Corresponding statistics are shown in Table S7

We also found that deep woody roots had higher conduit t/b values than those in shallow soil when above-ground biomass was burned every 4 years and lower conduit t/b values than shallow roots when burned every 20 years (Figure 1f). High conduit t/b (i.e. greater conduit cell wall thickness relative to diameter) confers hydraulic safety through mechanical strength (Hacke et al., 2001; Pratt et al., 2007). Since *C. drummondii* relies primarily on deep soil water, mechanical strength at greater depths might protect roots from hydraulic dysfunction if deep soil water becomes depleted, while allowing roots to regain functionality once water stores are replenished. Given that tallgrass prairie burned every 4 years has greater plant diversity than when burned every 20 years, and that much of this diversity is attributed to deep rooted shrubs and forbs (Ratajczak et al., 2012; Smith & Knapp, 2003), *C. drummondii* may experience greater competition for deep water in the 4-year burn treatment. If so, deep water stores are more likely to become depleted and deep roots with greater mechanical strength may provide a competitive advantage for *C. drummondii* in more diverse plant communities.

Also contrary to our predictions, we found that shallow grass roots generally had thinner, more numerous xylem conduits than deeper roots, which resulted in similar K_t across depths (Figure 1a,c; Tables S1 and S2). Thinner, more numerous root conduits could confer resistance to embolism (Wahl & Ryser, 2000) and benefit grasses in shallow soils

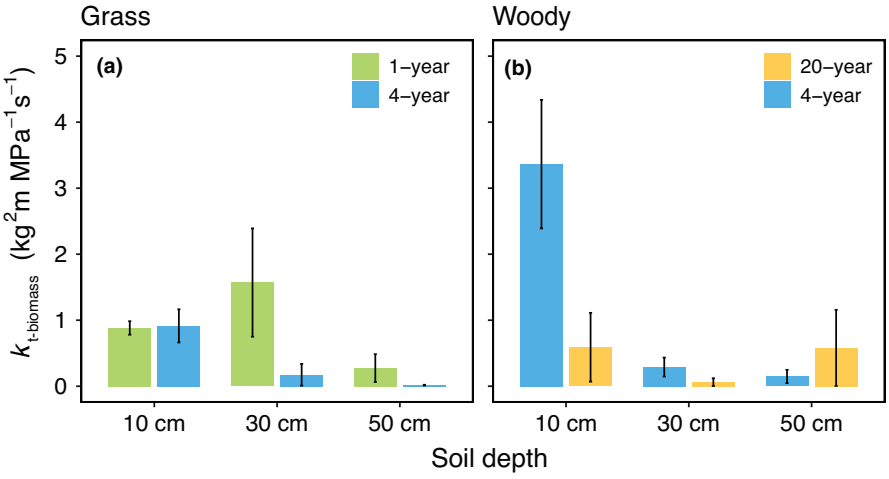

FIGURE 4 Mean (±1 *SE*) theoretical root conductance ($k_{t\text{-biomass}}$) measured in grass and woody roots across burn treatments (1-year, 4-year and 20-year burn intervals) and soil depths (10, 30 and 50 cm deep). Corresponding statistics are shown in Table S8

that experience fluctuations in water availability, as well as greater exposure to rapidly changing microclimate conditions. However, these anatomical differences did not alter the capacity for grass roots to move water efficiently across soil depths or fire treatments.

4.2 | Do relationships between anatomical traits that confer hydraulic safety versus efficiency vary by fire frequency or soil depth?

We were interested in characterizing relationships between root anatomical traits that are associated with hydraulic safety (high conduit t/b; greater conduit cell wall thickness relative to diameter) and efficiency (e.g. wide conduit diameter; high d_h and K_t) because these trade-offs can provide mechanistic insight regarding the hydraulic strategies utilized by different species and functional groups (Bouche et al., 2014; Hacke et al., 2006; Ocheltree et al., 2016; Wheeler et al., 2005). Traits that promote efficient water transport were negatively related to conduit t/b (hydraulic safety), but only in woody roots at locations burned every 20 years (Figure 2). Conversely, grass conduit diameter was not significantly related to conduit t/b in either burn treatment (Figure 2a) and both d_h and K_t were *positively* associated with conduit t/b in the 4-year burned roots (Figure 2b,c). This result is a unique example of trait relationships that may provide both hydraulic safety and efficiency at the anatomical level, as most studies show either weak trade-offs between safety and efficiency or low safety and low efficiency (Gleason et al., 2016). Why trait relationships varied between root functional types or environmental gradients is unknown but may be related to the complex multifunctionality of roots (Kramer-Walter et al., 2016) and/or external factors that can alter root traits such as mycorrhizal associations (Chen et al., 2016).

4.3 | Is root anatomy associated with integrative root traits?

We hypothesized that efficient water-use traits would be correlated at both the anatomical and integrative trait levels because root hydraulic efficiency can potentially impact above-ground plant growth (Hummel et al., 2007; Wahl & Ryser, 2000). However, neither root biomass nor root diameter were associated with anatomical traits for either functional group. We did find a negative relationship between woody SRL and conduit t/b (Figure 3), suggesting that hydraulic safety versus efficiency trade-offs may transcend multiple biological scales of inquiry. In other words, root systems with high SRL (i.e. those that are generally more efficient at acquiring water; Comas & Eissenstat, 2004; Reich et al., 1998) may develop at the cost of less conduit mechanical safety. This trade-off could occur because SRL has a negative relationship with root tissue density [RTD; Tissue Density = Dry Mass/(Root Length $\times \pi \times$ Root Diameter^2); Figure S1a; Freschet et al., 2021], which is associated with traits that could reduce conduit t/b (e.g. a larger proportion of cell wall within the stele; Wahl & Ryser, 2000). Indeed, we found that woody roots with large conduit t/b values had thicker conduit cell walls (Figure 2h), and that conduit t/b exhibited a positive (albeit non-significant) relationship with RTD (Figure S1b). Using this same logic, the lack of a relationship between grass conduit t/b and tissue density (Figure S1b) may explain the non-significant relationship observed between grass conduit t/b and SRL. These results highlight that anatomical traits can be used to enhance mechanistic understanding of commonly measured root economic traits such as SRL and that future research should investigate these relationships in situ across a broad range of plant functional groups.

4.4 | When scaled by root biomass, do root water-use traits impact the capacity for water uptake across treatment contrasts?

Although anatomical traits varied across soil depths and fire frequencies, these patterns did not impact the theoretical water conducting capacity (K_t) of individual roots (Tables S1 and S2). Once scaled by biomass, however, $k_{t\text{-biomass}}$ differed by depth for both woody and grass root systems. Shallow roots of both functional types had greater $k_{t\text{-biomass}}$ than deep roots, and this response was greater in woody roots (Figure 4). Our observation that grasses have greater $k_{t\text{-biomass}}$ at shallow and intermediate depths is not surprising given that they have extensive, fibrous roots concentrated primarily in shallow soil that maximize resource uptake (Nippert et al., 2012). High conductance due to the distribution of root biomass at these depths likely allows grasses to rapidly utilize available water, providing them a competitive advantage over co-occurring species that use water less efficiently (O'Keefe & Nippert, 2018). This advantage may be enhanced under water-limiting conditions because their thinner xylem conduits (compared to deeper roots; Figure 1a,c) likely confer resistance to embolism (Wahl & Ryser, 2000). Thus, dominant C_4 grasses may exhibit unique root trait combinations that allow for both safe and efficient water use in tallgrass prairie.

We did not expect shallow woody roots to also exhibit high $k_{t\text{-biomass}}$ because these shrubs rely on deeper water sources than grasses (Ratajczak et al., 2011) and should benefit from having deep roots with higher conductance (Wang et al., 2015). One explanation for this observation could be that shallow woody roots have a greater capacity to efficiently transfer water from saturated shallow soils to drier deeper soils immediately after a large precipitation event (i.e. exhibit 'inverse hydraulic lift'; Schulze et al., 1998). Although hydraulic lift is not a common phenomenon in this mesic tallgrass prairie (O'Keefe & Nippert, 2017), inverse hydraulic lift may be more prevalent, especially for woody plants with this unique conductance pattern. Shallow woody roots had greater $k_{t\text{-biomass}}$ in the 4-year burned watershed, where shrubs may experience greater competition for water with neighbouring plants and may benefit from root systems that can transport water to deeper soil when shallow soils are saturated. This phenomenon could potentially accelerate shrub growth and rates of woody encroachment under extended periods of drought.

5 | CONCLUSIONS

Overall, our results have important implications for understanding the water-use strategies of woody and grass functional groups in a dynamic tallgrass prairie. We showed three key findings: (a) Woody roots had high hydraulic conductance in shallow soils and greater mechanical strength in deeper soils, which may provide a competitive advantage in less frequently burned, more diverse plant communities; (b) shallow grass roots have unique trait combinations at the anatomical and root-system levels (thinner, more numerous conduits and higher $k_{t\text{-biomass}}$ compared to deeper roots) that likely allow these plants to rapidly use water but tolerate dry soils under multiple fire regimes; and (c) hydraulic safety versus efficiency trade-offs may translate between different hierarchical scales (i.e. from anatomical to integrative root traits). These results provide anatomical evidence to support traditional notions regarding woody and grass water use in tallgrass prairie (e.g. that grasses aggressively use shallow water) and also provide novel insight regarding functional strategies that may facilitate woody encroachment in less frequently burned tallgrass prairie (e.g. that deep woody roots may tolerate water depletion in diverse communities and have the ability to replenish these deep water stores with hydraulically efficient shallow roots). Future work should investigate these dynamics, as they may accelerate woody encroachment in tallgrass prairies under altered precipitation patterns associated with global climate change. Finally, our finding that anatomical trait relationships and their impact on root functional traits vary across landscape treatments highlights the need for additional studies of root trait dynamics in natural ecosystems.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and designed the methodology; K.O., S.B., R.K. and E.G.T. collected the data; K.O. analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.dr7sqvb0g (O'Keefe et al., 2021).

ORCID

Kimberly O'Keefe https://orcid.org/0000-0001-7799-0514

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