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THE EFFECTS OF PRECIPITATION, TREE SIZE, AND STAND DENSITY ON WATER
USE AND DROUGHT TOLERANCE OF EASTERN REDCEDAR IN OKLAHOMA, USA

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THE EFFECTS OF PRECIPITATION, TREE SIZE, AND STAND DENSITY ON WATER
USE AND DROUGHT TOLERANCE OF EASTERN REDCEDAR IN OKLAHOMA, USA

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Abstract

Plant woody encroachment and its effects on the local water budget have become of increasing concern globally as woody plants continue to expand their geographic range into grasslands at a faster rate each year. In the Great Plains Region of the United States, woody plant encroachment has begun to replace native grasslands at an accelerating rate. The replacement of native grassland species with woody plants, such as trees and shrubs, affects the local water budget due to increased rooting depth and altered landscape phenology. Eastern redcedar (ERC, *Juniperus virginiana*, L.) is an evergreen tree that remains physiologically active year-round and is considered one of the fastest encroaching species in the Great Plains, converting grasslands into closed canopy forests in as little as 60 years.

In Chapter 2 I aimed to identify the source of ERC water uptake, including groundwater, along a precipitation gradient in Oklahoma using stable hydrogen and oxygen isotopes from stem, soil, and groundwater samples. This will allow us to predict how ERC may respond to a changing climate that is predicted to be warmer and drier. As ERC expands its range, it is already thriving in areas that were historically unsuitable. In Chapter 3 I assess how ERC cellular and tree level water stress and stress tolerance varies with tree size in a sparsely versus highly encroached stand. Further, in chapter 4, I quantify transpiration of ERC at the tree and canopy level among ERC of varying size and density using Granier style thermal heat dissipation probes. As the replacement of native grasses with woody plants becomes increasingly common, it will be important to understand how the function of encroaching plants is shaped by environmental pressures.

Chapter 1: Introduction

1.1 Background

Drought and woody plant encroachment into grasslands has led to growing concerns about changes in the local water balance. Woody encroachment, the process in which woody plants replace grasslands is known to occur globally and can alter the local water balance by tapping into groundwater reserves and reducing recharge (Caterina et al., 2013; Huxman et al., 2005). Additionally, as canopy cover increases so does tree transpiration and evaporation of soil moisture (Jasechko et al., 2013; Schlesinger and Jasechko, 2014; Zhang et al., 2001).

One predominant woody species known to encroach grasslands in the Southern Great Plains is eastern redcedar (*Juniperus virginiana* L., ERC) (Knapp et al., 2008b; Tennesen, 2008). Previous studies have examined ERC water use and drought tolerance traits, however, many have been conducted in mesic environments in the north and eastern US and in karst topography to the south in Texas. I aimed to examine ERC water use and drought tolerance strategies from the cell to the stand level in a region (central Oklahoma) at the western boundary of the range of not only ERC, but of trees as the dominant vegetation.

1.2 Objectives

In arid and semi-arid environments groundwater is a key component of the local water budget and is critical to the survival of both people and plants. Groundwater recharge is controlled by soil texture and the amount of precipitation a landscape receives; however it can also be impacted by vegetation type. Precipitation is the largest input to the water budget and at the same time, largely dictates the kind of vegetation that can occur in a given area. In this study, I aim to increase the knowledge of how ERC will respond to variation in climate, such as

prolonged drought, and an overall increase in tree density as a result of increased encroachment. ERC is encroaching into grasslands at an unprecedented rate for a species that historically in the Great Plains region was confined to growing on rock outcrops and hillsides.

The specific aims of this study were to:

1. Identify sources of water uptake by eastern redcedar across a precipitation gradient in Oklahoma.
2. Examine how cellular and tree level response to drought and drought tolerance traits vary with tree size in two adjacent sites with contrasting canopy density.
3. Determine total water use through Granier-style thermal heat dissipation probes to quantify transpiration at the tree and stand level in two adjacent sites with contrasting canopy density.

1.3 Organization of the Dissertation

This dissertation consists of one introductory chapter, with three data chapters. Chapter 2 will be submitted to the American Geophysical Union's *Water Resources Research* journal. Chapter 3 will be submitted to *Functional Plant Ecology* section of *Frontiers in Plant Science*. Chapter 4 is expected to be submitted to Elsevier's *Rangeland Ecology and Management* Journal.

Chapter 2: Seasonal water source and groundwater use by eastern redcedar using stable isotopes across Oklahoma's precipitation gradient

This study examined the source of water uptake by eastern redcedar across a precipitation gradient in Oklahoma to determine the effects of groundwater uptake under different precipitation regimes. In order to better assess the effects of eastern redcedar on local water budgets we aim to answer three questions. (1) How does stable deuterium and oxygen isotope

composition vary spatially and temporally in potential source (groundwater, soil water) and stem waters of eastern redcedar? (2) Does eastern redcedar water source utilization vary across a precipitation gradient or climatological season? And (3) what proportion of eastern redcedar water uptake is derived from ground water?

Chapter 3: Effect of tree size and stand density on water stress tolerance of eastern redcedar

For this study I examined cellular and tree level responses to drought at the University of Oklahoma's Kessler Atmospheric and Ecological Field Station (OU KAEFS) in McClain County, OK located in a semi-arid region in Oklahoma. I examined 1) How does water stress vary across ERC individuals of different sizes (heights) and between stands with different levels of encroachment (density)? 2) Does water stress tolerance of ERC individuals vary with size and encroachment level? And 3) Do smaller ERC individuals utilize a more conservative hydraulic safety strategy?

Chapter 4: Eastern redcedar tree size and stand density effects on transpiration

This study quantified the differences in sapflux, tree level transpiration and canopy level transpiration at OU KAEFS in eastern redcedar individuals of two contrasting canopy types. We aimed to answer three questions: 1) do sapflux (J_s ; $\text{g m}^{-2} \text{s}^{-1}$) and transpiration rates (E_t ; kg d^{-1}) vary with encroachment level? 2) Does whole canopy transpiration (E_c ; $\text{kg m}^{-2} \text{d}^{-1}$) differ between sites? 3) Does competition or other density effects mitigate the potential increase in whole canopy transpiration as tree density increases?

Chapter 2: Seasonal water source and groundwater use by eastern redcedar using stable isotopes across Oklahoma's precipitation gradient

Abstract

In semi-arid environments groundwater is a key component of the local water budget, with evapotranspiration consuming nearly all precipitation inputs, and heavily affecting groundwater recharge. Woody encroachment in semi-arid environments – the replacement of native grasses with woody trees and shrubs – is increasing pressure on groundwater resources due to the deeper roots and in some cases evergreen nature of the woody plants. Using stable hydrogen (δD) and oxygen ($\delta^{18}O$) isotopes we assessed the effects of the commonly encroaching eastern redcedar (ERC) tree on local water budgets by partitioning stem water into potential water sources in each climatological season. At three locations along Oklahoma's precipitation gradient, we examined (1) How does stable isotopic composition vary temporally in source waters; (2) Does the source of ERC water uptake vary temporally and across a precipitation gradient and; (3) What proportion of ERC water uptake is derived from groundwater? The isotopic composition of soil water had a broad range of values (-144.1 to -25.3 and -14.7 to 0.7 ; δD and $\delta^{18}O$ respectively). Although proportion groundwater use varied between 3-31%, it did not vary significantly by season ($p=0.81$) or across the precipitation gradient ($p=0.23$), with differences only between the wettest and driest sites during the summer ($p<0.05$). These results indicate relatively low groundwater use, contrary to what has been previously hypothesized. While ERC is certainly intercepting precipitation and affecting groundwater recharge, direct groundwater use was surprising low. These results contribute to a better understanding of how ERC, and woody encroachment in general, affect local water budgets.

2.1 Introduction

Woody encroachment, the process in which woody plants replace grasslands is known to occur globally and can alter the local water balance by tapping into groundwater reserves and reducing recharge (Caterina et al., 2013; Huxman et al., 2005). As canopy cover increases so does transpiration (T) and evaporation (E) (Jasechko et al., 2013; Schlesinger and Jasechko, 2014; Zhang et al., 2001). One such woody species known to encroach grasslands in the Southern Great Plains is eastern redcedar (*Juniperus virginiana* L., ERC) (Knapp et al., 2008b; Tennesen, 2008). ERC encroachment and water use has been studied thoroughly in areas that experience prolonged drought such as arid to semi-arid environments, but also in mesic regions (Zou et al., 2010; Zou et al., 2014). However, many of these studies focus on total water uptake or groundwater and stream water recharge, but they do not identify the proportional contribution of various water sources to water uptake by ERC.

In arid and semi-arid environments groundwater is a key component of the local water budget and is critical to the survival of both people and plants. Groundwater recharge is controlled by the amount of precipitation a landscape receives both in terms of seasonality and spatial distribution (citation). Precipitation is the largest input to the water budget and largely dictates the vegetation type for a given area (Morecroft et al., 2004; Nadezhdina et al., 2010; Petrie and Brunsell, 2012). Evapotranspiration (ET) is the largest output to the water budget, and in arid and semi-arid environments ET is nearly equal to precipitation (Turner, 1991; Zhang et al., 2001). Transpiration alone can range from 55-80% of ET in deciduous forests to semiarid regions (Schlesinger and Jasechko, 2014). Typically, in these regions, when vegetation type transitions from grassland to woodland, ET is increased while soil moisture and groundwater levels, as well as recharge, are reduced due to an increase in above ground biomass and deeper root systems (Huxman et al., 2005; Wine et al., 2015).

Stable isotopes of oxygen ($\delta^{18}\text{O}$) and hydrogen (δD) are commonly used to identify water sources of woody plants (Dawson et al., 2002; Ehleringer and Dawson, 1992; Jackson et al., 1999). Because it is assumed there is no isotopic fractionation of hydrogen and oxygen molecules during plant root water uptake (Allison et al., 1984; Dawson et al., 2002), the isotopic signature of stem water can be compared with the signatures of potential source waters to determine the contribution of various sources. As hydrogen and oxygen experience varying levels of evaporative effects, specifically $^2\text{H}:^1\text{H}$ (deuterium; δD and $^{18}\text{O}:^{16}\text{O}$, these isotopes will become enriched with more of the heavy isotope in deeper layers of the soil (Ehleringer and Dawson, 1992). As such, the ratio of the heavy (^2H , ^{18}O) to lighter (more common ~99%) isotope (^1H , ^{16}O) can then be used to identify the water source in combination with soil water and groundwater (Jasechko et al., 2013). Ehleringer and Dawson (1992) showed that plants with the ability to switch between shallow and deep soil water sources, such as ERC, have an advantage in regions with limited soil moisture availability (Grossiord et al., 2017). This dual rooting strategy can be a substantial advantage to host plants, but also surrounding vegetation through hydraulic redistribution, during periods of drought (Nadezhdina et al., 2010; Yoda et al., 2012). However, not all woody plants have this ability. Both the precipitation gradient in Oklahoma and ERC encroachment provide a unique opportunity to examine how stable isotopes (deuterium and oxygen), as well as deep water uptake (groundwater), vary spatially and temporally.

In order to better assess the effects of eastern redcedar on local water budgets we aim to answer three questions. (1) How does stable deuterium and oxygen isotope composition vary spatially and temporally in potential source (groundwater, soil water) and stem waters of eastern redcedar? (2) Does eastern redcedar water source utilization vary across a precipitation gradient

or climatological season? And (3) what proportion of eastern redcedar water uptake is derived from ground water? We hypothesize that (1) soil and stem water will be more isotopically enriched in the western site, with enrichment decreasing eastward as precipitation increases, (2) eastern redcedar utilization of groundwater vs. soil water will be higher in the western site and during dry summer months, with groundwater proportions decreasing to the east and during Spring?, and (3) groundwater will make up a significant proportion of water taken up by eastern redcedar across all sites.

2.2 Materials and Methods

2.2.1 Sampling Approach

Three sampling sites were established along a precipitation gradient in Oklahoma, USA. Within each site, 12 trees (8 at Dewey County), were selected to be resampled each climatological season. Samples were taken in Dewey County, Kessler, and Blue River during the summer 2015 (19 Aug, 21 July, 13 Aug), fall (10 Nov, 12 Nov, 3 Nov), winter (10 Feb, 5 Feb, 15 Feb), and spring 2016 (13 May, 11 May, 18 May), respectively, to determine and partition source water into four categories: groundwater, and soil water 0-10 cm, 10-20 cm, and 20-30 cm in depth. Sample trees were chosen by establishing three parallel transects 50 meters apart, starting ~ 10 m from a perennial water source (stream or reservoir), and selecting trees 30m apart along each transect. Transects were established moving away from a surface water source to examine possible effects on tree water source uptake from these water bodies. Only two transects (with a total of eight trees) were established at the Dewey County site. Water source of each tree was then determined using stable hydrogen and oxygen isotopes.

2.2.2 Site Descriptions

Dewey County Wildlife Management Area (Western Site)

Located 36.000299 lat. and -99.024779 long. outside Taloga in Dewey County, Oklahoma and positioned above the Rush Springs aquifer sits the Canadian River (Figure 1). The soils are classified as Lincoln fine sandy loam, with excessive draining capacity, rapid permeability, low runoff and two-meter depth to groundwater (NRCS, USDA). The average long-term (15 yrs.) precipitation is 710.7 mm with the majority occurring in the spring and summer months with an average annual temperature of 15.0 degrees Celsius (Table 1) (mesonet.org).

Kessler Atmospheric and Ecological Field Station (Central Site)

Located 34.983598 lat. and -97.529819 long. in Purcell in McClain County, Oklahoma and located over the Central Oklahoma aquifer sits the Finn Creek Site 21 Reservoir (Figure 1). The soils are classified as a Nash-Lucien complex, with well-draining, high permeability, high run off and depth to groundwater that is less than two meters in depth (NRCS, USDA). Average long-term precipitation is 908.3 mm with the majority occurring in the spring and summer months with an average annual temperature of 16.1 degrees Celsius (Table 1) (mesonet.org).

Blue River Public Fishing and Hunting Area (Eastern Site)

Located 34.32343 lat. and -96.594221 long. outside Tishomingo in Johnston County, Oklahoma and positioned over the Antlers aquifer sits the Blue River (Figure 1). Soils are classified as a Claremore-rock Outcrop Complex, with well-draining, moderate permeability, moderate runoff, and depth to groundwater is approximately one meter deep (NRCS, USDA). Average long-term precipitation is 998 mm with the majority occurring in the spring and summer months with an average annual temperature of 16.7 degrees Celsius (Table 1) (mesonet.org).

Sampled tree height, diameter at breast height (DBH), and canopy cover for each site was measured (Table 2).

2.2.3 Sampling methods

Stem Water Samples:

One stem segment per individual was sampled seasonally at breast height from the south-facing side of the tree. Each stem segment selected was free of major side branches and stems were immediately trimmed of any leaves, the bark layer was removed, placed into a 15 ml sterile centrifuge tube, capped (GeneMate), sealed with parafilm and placed in an ice chest until stored in a freezer in the lab later the same day.

Soil Water Samples:

Soil samples were taken at 3 locations along each transect and at three depths (0-10, 11-20, and 21-30 cm), in order to characterize soil water signatures at different depths. Soils were sampled using a 15 x 5 cm soil corer (AMS, Inc.), separated into the desired depths and homogenized. Each sample was then placed in a centrifuge tube, capped, sealed with parafilm and placed in an ice chest until stored in a freezer in the lab later the same day.

Water Samples:

Groundwater, surface water, and precipitation were collected throughout the study period. Groundwater was drawn up from a well at each site by either pump or windmill. Surface water was collected at a depth of approximately 0.61 meters minimize evaporative effects. Precipitation was collected at the Kessler and Blue River sites in a mason jar with paraffin oil, which was placed before a precipitation event and collected (and sealed) no later than 24 hours

after the precipitation event ceased. All water samples were collected using a 15 ml syringe. Samples were pushed through a 0.7-micron glass fiber syringe filter (25 mm, Sterlitech Corp.) into a 2 ml clear glass vial (PTFE/Silicone, 9 mm, Supelco), sealed with parafilm and stored in a refrigerator in the lab at 5° C until sent for analysis.

The local meteoric water line (LMWL) was derived by (Jaeschke et al., 2011), using 13 years of precipitation isotopic composition data.

2.2.4 Water Extraction and Isotope Composition Analysis

All stem, soil, and water samples were mailed overnight with cold packs to the Duke Environmental Isotope Laboratory (Durham, North Carolina, USA) for water extraction and analysis. Water from stem and soil samples was extracted using cryogenic vacuum distillation. To determine isotopic composition, all water samples were then analyzed using a ThermoFinnigan thermochemical elemental analyzer (TC/EA) to determine $^2\text{H}: ^1\text{H}$ (deuterium; D) and $^{18}\text{O}: ^{16}\text{O}$ ratios. Isotope ratios are expressed in conventional δ notation and referenced to the Vienna-SMOW standard for $\delta^{18}\text{O}$ and δD . The precision of these samples was +/- 1.5 and +/- 0.1 per mil. at one std. dev. for ^2H and ^{18}O , respectively.

2.2.5 Water Source Partitioning and Statistical Analysis

All water source partitioning was conducted using the Siar V4 package in R (Parnell et al., 2010). Partitioning was conducted using a Bayesian model with 500,000 iterations and Gaussian likelihood. The means of the model results are presented here. Multivariate Analysis of Covariance was performed to compare the relationships of site characteristics to δD , $\delta^{18}\text{O}$, and to the ratio of $\delta\text{D} : \delta^{18}\text{O}$. All statistics were performed using RStudio. All boxplots were made in RStudio while regressions were calculated in Sigmaplot Version 10.0.

2.3 Results

2.3.1 Temporal and spatial variation in isotopic signatures of potential source waters

Sampled groundwater and precipitation clustered near the Norman local meteoric water line (LMWL; from Jaeschke et. al 2011), defined as the relationship between hydrogen and oxygen isotopes at a site (Hughes and Crawford, 2012). The composition of surface waters varied between sites – from slightly enriched to very depleted values – with the western site surface water clustered along the LMWL due to its spring fed nature and the location of sampling near one of the main springs. Although soil water isotopic composition values are found along a broad range of values ($\delta^{18}\text{O}$ from -14.7 to 0.7 and δD from -144.1 to -25.3), the soil water of the western site is generally more isotopically depleted than the other sites and falls closer to the LMWL.

In all sites, groundwater falls along the LMWL (Fig. 3). In all sites, winter values of stem and soil water were most depleted compared to other seasons, with the largest seasonal difference occurring at the western site. Eastern site's surface water and groundwater fall along the LMWL with fall precipitation just below the LMWL. The average isotopic

2.3.2 Temporal and spatial variation isotopic signatures of stem water

Composition of soil δD and $\delta^{18}\text{O}$ varied by season ($p < 0.001$, $p < 0.001$), while only $\delta^{18}\text{O}$ varied across sites ($p = 0.004$) (Figure 2B). Isotopic differences in soil water are driven primarily by summer and winter composition values ($p < 0.001$, $p < 0.001$), δD and $\delta^{18}\text{O}$, respectively.

Differences in isotopic composition in stem water of δD and $\delta^{18}\text{O}$ were driven by season ($p < 0.001$, $p < 0.001$) rather than site ($p = 0.929$, $p = 0.499$) (Figure 3). There was no effect of tree height on the isotopic composition of stem water ($p = 0.526$, $p = 0.599$).

2.3.3 Stem water source partitioning and proportional ground water uptake

The proportion of groundwater uptake by ERC did not vary by season ($p= 0.81$) or across sites ($p= 0.23$) (Figure 4), with the exception of summer groundwater uptake in the western site compared to the eastern site ($p< 0.05$). There was no difference in summer groundwater use between either of these sites and the central site ($p= 0.839$). The proportion of ground water use in the western site varied between 3% and 31% across seasons; at the central site, groundwater use varied between 3% and 9%; and at the eastern site groundwater use varied between 5% and 10% (Table 3).

2.4 Discussion

The isotopic composition of δD and $\delta^{18}O$ in water sources is driven by fractionation as a result of evaporative effects. Summer and winter sampling dates showed the greatest difference in isotopic enrichment of stem water. While varying degrees of isotopically depleted source water was being taken up by ERC at all sites, there was not a significant difference in groundwater use between sites within season. Groundwater use across sites and season varied between 3% and 31% and was only significantly different in the most spatially separated sites, the western and eastern sites during the summer. Overall, groundwater use was much less than hypothesized.

The isotopic composition of soil water is most isotopically enriched in the summer, and most depleted during the winter at all sites. While we acknowledge that juniper roots extend past our maximum sampling depth of 30cm, we feel this was deep enough to separate deep (ground

water) and shallow (0-30cm) water sources. This shift in isotope composition is driven, in part, by the differences in seasonal input of precipitation with the largest amounts of precipitation occurring during the summer and the least amount occurring in the winter (Friedman et al., 1964; Knapp et al., 2020). The observed patterns of seasonal variation were as hypothesized as was also confirmed in Benettin et al. 2018 , however, variation within season but among sites was also expected as the sites are located along a precipitation gradient in which the lowest amount of precipitation occurs in the northwest portion of the state and increases to the southeast. However, this expected spatial variability did not occur, which may have been due to the occurrence of above average precipitation during the sampling year, resulting in greater soil water availability (and less water stress) than is typical.

The isotopic signature of stem water was similar to soil water, indicating that soil layers are the source of most the water taken up by the trees with groundwater accounting for only a small proportion in most cases. The high evaporative pressures during the hot summer months result in isotopic enrichment of source waters (Ehleringer and Dawson, 1992). Thus, we see variation among seasons, but not among sites along the precipitation gradient as expected. Given the dominant role of soil water in contributing to stem water, the seasonality expressed in stem water reflects the seasonal changes in isotopic composition of soil water. Roden et al. 2005 found similar results with Douglas-fir and pine across a precipitation gradient that found no differences in $\delta^{13}\text{C}$.

Trees are deep rooted and capable of transpiring water from deep within soil layers and underground aquifers (Dawson et al., 2002). In many parts of the world precipitation is highly seasonal including some places with bimodal (summer/winter) rainfall patterns. In areas undergoing woody plant encroachment, deep-rooted plants can access deeper source waters than

most native grasslands species, in turn altering the local water budget. In this study, we found individuals simultaneously utilizing water from both shallow and deep-water sources with varying amount of groundwater water dependence. As juniper canopy cover increases, the stands have the ability to completely transpire up to 99.5% of precipitation during a dry year (Caterina et al. 2013). The ability for roots to actively uptake shallow soil water and deeper water sources is especially important in areas that receive patchy summer precipitation (Ehleringer and Dawson, 1992), such as Oklahoma.

Plants adapted to this type of rooting strategy develop a dimorphic root system, which allows plants to take advantage of precipitation in shallow soil zones while also tapping into deeper reserves between rainfall events and during times of drought (Eggemeyer et al., 2009). Plants sampled in this study received above normal precipitation during the sampling year, which may have contributed to an increase in shallow (<30 cm) soil water and decreased groundwater use. Contrary to our findings, Grossiord et al. 2017 utilized a warming and reduced precipitation regime to study *Juniperus monosperma* water source uptake variation and found that during dry summers junipers shifted water uptake to shallow sources, while during normal and wet summer junipers increased deep water uptake. It is important to note that in this study *J. monosperma* had no access to groundwater and utilized summer monsoon rainfall as a primary precipitation source. However, McCole and Stern 2007 found that during wet periods junipers used a shallower water source as compared to drier periods. During the study, the three sampled sites received 9.3%, 21.8%, and 38.1% more precipitation than the 15-year average for Dewey County, Kessler, and Blue River, respectively (Mesonet.org). Therefore, it is plausible that the trees were not under severe water stress. Dewey County received the lowest deviation from normal precipitation and used more groundwater than any other site.

Other studies examining the effects of woody encroachment on the water budget have focused on the effects of plant removal along streams (Zou et al., 2014), changes in local ET, and total transpiration rates. Plant removal along stream banks has had mixed results. Zou et al. 2010 showed an increase in stream flow with the removal of woody plants in subalpine and mixed conifer forests, while also demonstrating little to no effect in pinyon-juniper woodland. Although average tree size (height, DBH, canopy size) were larger at the eastern site, regression analysis showed no significant trends between tree size and isotopic signature or groundwater use (all $p > 0.05$). In another study, (Dawson and Ehleringer, 1991) showed that streamside trees were not using stream water even though they theoretically had access via roots. It has been shown that an increase in forest cover increases local ET by increasing the amount of tree canopy surface area intercepting precipitation and increasing transpiration, thus decreasing groundwater recharge (Honda and Durigan, 2016; Zou et al., 2015). This study supports the idea that groundwater recharge is most likely affected, although not necessarily directly due to groundwater uptake, but through interception of precipitation and uptake of water in shallow soil layers and reduction in aquifer recharge.

These results show relatively low groundwater use and are at odds with previous studies predictions based solely on extrapolating sap flow data. Prior studies have concluded from significant transpiration occurring in ERC individuals that they must be using groundwater. While this is a reasonable assessment of how ERC affects groundwater as a whole, i.e. groundwater recharge, it is not a direct observation of groundwater withdrawal. The results of this study could have important implications when regarding the local water budget and conservation issues in Oklahoma, in particular, but also more broadly in the Great Plains region.

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Tables and Figures

Table 2.1: Environmental, soil, and ground water depth conditions for each site. Environmental data was retrieved from Oklahoma Mesonet Data. Soil characteristics were supplied by USDA NRCS (www.nrcs.usda.gov)

Site	Precipitation (mm) (annual)	Precipitation (mm) (15 yr. mean)	Temperature (°C) (annual)	Temperature (°C) (15 yr. mean)	Soil Characteristics	Depth to Ground Water
DC	783.3	710.7	15.7	15	Excessive Draining	< 3 m
KS	1161.0	908.3	16.9	16.1	Well-Draining	< 2 m
BR	1612.0	998.0	17.1	16.7	Well-Draining	< 1 m

Table 2.2: Average tree height, diameter at breast height (DBH), and canopy cover for each site as well as the range of each physical characteristic of all sampled trees.

Site	Height (m) (range)	DBH (cm) (range)	Crown Size (m²) (range)
DC	4.83 (3 -6.9)	12.48 (5.3 – 22)	16.98 (4.45 – 41.85)
KS	6.08 (3.8 – 7.3)	17.8 (8.1 – 30.9)	31.51 (11.55 – 82.35)
BR	10.18 (6 – 16)	27.88 (6.6 – 59.2)	43.47 (9.62 – 96.41)

Table 2.3: Partitioned groundwater use (%) for each site and season.

Site/Season	Summer '15	Fall '15	Winter '15	Spring '16	Annual Site Average (%)
DC	31	13	3	15	15.5
KS	9	6	3	9	6.75
BR	5	10	7	6	7
Season Average (%)	15	7.25	3.25	10	

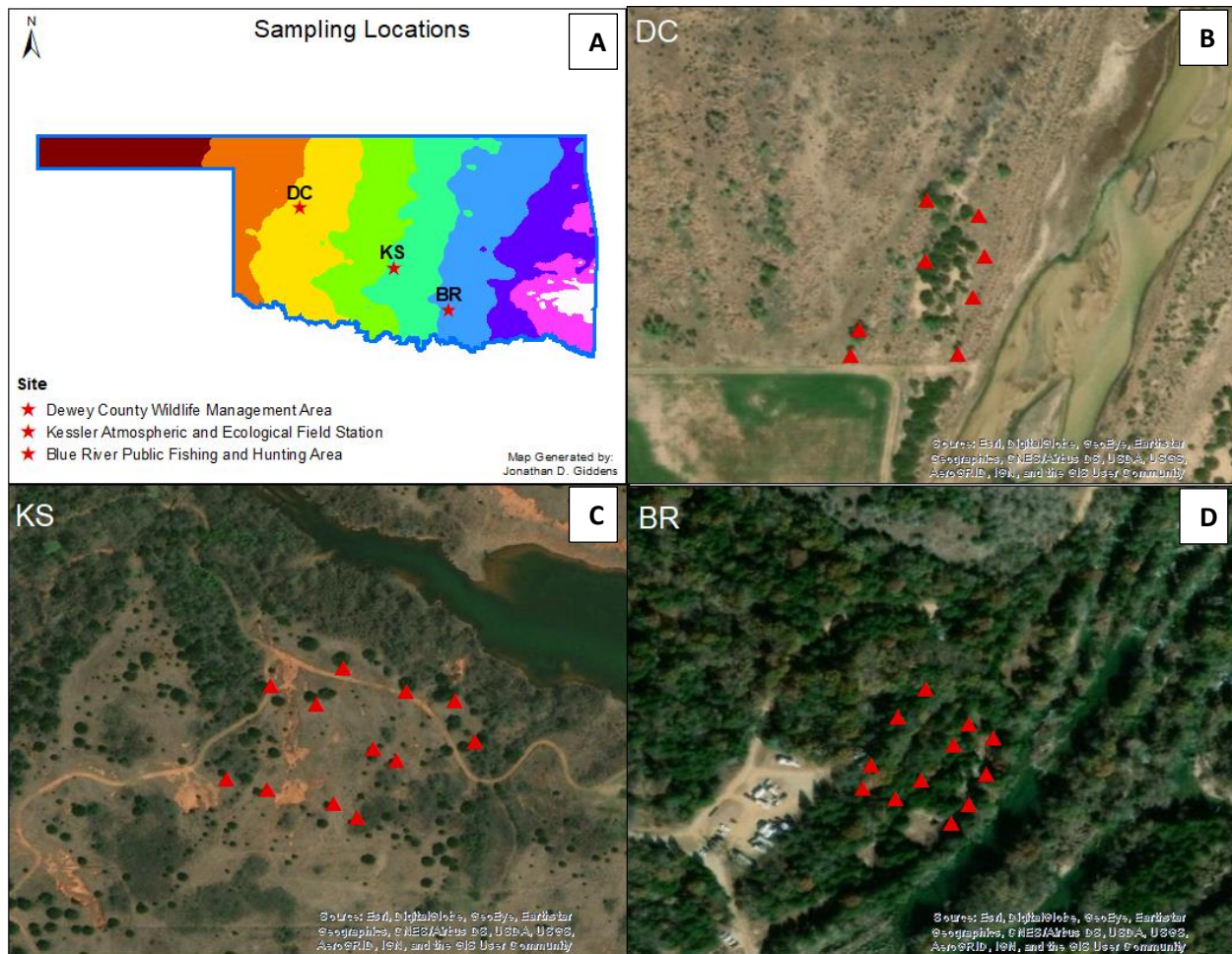


Figure 2.13: Panel A represents the site locations in relation to the precipitation gradient which increases from NW to SE. Colors of red to blue indicate less precipitation to higher precipitation. Sites Dewey County (DC), Kessler (KS), and Blue River (BR) are located 36.000299 lat. -99.024779 long., 34.983598 lat. -97.529819 long., and 34.32343 lat. -96.594221 long., respectively. Panels B, C, D, are demarcating the sampled tree location at each site indicated by triangles.

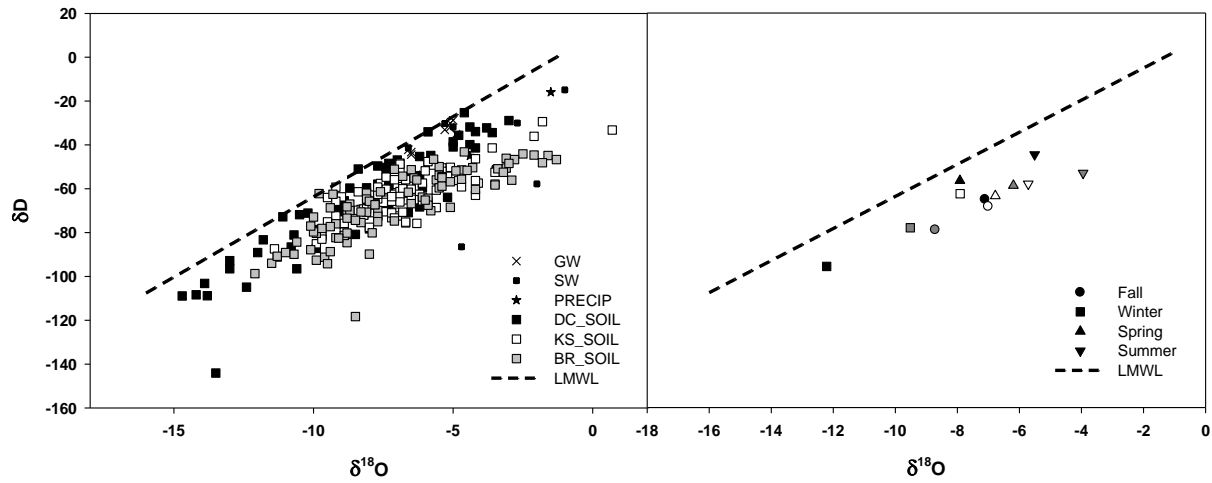


Figure 2.2: In both panels, Dewey County (DC) is depicted as black symbols, Kessler (KS) is depicted as white symbols, and Blue River (BR) is depicted as gray symbols. Panel A shows all sampled soil, groundwater, surface water, and precipitation from all sites and seasons. The local meteoric water line (LMWL) was calculated using Jaeschke 2011 and is shown as a dashed line. Panel B represents average soil water values across sites within seasons. The seasonal values are represented by circles, squares, triangles, and upside down triangles for fall, winter, spring, and summer, respectively.

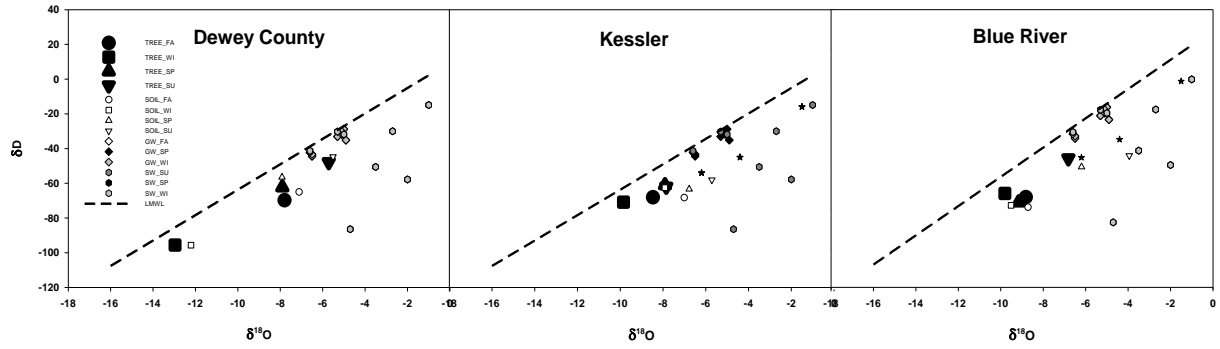


Figure 2.3: Average source water isotopic signature from tree, soil, groundwater, surface water, and precipitation samples for each site and season.

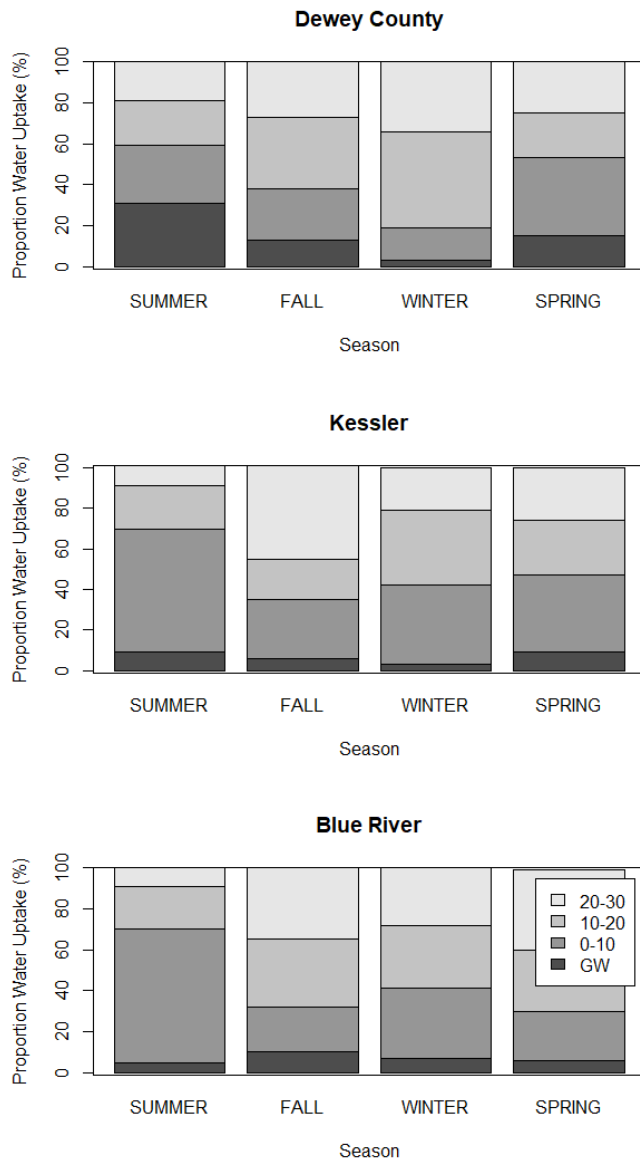


Figure 2.14: Source water partitioning by site and season. The sources are comprised of three soil depths and groundwater.

Chapter 3: Effect of tree size and stand density on water stress tolerance of eastern redcedar

Abstract

Encroachment of woody plants into grasslands is a relatively new phenomenon with numerous ecological, hydrological, and societal impacts. Changes in climate, atmospheric carbon dioxide, over-grazing, and fire disturbance have all been identified as major drivers of the increase in woody plant abundance. However, for encroachment to occur particular woody species must be able to establish themselves and survive in environments unfavorable to most woody plants. This study took place at the Kessler Atmospheric and Ecological Field Station in McClain County, Oklahoma during the period of 2017-2019 in two sites with differing levels of eastern redcedar (*Juniperus virginiana* L.) encroachment. We examined 1) How does water stress vary across ERC individuals of different sizes (heights) and between stands with different levels of encroachment (density)? 2) Does water stress tolerance of ERC individuals vary with size and encroachment level? And 3) Do smaller ERC individuals utilize a more conservative hydraulic safety strategy? We found that although soil moisture varied significantly between sites, with the high-density site 30% drier on average, there were no differences in water stress (as assessed through predawn and midday water potential) between high and low density sites. There were, however, indications that smaller trees (<3m in height) have a less varied, more conserved hydraulic approach to water stress. In particular, smaller trees exhibited higher, less variable shoot saturated water contents in the high-density site, allowing a larger margin of water loss before reaching turgor loss. These results may give insight into how ERC individuals are able to encroach into and establish in typically unfavorable locations and how they are able to overtake grasslands in the Great Plains region.

Effect of tree size and stand density on water stress tolerance of eastern redcedar

3.1 Introduction

Encroachment of woody plants into grasslands, rangelands and savannahs is a global phenomenon with ecological, hydrological, and societal impacts (Archer et al., 1988; Briggs et al., 2005; House et al., 2003; Knapp et al., 2008a). These ecosystems are representative of more than 40% of the terrestrial landscape globally (Browning et al., 2008; Chapin et al., 2001) and play critical roles in the global carbon cycle and agronomic activities such as food and livestock production (Amthor et al., 1998; Schimel et al., 1994; Van Auken, 2000). While grasslands have been established for over 10,000 years in central North America, the process of woody plant encroachment into these areas, and the subsequent conversion to woodland and forests, has only been documented over the past 160 years (Knapp et al., 2008a; Lauenroth et al., 1999; Van Auken, 2009) driven by natural and anthropogenic disturbances (House et al., 2003). Changes in climate, atmospheric carbon dioxide, over-grazing, and fire disturbance have all been identified as major drivers of the increase in woody plant abundance (Briggs et al., 2005) causing changes in plant diversity and richness, fauna habitat, carbon cycle, and the hydrologic cycle (Honda and Durigan, 2016; Huxman et al., 2005; Ratajczak et al., 2012; Veldman et al., 2015).

The Great Plains region in North America is experiencing woody encroachment at an accelerating rate (Briggs et al., 2005; Huxman et al., 2005; Knapp, 2008; McKinley and Blair, 2008). Species such as *Cornus drummondii* and *Juniperus virginiana* are among those currently exhibiting high rates of encroachment into areas that are historically grasslands and rangelands (Engle et al., 2008). Factors such as fire suppression, over-grazing, and absentee landowners

have contributed to the increase in woody plants where they would generally have not been found (Knapp et al., 2008b; Van Auken, 2000). In Oklahoma alone, woody encroachment has been increasing exponentially equating to nearly 300,000 acres of converted land annually, resulting in ecological and economic consequences (citations). In southern Oklahoma, another juniper species, the Ashe Juniper (*Juniperus ashei*), is of increasing concern as it expands its range by encroaching into grasslands from the species historic range restrictions of limestone rock outcrops. Throughout the state of Oklahoma and the central great plains, a related species *Juniperus virginiana* (eastern redcedar, ERC) is an even more widespread problem, expanding at a rate of 8% annually between 1984-2010 (Wang et al., 2017), costing the state ecologically by altering habitats and ecosystems and economically. According to the redcedar task force formed by the Oklahoma Secretaries of Agriculture and Environment, Oklahoma suffered \$218 million in economic losses in 2002 alone.

Soil moisture has been shown to be reduced by the presence of eastern redcedar (Duesterhaus, 2008; Kerhoulas et al., 2013; Owens et al., 2006; Zou et al., 2015). Much of this is due to canopy interception and evaporation of precipitation. In a tall grass prairie in central Oklahoma, USA, it was shown that high density stands intercept 1.6 times the amount of precipitation as those in low density stands (Zou et al., 2015). Increasing expansion of eastern redcedar, along with increasing occurrences of high density redcedar stands, intensifies competition for limited amounts of available water in the soil (Kadmon, 1995). Expanding populations of woody species, such as eastern redcedar, into grasslands is only expected to increase the heterogeneous pattern of soil moisture levels in response to precipitation (Breshears, 2006).

Trees are able to withstand periods of inadequate soil moisture by limiting the risk of leaf dehydration through controlling minimum leaf water potential (Ψ_{leaf}) using a continuum of strict control (isohydry) to little control (anisohydry) of stomates. In the case of trees utilizing a more isohydric response, minimum water potentials would not vary greatly due to the stomatal control of the individual. Stomatal closure helps maintain leaf turgor but comes at the expense of lower carbon intake needed for photosynthesis. Meanwhile more anisohydric species will exhibit less strict stomatal control resulting in more negative Ψ_{leaf} and a greater chance of hydraulic failure due to declining leaf turgor. Species that exhibit a wider range of Ψ_{leaf} have a greater ability to alter leaf hydraulic properties in response to soil moisture conditions (Johnson et al., 2018). The ability for trees to alter leaf hydraulic properties is key to survival in areas that experience a range of environmental and soil moisture conditions. The adjustment of leaf turgor loss point at a given water potential (Ψ_{TLP}) has long been used as a metric of a tree's ability to tolerate periods of low soil moisture. Individuals with greater ability to adjust Ψ_{TLP} will have an advantage during times of water stress.

Similarly, osmotic potential at full turgor is another useful metric when determining how a leaf (or shoot in the case of ERC) is able to adapt to changing environmental conditions. Osmotic potential is important in turgor maintenance (Saxe et al., 1993). The adjustment of solutes, or osmotic potential, is widely known as an adaptive response to water stress (Callister et al., 2006). As tree height increases the more plasticity in osmotic potential can be observed as well as more negative values overall (Woodruff et al., 2004). As trees grow larger and competition for resources increases, the need to vary hydraulic parameters to changing abiotic drivers also increases. It is known that as a tree increases in height the water potentials experienced by leaves become more negative, thus requiring more energy to adjust the osmotic

potential to transport water to the leaves (Friend, 1993). One way in which that occurs is by increasing the solute concentration and making the osmotic potential more negative (Friend, 1993; Zach et al., 2010). It has been demonstrated that trees exhibiting a more anisohydric response have the most plasticity in leaf hydraulic parameters (Woodruff et al., 2009). Meinzer et al. (2014) hypothesized that anisohydric species must be able to adjust Ψ_{TLP} and osmotic potentials to compensate for their relatively limited stomatal control.

Unfavorable conditions, both biotic and abiotic, can inhibit the function and survival of both large and small trees (citations). To overcome the difficulties presented by a given environment, trees may utilize either conservative or more plastic hydraulic responses. Plasticity refers to the ability of an individual to shift its phenotype to a wide range of environments, nutrients, and competition. The more plastic the physiological responses are the more likely the individual will survive in a variable environment. In habitats with low resource supply, associated with slow growth, individuals generally favor a more conservative approach. In heavily encroached areas there is great competition for light, water, and nutrients, which suggests a more conservative strategy would be advantageous. However, as trees get larger, they become more plastic in their response (Zach et al., 2010). Smaller trees have shallower roots making it more difficult to uptake sufficient water especially during times of water stress. Smaller trees also have smaller tracheids, thus smaller pores, which leads to less xylem conductivity but also less embolism (Woodward, 1993). Assuming year-to-year variability is mainly related to the climatic environment, we should expect trees facing the same conditions will respond in a concerted way (Fritts, 1976). However, this is not always the case, especially when examining the continuum of hydraulic properties and Ψ_{leaf} representing variation between isohydry and anisohydry (Meinzer et al., 2016).

In this study we aim to answer the following questions about the plasticity of ERC drought tolerance strategies in two areas with different magnitudes of encroachment. 1) How does water stress vary across ERC individuals of different sizes (heights) and between stands with different levels of encroachment (density)? 2 Does water stress tolerance of ERC individuals vary with size and encroachment level? And 3 Does hydraulic strategy safety vary with individual tree size? In order to investigate these questions, we used a multitude of ecophysiological approaches both in the field and in the laboratory.

3.2 Materials and Methods

3.2.1 Site description

This research took place at the 146-hectare Kessler Atmospheric and Ecological Field Station (KAEFS) and the University of Oklahoma, Norman, OK (OU). Located at 34.983598 lat. and -97.529819 long. in Purcell in McClain County, Oklahoma. The soils in the low encroachment site are classified as a Nash-Lucien complex with well-draining, high permeability, and medium run off (NRCS:, USDA). Soils in the high encroachment site are classified as Stephenville and Newalla soils with moderately deep, well drained soils. Average long-term precipitation at KAEFS is 908.3 mm with the majority occurring in the spring and summer months, and the average annual temperature is 16.1 degrees Celsius (mesonet.org; Table 1).

The two sites are adjacent to each other and are defined by the current level of woody encroachment. One site is defined as heavily encroached (high density, HD) which represents a forest environment 769 m² in area consisting of a closed canopy with lower branch die-off. We

expected this environment to be more humid with lower temperatures and lower soil moisture due to canopy precipitation capture. The adjacent site is characterized as low encroachment (low density, LD) with widely spaced individuals 1,527 m² in area having canopies that nearly reach the ground with no die-back. The environment was expected to be less humid with higher temperatures and higher soil moisture.

3.2.2 Species Description

Eastern redcedar is a monoecious, evergreen tree capable of living up to 300 years. ERC is capable of growing up to 15 meters in height, although that is typically only found in deep, fertile soil (Adams, 2011). Roots can reach depths of 6 meters and can spread laterally up to 5 meters (Adams, 2014). In areas with shallow soil or in arid to semiarid environments, height is typically reduced. Soil type nor soil depth appear to have little to no effect on the species' ability to be present in a given area but can have effects on characteristics such as tree height and canopy size (Ganguli et al., 2016). The typical ERC canopy is dense and extends to the ground in areas with high sun exposure and little competition. While ERC canopies in a high density area with high competition, such as a closed canopy ERC stand, will have branch dieback at the base of the tree (Caterina, 2013).

3.2.3 Soil texture analysis

Soil texture was analyzed from six locations within each site from samples cored to 15 cm. Three replicate samples and a control were analyzed from each core and then averaged (Table 2). The protocol for this method was adapted from Ashworth et al. (2001). First, 100 ml 1N Na(PO₃)₄ was mixed with 50 g homogenized soil and 250 ml DI water in a glass jar with a lid. The sample was vigorously shaken for 1-2 minutes to break up any soil conglomerates, and

then poured into a 1000ml glass cylinder. This process was repeated two additional times. The control cylinder was filled with 100 ml $\text{Na}(\text{PO}_3)_4$ and all 4 cylinders were then filled to 1000 ml with room temperature de-ionized water. Each cylinder was then capped and rotated end-over-end for 1-2 minutes until no soil was visible on the bottom. As soon as the soil was in suspension the cylinder was placed on a flat surface, a hydrometer was gently inserted, and a reading was taken from the hydrometer after 40 seconds had elapsed. This process was repeated once more for each cylinder, and the readings were averaged. After both 40 second readings were completed, the cylinders were left undisturbed for 24 hours before the final hydrometer reading was taken. Final calculations of clay, sand, and silt were then made using Ashworth et al. (2001).

$$\% \text{ Clay} = (100/\text{ODwt})(\text{R24h-Blank})$$

$$\% \text{ Sand} = 100 - (100/\text{ODwt})(\text{R40sC-Blank})$$

$$\% \text{ Silt} = 100 - \% \text{ Sand} - \% \text{ Clay}$$

Where ODwt is the oven dried weight, R24h is the hydrometer reading g L^{-1} at 24 hours, R40s is the hydrometer reading at g L^{-1} at 40 seconds, R40sC is the average of the two 40 second readings, and Rblank is the reading from the control cylinder with 100 ml $\text{Na}(\text{PO}_3)_4$. Sand is defined as soil particles >50 microns $<2\text{mm}$, silt as soil particles >2 microns $<2\text{mm}$, and clay as soil particles <2 microns.

The results of the texture analysis were then run through the USDA Agricultural Research Services Soil Water Characteristics program based on Saxton and Rawls (2006). This program, driven by soil texture, calculates soil properties such as wilting point, field capacity, and soil saturation.

3.2.4 Soil Moisture

Soil moisture measurements were taken approximately monthly during 2017-2019 using the HydroSenseII water content sensor (Campbell Scientific, Logan, UT) with 20 cm rods. Soil moisture measurements were taken repeatedly from the same 16 locations (8 per site). Additional continuous soil moisture measurements were collected during 2018 using soil moisture probes (CS650 30cm, Campbell Scientific) placed in both sites (4 per site). These data were recorded every 30 minutes on a datalogger (CR1000, Campbell Scientific), through October 2018 when all sensors were destroyed by wildlife.

3.2.5 Water Potential

Water potential was taken predawn (400-600) and at midday (1200-1400) twice in 2017 (Sep., Oct.), three times in 2018 (Apr. Jun., Jul.), and twice in 2019 (Mar., May.) from both sites (10 individual trees per site). Shoots were selected from fully sun exposed south side of the tree at breast height and immediately placed into a pressure chamber (1505D-EXP, PMS Instrument Company, Albany, OR) to determine shoot water potential (Johnson et al., 2018; Scholander et al., 1965).

3.2.6 Stem Conductance and Embolism

Stems were sampled to determine maximum stem conductance (K_{max}) and percent native embolism during spring 2017 and summer 2018. Samples taken in 2017 were collected mid-morning, while the 2018 samples were collected predawn. All samples were immediately sealed in a plastic bag with a wet paper towel and placed in a cooler. This difference in collection methods restricted us to only comparing results for native embolism between sites (for each sampling date), whereas both comparisons could be made for K_{max} due to this metric being

measured after stems had been under vacuum in a clear polycarbonate desiccator (Bel-Art SP Scienceware, Wayne, NJ) with 0.2 micron ultrapure water (ELGA PF2XXXXM1, Lane End, High Wycombe, UK) for 12-24 hours to refill any embolized xylem (Baker et al., 2019). Stem conductance was measured using the hydraulic conductivity apparatus as described by Jacobsen (2011) and first developed by Sperry et al. (1988). Water traveled through the apparatus to the stem by way of a gravitational pressure head and was then collected in a container on a high precision balance (Sartorius Entris 22-1S, Wood Dale, IL, USA). The flow rate was calculated using the ConductR software in R, the R-based conductivity program (<https://mcculloh.botany.wisc.edu/methods/>). Native embolism was determined by dividing the flow rate of samples directly from the field by the rate of flow from the rate of K_{max} after the xylem had been refilled in degassed, ultrapure water under vacuum for 12-24 hours.

3.2.7 Pressure-Volume Curves

Shoots were collected for pressure-volume curves on two days in 2018 (18 and 24 July) and two days in 2019 (6 June and 18 September) to determine differences in leaf level drought tolerance traits between individuals both within and between sites. Shoots were excised at breast height and from the south sun facing side of the tree when possible; some samples had to be collected at lower (small trees) or higher heights (trees with lower branch dieback in the HD site). Once samples were collected, they were sealed in a plastic bag with a wet paper towel and placed in a cooler until later that day when they were stored in the lab fridge until measurements could be completed (within 1-2 days). These stems were not rehydrated due to the possible inclusion of artifacts which could shift the turgor loss point (Meinzer et al., 2016). According to Meinzer et al. (2014) this may have to do with how species regulate their water potential and characteristic of species on the anisohydric spectrum of the iso-anisohydric continuum. The

“push” or “squeeze” method (Leuschner et al., 2019; Májeková et al., 2019; Tyree and Jarvis, 1982) was used to generate increasing pressure at 0.2 MPa steps (starting with the initial water potential until the tail of the curve was linear) using a pressure chamber (1505D-EXP, PMS Instrument Company, Albany, OR). The sample was weighed using a precision balance (Sartorius Entris 22-1S, Wood Dale, IL, USA) to 0.001g between each pressure step.

3.2.8 Data analysis

Statistics were performed in SigmaPlot 10.0 and in RStudio (R Development Core Team). ANOVA was used to test for differences in soil moisture, shoot water potential, and drought tolerance properties between sites. Where differences were found with ANOVA, t-tests were used to examine further. Relationships of pre-dawn water potentials and P-V parameters with tree height were evaluated through a series of linear regression analysis. F tests were performed to determine whether these regressions should be treated fitted separately for each site or treated as one data set. Residuals from these regressions were also evaluated against soil moisture to ensure comparability of values generated on different sampling days. Soil texture and subsequent soil properties were analyzed at the site level using analysis of variance and t-tests to test for soil property differences.

3.3 Results

3.3.1 Soil moisture and degree of water stress in low and high density sites

Soil moisture volumetric water content (VWC; %) varied significantly between 4% and 35% across sites and years ($p < 0.001$, $p < 0.001$; Figure 1a). Across the study period, VWC in the LD site ranged from 8 to 35% and from 4 to 29% in the HD site. VWC in LD and HD sites differed on all sampled dates ($p < 0.005$), with the exception of July 2018 and 2019 ($p > 0.05$).

Average VWC in the LD site was 28% higher than in HD (average VWC 26% vs. 19). Soil moisture relative water content (RWC; %) ranged from 14 - 100% in the LD site and 0 - 83% in the HD site (Figure 1b). The average difference in RWC between sites across the study period was 43%.

During the study predawn water potentials values ranged from 0.39-0.91 MPa and 0.31-1.08 MPa in the HD and LD sites, respectively. Midday values ranged from 0.75-2.3 MPa and 0.89-2.35 MPa in the HD and LD sites, respectively (Figure 2a). Predawn water potentials differed between the sites on two sampling dates: Sep 2017 and Mar 2019 ($p < 0.001$ and $p = 0.01$, respectively). Midday water potential differed between sites on sampling dates Mar and May 2019 ($p = 0.037$, $p = 0.004$, respectively). Adjusted water potential differed between sites on sampling dates Sep 2017, Mar and May 2019 ($p = 0.009$, $p < 0.001$, $p = 0.003$, respectively; (Figure 2b). There were no differences found ($p > 0.05$) on any other sampling date. There were no significant correlations between predawn or midday water potential and tree height in either the LD or HD sites ($p > 0.05$). Additionally, there were no significant correlations between water potential and soil moisture at either site, nor were there any correlations between water potential x height and soil moisture (all $p > 0.05$).

Despite some site differences in water potentials, native embolism did not differ between sites in either year ($p = 0.44$, $p = 0.88$, respectively), or across years (HD $p = 0.46$, LD $p = 0.83$). Average native embolism in 2017 was 11% and 18% for the HD and LD sites, respectively, and 16% for both sites in 2018 (Figure 3a). Maximum stem conductivity (K_{max}) did not differ between the HD and LD sites in spring 2017 or summer 2018 ($p = 0.35$, $p = 0.17$, respectively), however K_{max} differed between years in both sites ($p = 0.002$, $p < 0.001$, respectively; (Figure 3b).

Average K_{max} in the LD site was 0.30 in 2017 vs. 0.18 $\text{g s}^{-1} \text{MPa}^{-1} \text{mm}^{-1}$ in 2018, and 0.44 vs. 0.21 $\text{g s}^{-1} \text{MPa}^{-1} \text{mm}^{-1}$ in the HD sites.

3.3.2 Leaf hydraulic characteristics in low and high density sites

Pressure-volume curves indicated significant correlations between height and four parameters: saturated water content (SWC, MPa), osmotic potential at full turgor (P_o , MPa), water potential at turgor loss point ($\Psi_{t_{lp}}$, MPa), and capacitance at turgor loss point ($C_{t_{lp}}$, MPa; Figures 4a-d). Full vs. reduced model F-tests revealed that the LD and HD sites had significantly but differing relationships between tree height and SWC and tree height and $C_{t_{lp}}$ ($r=0.42$, $p=0.009$; $r=0.53$, $p=0.005$, respectively). In the HD site SWC and $C_{t_{lp}}$ both declined exponentially with increasing tree height ($r=0.68$, $p=0.002$; $r=0.63$, $p=0.006$, respectively); however, in the LD site these parameters remained fairly constant with tree size ($r=0.58$, $p=0.16$; $r=0.30$, $p=0.23$, respectively). Both sites had the same exponential decline of P_o and $\Psi_{t_{lp}}$ as tree height increased ($r=0.17$, $p=0.0086$; $r=0.3524$, $p<0.001$, respectively). Relative water content at turgor loss point ($RWC_{t_{lp}}$), modulus of elasticity (ϵ), and capacitance at full turgor (C_{ft}) did not have any relationship with tree height or by site (all $p>0.005$; Figures 5a-c).

3.4 Discussion

This study aimed to examine differences in ecophysiological strategies of eastern redcedar (*Juniperus virginiana* L.) trees across a range of height sizes in managing water stress in two contrasting canopy types: a high density eastern redcedar site (HD) and an adjacent site with low density of eastern redcedar (LD) over a period of three years with variable soil moisture conditions. We found several drought tolerance properties, as derived by pressure-volume curves, were significantly different between sites and negatively correlated with tree height.

Shoot saturated water content and capacitance at turgor loss point both decreased with height in the HD (but not LD) site, while osmotic potential at full turgor and water potential at turgor loss point showed a similar decrease with height in both HD and LD sites. In contrast, we found that although the soil moisture differed significantly between sites on almost all dates, there was no difference in predawn or midday water potentials apart from a few sampling dates (Sep 2017, Mar 2019 and Mar and May 2019) for predawn and midday water potential, respectively, when RWC was high. Adjusted water potentials differed Sep 2017, Mar and May 2019 also when soil RWC was high.

While tree height seemingly had no effect on shoot water potential it did have an impact on measured pressure-volume parameters that control important leaf biophysical properties (Johnson et al., 2018) such as saturated water content, osmotic potential at full turgor, water potential at turgor loss point, and the capacitance at turgor loss. The impact of tree height on all four parameters is strongest in the shortest trees (< 3 meters), with these trees also showing less variation within each parameter. This suggests that smaller trees may be more conservative in their hydraulic and biophysical leaf properties. Turgor loss has been shown to be a strong predictor of plant drought response strategies among many tree species (Blackman, 2018). Variability observed among a species' turgor loss point is driven mainly by the osmotic potential rather than cell wall elasticity (Knipfer et al., 2020; Lenz et al., 2006; Májeková et al., 2019). This relationship between osmotic potential and turgor loss point may help to explain why this study found no significant differences in cell wall elasticity between sites nor a significant correlation with height.

Available soil moisture has an integral role in tree survival and mortality, with particular importance in semi-arid regions that receive frequent periods of drought. Precipitation plays an

obvious role in soil moisture content, however tree and stand canopies, as well as litter, can significantly reduce precipitation reaches ground. Soil moisture has been demonstrated to be lower under canopies of both forest and grassland species (Breshears et al., 2008; Duesterhaus, 2008; Kerhoulas et al., 2013; Owens et al., 2006; Zou et al., 2015). Jia et al. (2017) concluded that woody crops, particularly *Caragana korshinskii* in the Loess Plateau of China, reduce soil moisture at higher levels than in neighboring croplands due to canopy interception. Canopy interception of encroaching junipers in a tallgrass prairie in central Oklahoma, USA has been shown to be as much as 1.6 times larger in HD (closed stands) and compared to LD (open stands) (Zou et al., 2015). The decline in soil moisture under tree canopies due to canopy interception can largely be attributed to precipitation being exposed on leaves to evaporation for longer periods of time leading to higher evapotranspiration rates (Kerhoulas et al., 2013).

While this study did not directly measure canopy interception rates, it is clear from soil moisture data that differences between HD and LD sites can be attributed to canopy cover, as we found an average reduction of 28% VWC in the HD site compared to the LD site during the three-year study period. This is consistent with other studies that found eastern redcedar canopies intercepted precipitation amounts of 52% in Kansas and 36.3% in Oklahoma (Duesterhaus, 2008; Zou et al., 2015), although extremes as low as 7% and as high as 79% interception have also been reported (Slaughter, 1997; Thurow and Hester, 1997). The intensity of precipitation events also plays a large factor on how much moisture reaches the soil (Owens et al., 2006; Zou et al., 2015). Interception has been observed to be as high as 96% in *Juniperus ashei* forests in Texas, USA when precipitation amounts are less than 2.54 mm (Owens et al., 2006). However, Owens et al. (2006) also showed that interception was decreased to 50% when precipitation events were greater than 11 mm. Throughout 1 January-31 December 2018 our field site received

precipitation greater than 11mm a total of 18 days, while 1 January-31 December 2019 received 19 days above 11mm (Mesonet.org).

We found that while volumetric soil moisture was different between sites, the amount of water stress as indicated by predawn and midday water potential did not differ within or between sites even when tree height is accounted for. Our findings are contrary to Bowker et al. (2012) which found in Arizona that Junipers located in grasslands had a 30% lower water potential and approximately 45% higher mortality rate as compared to those located in woodlands. Differences in findings could be due to the difference in soil type (rocky volcanic (AZ) vs. sandy loam (OK)) or due to the extreme anisohydric nature of *Juniperus monosperma*, among the most drought tolerant of all North American *Juniperus* species (Mueller et al., 2005), possibly due to its extensive rooting system reported to reach as far as 61 meters below the surface (Stone and Kalisz, 1991). While eastern redcedar is considered to be anisohydric, we did not see large shifts in water potential, a typical anisohydry response, during any sampling period. This could be attributed to the abnormally wet years observed during the study period or due to location differences. The higher levels of sand in the soil texture in our study site as compared to that in Bowker et al. (2012) may aid in decreasing plant water stress due to lower soil matrix potential, which is the ability for soil particles to hold on to water, and higher drainage rates. Indeed, in water limited environments primary production has been found to be positively correlated with abundance of sand (inverse texture hypothesis) (Noy-Meir, 1973) even at relatively low amounts (Yang et al., 2009).

Native embolism did not differ between sites within year or across years although differences were seen in K_{max} within site between years. Within years we saw no differences in K_{max} between sites. This indicates it was most likely abiotic drivers, such as precipitation and

soil moisture, that drove the differences rather than the density of individuals at the site level. One possible explanation for not observing differences in native embolism or site differences in K_{max} is that every attempt was made to take branch samples from the same height. Zach et al. (2010) notes that increased tree height typically includes larger vessels capable of maintaining a higher level on stem conductivity (Potheir et al., 1989). If we had sampled from significantly different heights within the tree we may have seen more variation in embolism and conductivity.

Interestingly, no significant differences or correlations were found in shoot relative water content at turgor loss point between sites or with tree height despite water potential at turgor loss point being significantly correlated with tree height. This may be explained with the addition of the relationship between relative water content and osmotic potential. Tyree and Karamanos (1981) found that the osmotic potential at full turgor is relatively unchanged while leaf water content changes. This is also reflected in this study where there is no change in shoot relative water content when compared to tree height (Figure 5). The ability to maintain high levels of shoot water content may provide clues as to why seedlings are able to establish and survive under lower soil moisture conditions in the HD site while also having a higher (less negative) turgor loss point and osmotic potential. The relatively conservative patterns of drought tolerance properties by smaller trees is noteworthy while high degrees of variation among taller trees (>3m) was observed. In this regard, one potentially impactful parameter, as derived from PV curves, was shoot saturated water content. High levels of shoot saturated water content were observed in smaller trees in the HD site allowing for higher tolerance to low soil moisture levels before reaching turgor loss. The maintenance of cell turgor is integral to a plant's ability continue to grow and maintain gas exchange (Tyree and Jarvis, 1982). Therefore, a greater margin between saturated water content and water content at turgor loss among smaller eastern redcedar

trees have a compensating ability in low soil moisture conditions to mitigate negative hydraulic effects. It may be possible to expand this concept to other plants with similar drought tolerance strategies and similar positions along the iso-anisohydry continuum.

Trees can also utilize morphological and anatomical approaches to adjust to changing water availability by regulating active sapwood area. By reducing the amount of active sapwood, trees can reduce hydraulic tension, modify crown or root morphology in order to regulate water demand and access or increase water storage within the stem (Tyree and Sperry, 1989). Trees adjust the leaf area to sapwood area ratio ($A_l:A_s$) by decreasing ($A_l:A_s$) as tree height increases (McDowell et al., 2002), and is often dependent on soil water availability (White et al., 1998). Taller trees also develop wider tracheids (the water transporting cells of conifers) in order to conduct more water to the upper parts of the tree. It has been shown that as tracheids increase in cross-sectional width, as tree height increases, the conductivity increases four-fold (Sperry et al., 2006; Zimmermann, 1983). However, this can have a negative effect in dry conditions as wider tracheids are more vulnerable to cavitation. Smaller trees have narrower tracheids making it less vulnerable to cavitation (Tyree and Sperry, 1989) which may help explain how seedlings/saplings are able to establish themselves despite competition for water from other trees.

This study revealed that while soil moisture was on average 28% lower in the HD site, water stress did not vary between ERC individuals of different sizes or different levels of encroachment. Furthermore, water stress tolerance did not vary by encroachment magnitude however smaller trees (< 3m) tended to exhibit more stress tolerance properties such as Ψ_{tlp} , P_o , and SWC_{ft} . It does appear that these water stress tolerance properties are less varied (more conserved) in smaller trees in both sites and levels of encroachment. This may help to explain

how ERC individuals are able to survive in unfavorable conditions while still establishing a new population or stand and suggests that dense stands will not increase mortality due to hydraulic failure.

Tables and Figures

Table 3.4 Average Annual Temperature (c) and Relative Humidity (%). Total annual rainfall (mm). 15 yr. avg 2004-2019.

	2017	2018	2019	15 yr. avg
Temperature (°C)	16.7	16.1	15.6	16.1
Humidity (%)	69	69	71	66
Rainfall (mm)	917	1124	1133	928

Table 3.5 Soil characteristics by site. N=12 (36 with 3 replicates of each sample), 6 sample locations per site.

Soil Characteristics	High Density Average (Range)	Low Density Average (Range)	2-Tailed T-Test
Sand (%)	49.28 (40.67-61.0)	50.22 (41.67-59.0)	P=0.81
Clay (%)	9.06 (5.0-11.33)	11.22 (5.0-17.67)	*P=.015
Silt (%)	41.67 (34.0-49.67)	38.56 (30.33-48.67)	P=0.46
Texture Class	Loam	Loam	
% VWC	9.36 (8.90-10.13)	15.57 (9.83-21.16)	***P<0.005
% H2O (g)	5.90 (5.40-6.20)	9.22 (6.50-11.40)	**P=0.004
Wilting point (% vol)	7.42 (5.30-8.70)	8.90 (5.40-12.60)	P=0.26
Field Capacity (% vol)	20.87 (16.50-23.60)	20.30 (13.30-25.70)	P=0.71
Saturation (% vol)	45.85 (45.50-46.10)	45.53 (45.10-43.20)	P=0.23

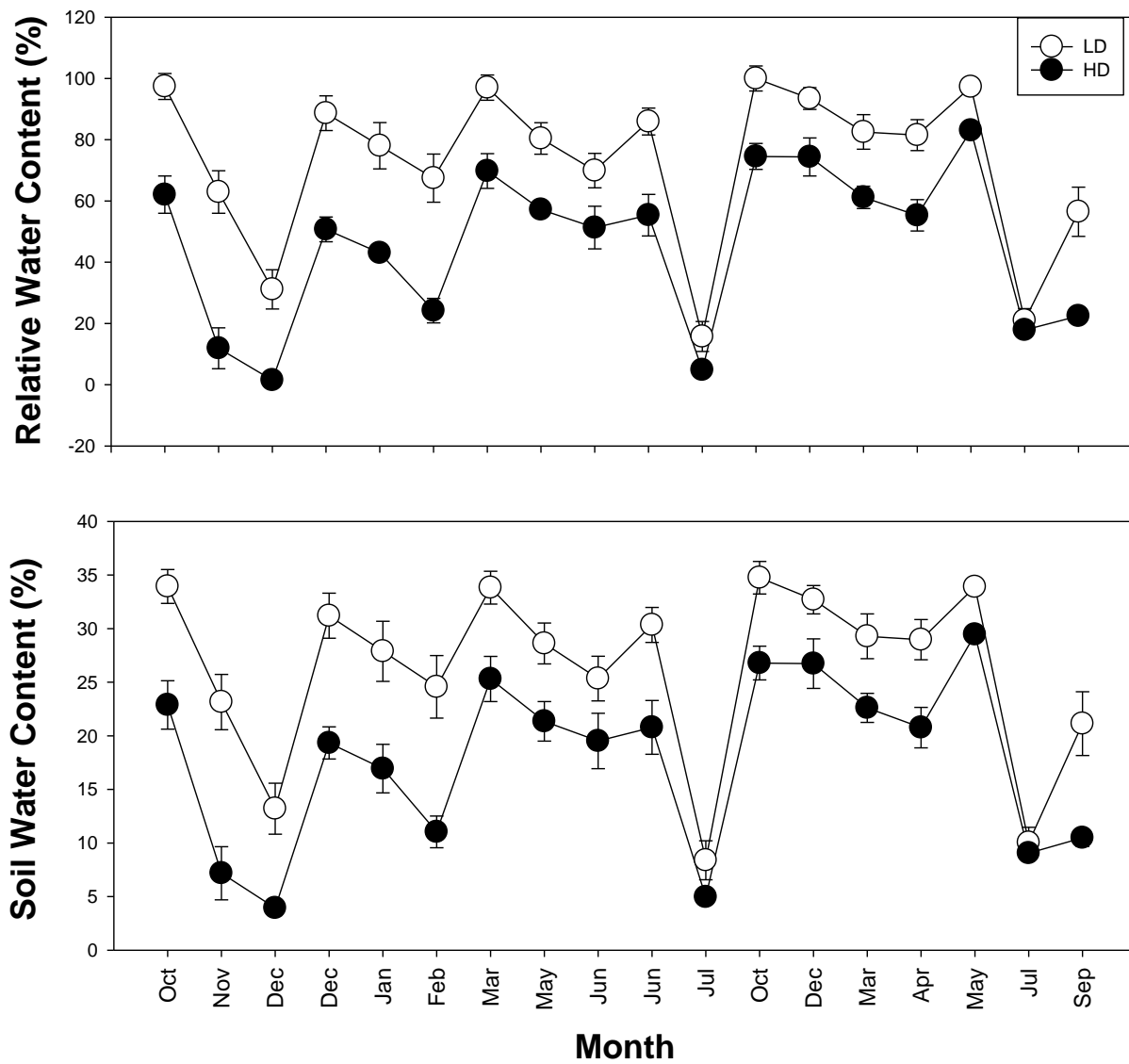


Figure 3.15 Saturated and relative water content measurements for 2017-2019. Each sampling date n=6 per site and averaged. Bars represent standard error.

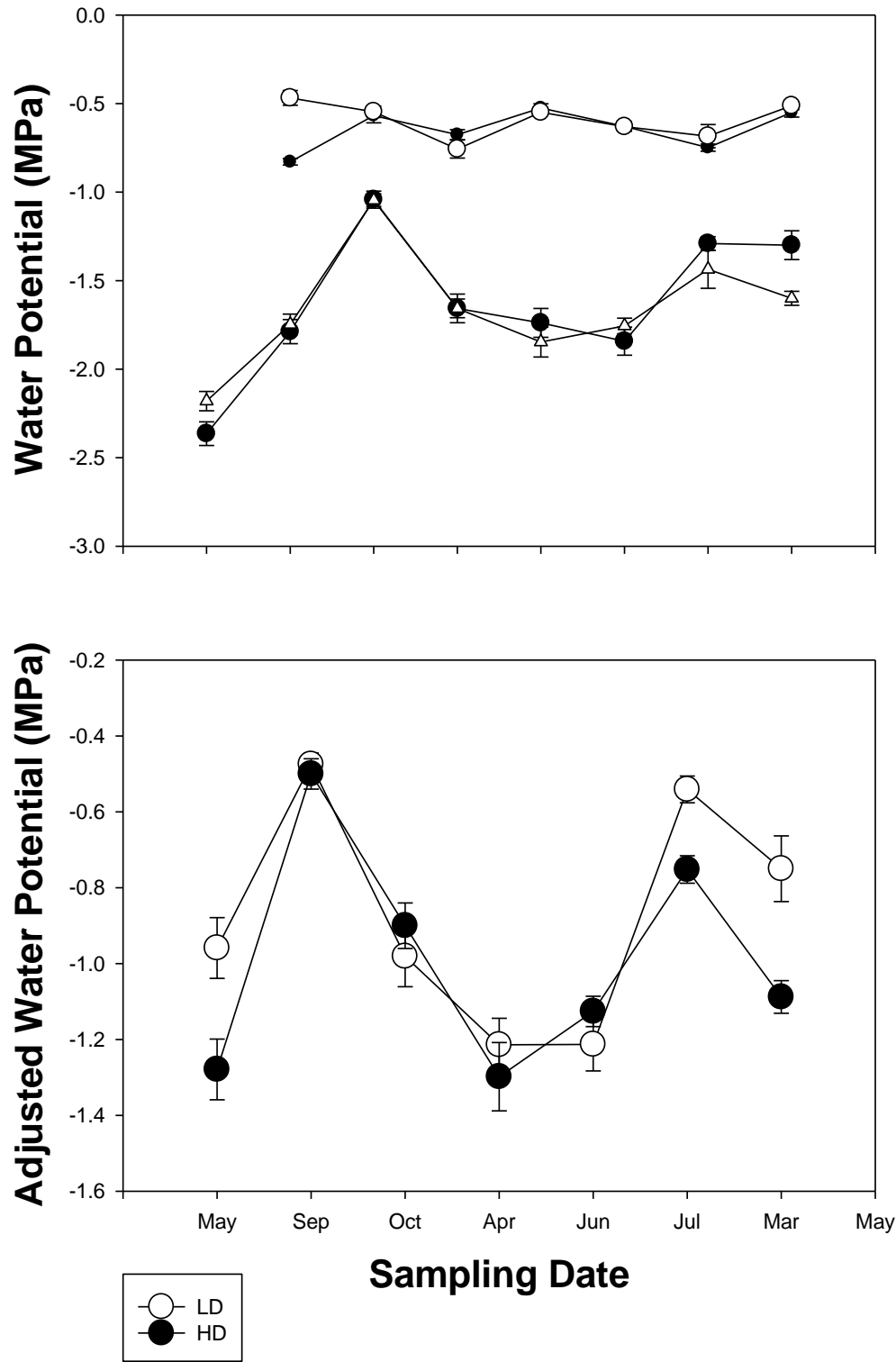


Figure 3.16. Panel A shows Predawn and Midday water potentials. In panel B adjusted water potential is shown. Sampling dates ranged from May 2017 to March 2019. Bars represent standard error.

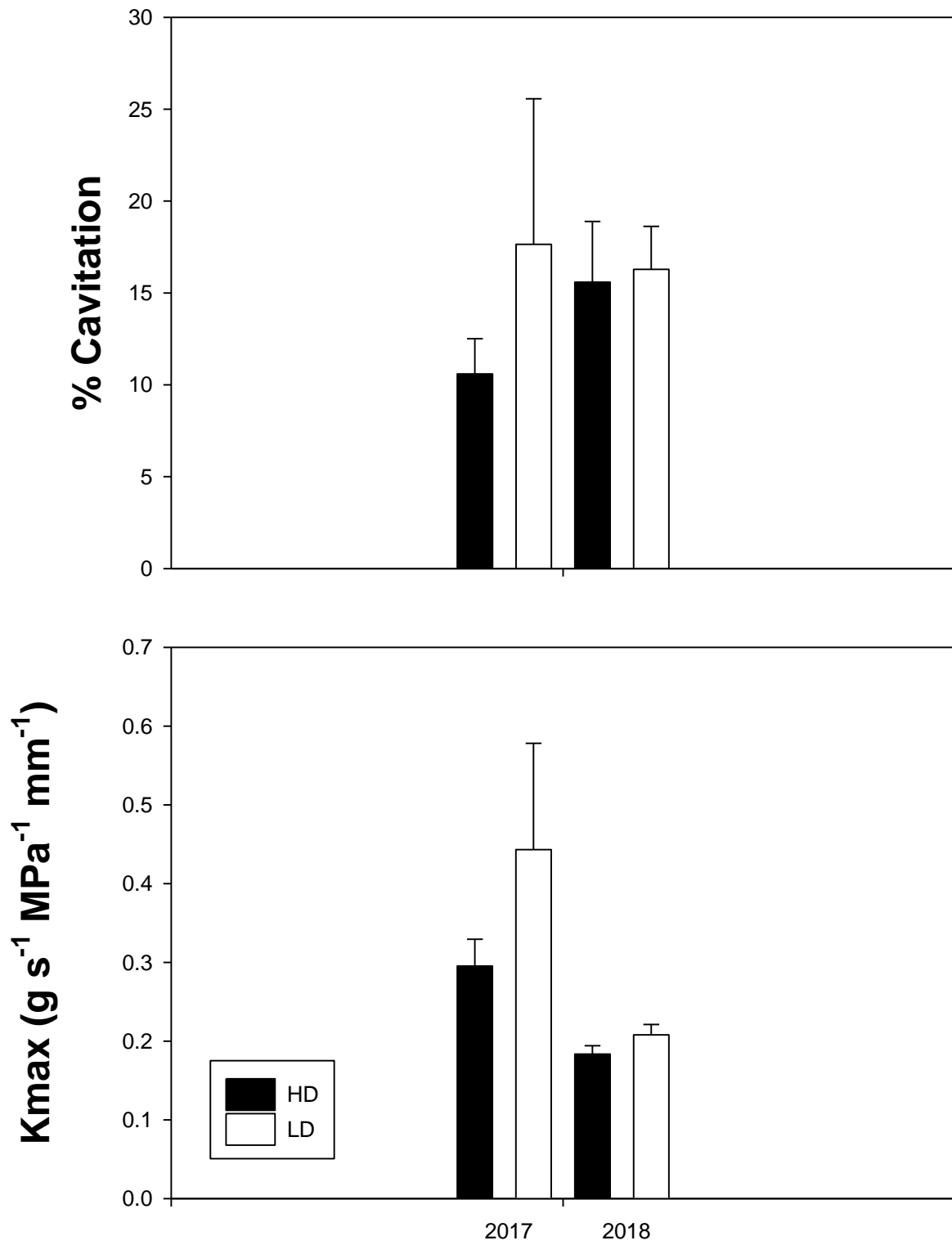


Figure 3.17. Maximum stem conductance (K_{max}) and % cavitation for years 2017 and 2018. Bars represent standard error.

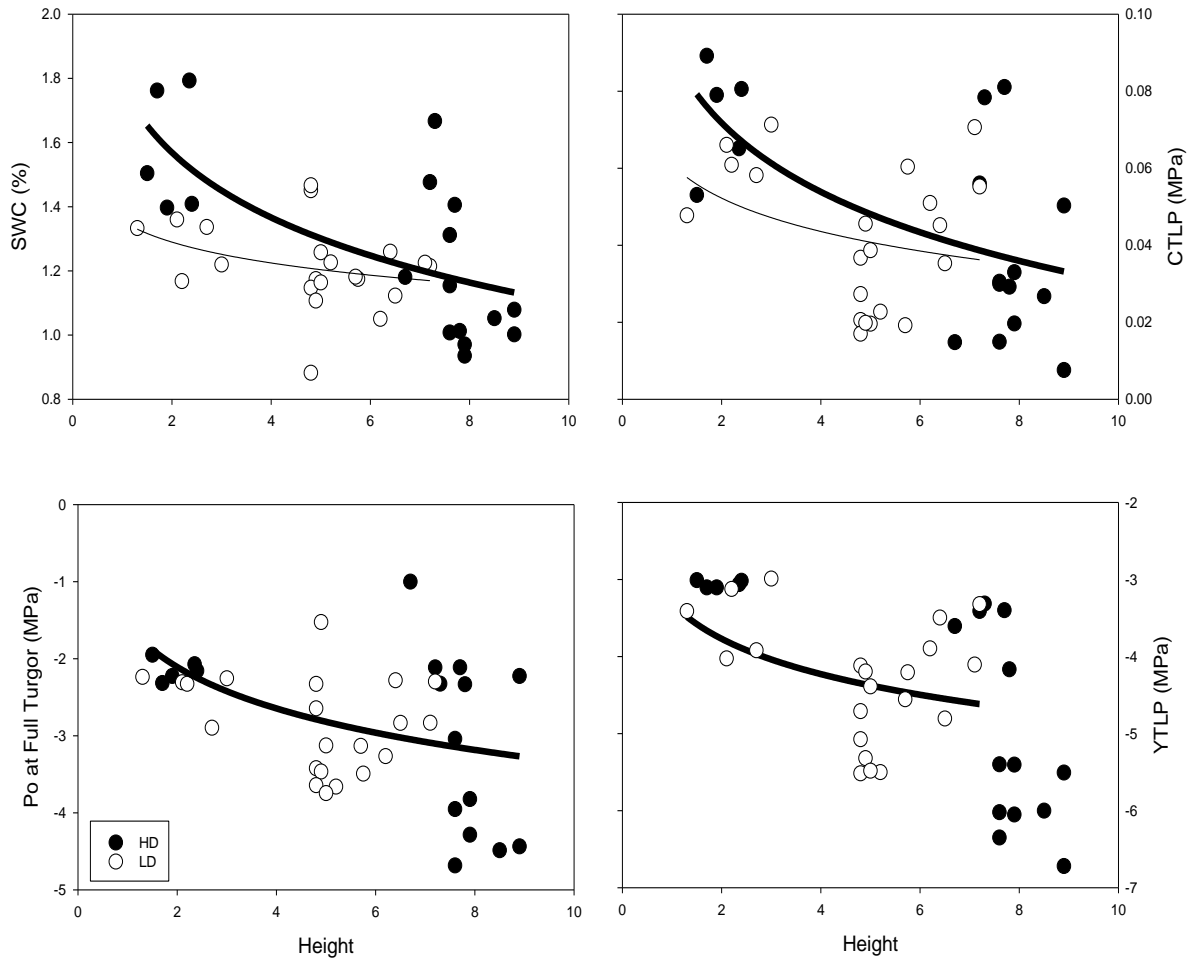


Figure 3.18. Saturated water content (5), water potential at turgor loss point (MPa), osmotic potential at full turgor (MPa), and capacitance at turgor loss point (MPa) for individuals in both sites. Individuals located in the HD site are represented by dark circles while the LD site is represented by open circles. Data from 2018 and 2019 have been aggregated. The thick line represents statistical significance < 0.05 .

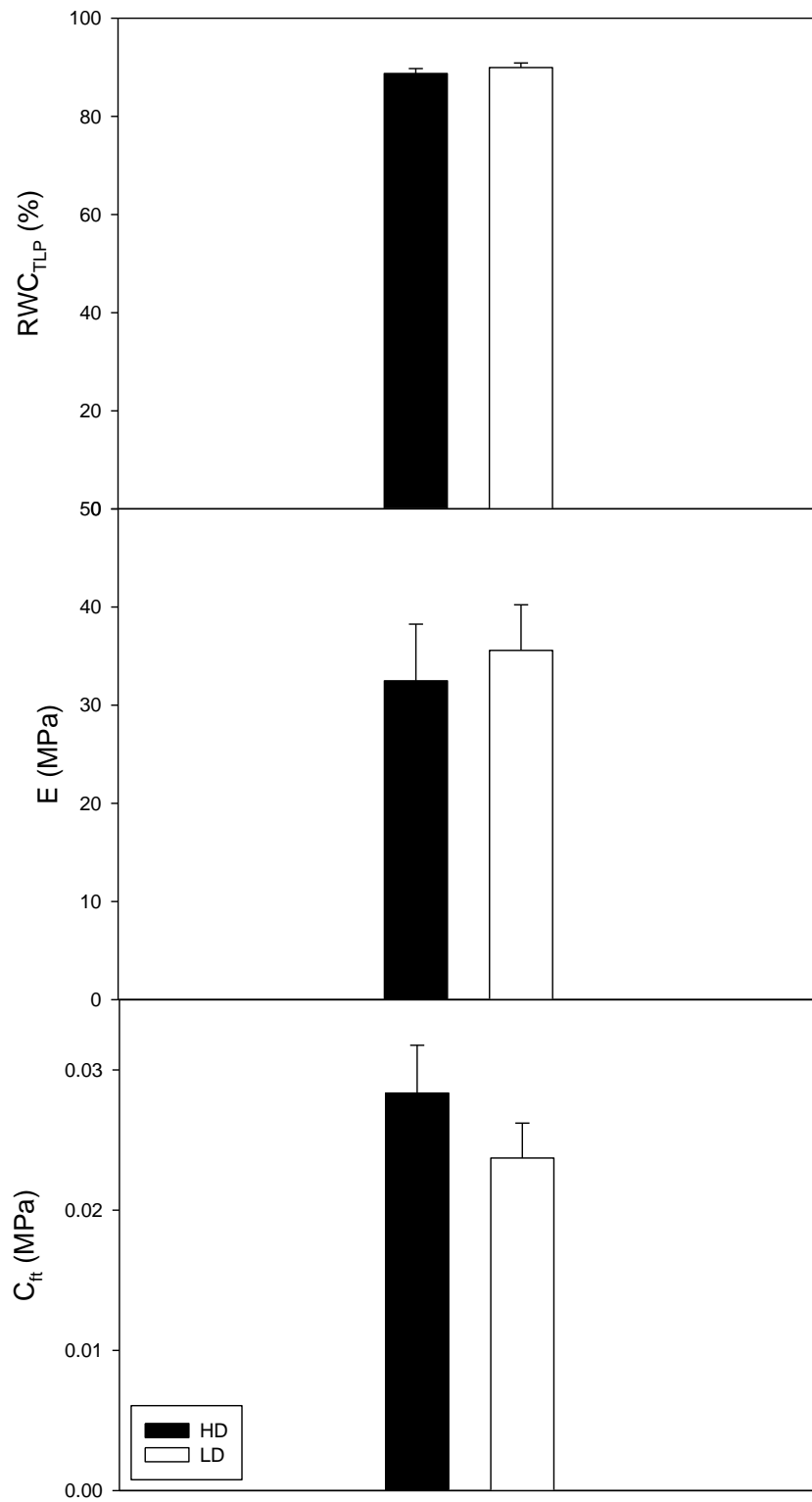


Figure 3.19. Relative water content and turgor loss point, modulus of elasticity, and capacitance at full turgor. Bars represent standard error.

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Chapter 4: Eastern redcedar tree size and stand density effects on transpiration

Abstract

Landscape conversion via woody encroachment into grasslands is likely to alter local water budgets and have a multitude of ecological consequences. In the southern Great Plains Region, USA, eastern redcedar (ERC) is the main species that is rapidly expanding into grasslands. This study examined sapflux and transpiration in two adjacent ERC stands (high density and low density) in central Oklahoma. This study was conducted during 2018-2019 in McClain County, OK at the University of Oklahoma Kessler Atmospheric and Ecological Field Station. We found that sapflux (J_s , $\text{g m}^{-2} \text{s}^{-1}$) rates were 12% higher in the low density stand during the 2018-2019 study period. However, due to larger tree sizes and higher tree density, tree (E_t , kg d^{-1}) and canopy level (E_c , $\text{kg m}^{-2} \text{d}^{-1}$) transpiration were 49% and 54% higher in the high density stand than in the low density stand. The results of this study suggest that as eastern redcedar continues to expand into grasslands increasingly large amounts of water will be transpired by the trees, limiting the amount of soil moisture available for other organisms such as grasses.

Eastern redcedar tree size and stand density effects on transpiration

4.1 Introduction

Changes in water use of trees under climate change and landscape conversion from grasslands to woodlands are of concern as they may alter local water budgets. In the case of woody plant encroachment, changes in vegetation composition often result in increased rates of transpiration due to altered phenology, greater rooting depth, and increased above ground biomass and leaf area (Caterina et al., 2013; Caterina, 2013; Huxman et al., 2005; Jasechko et al., 2013; Schlesinger and Jasechko, 2014; Zhang et al., 2001). *Juniperus* spp. are among the woody plants that are rapidly encroaching into the grasslands of the southern Great Plains, with *Juniperus virginiana* as the most prevalent species in that genera (Knapp et al., 2008b; Tennesen, 2008). *J. virginiana* L. (eastern redcedar, ERC) has encroached into millions of hectares in the Great Plains including Nebraska, Kansas, and Texas, including over 5 million hectares in Oklahoma alone (Engle et al., 2008). Satellite image analysis estimated that eastern redcedar coverage in central Oklahoma increased 8% per year during the period from 1984-2010 have per year (Wang et al., 2017), and that an open grassland can be converted into a closed-canopy forest in approximately 40 years (Briggs et al., 2002).

The replacement of native grasses with evergreen ERC alters the phenology of landscapes due to year-round water use resulting from continued physiological activity. Moreover, ERC woodlands accumulate more than 20 times the aboveground biomass annually than native grasslands (Briggs and Knapp, 1995; Knapp et al., 2008b; Lett et al., 2004; Norris et al., 2001) and can access deeper sources of water than most grasses due to a deeper rooting system (Eggemeyer et al., 2009; Huxman et al., 2005; Tennesen, 2008). ERC is considered drought tolerant and is more towards the anisohydric end of the iso-anisohydry continuum,

prioritizing gas exchange over stomatal control even during periods of low soil moisture (Bihmidine et al., 2009; Eggemeyer et al., 2009; Meinzer et al., 2014). In addition, ERC takes advantage of a dual rooting strategy with both shallow roots and a deep tap root. During periods of low soil moisture, ERC can take up water from deep soil layers to avoid hydraulic disfunction (Eggemeyer et al., 2009; Eggemeyer et al., 2006).

Sap flow measurements provide a unique way to directly measure plant water use and the effects on the local water balance (O'Keefe et al., 2020). Sap flow technology was originally developed in the 1930's (Čermák et al., 2004; Huber, 1932; Huber and Schmidt, 1936), with the thermal heat dissipating (THD) probe method developed by Granier in the 1980's (Granier, 1985; 1987). This technique continues to have wide-spread use in urban forests, native and plantation forests, and grasslands (Caterina et al., 2013; McCarthy, 2010; O'Keefe et al., 2020; Pangle et al., 2015; Pataki, 2011). Using this approach, it is possible to quantify and then scale sap flow from individual trees to canopy level using diameter at breast height (DBH) (Vertessy et al., 1995), sapwood area and depth (Dunn and Connor, 1994; McDowell et al., 2002), and crown area (Hatton et al., 1995). Previous studies have shown that ERC transpiration is variable and is sensitive to soil moisture and precipitation (Awada et al., 2013; Caterina et al., 2013; Landon et al., 2008; Leffler et al., 2002; Owens, 1996), thus additional measurements of water use under a variety of conditions will provide a better understanding of changes in the water balance due to ERC encroachment.

In this study we examined how sap flux at the tree and canopy level differ between two canopy types, one with a high level of ERC encroachment (high density; HD site) and an adjacent site with a low level of ERC encroachment of (low density; LD site). We aim to answer three questions: 1) do sapflux (J_s ; $\text{g m}^{-2} \text{s}^{-1}$) and transpiration rates (E_t ; kg d^{-1}) vary

between two encroachment levels? 2) Does whole canopy transpiration (E_c ; $\text{kg m}^{-2} \text{d}^{-1}$) differ between sites? 3) Does competition or other density effects mitigate the potential increase in whole canopy transpiration as tree density increases? These data will give insight to how ERC water use will affect the local water budget as woody plant encroachment continues to increase in density.

4.2 Materials and Methods

4.2.1 Site Description

This research took place at the University of Oklahoma Kessler Atmospheric and Ecological Field Station (KAEFS) in Purcell, OK. The field station consists of 146 hectares, and includes both actively managed, mostly by grazing cattle, and unmanaged regions. The unmanaged area, which has been unaltered for over 50 years, is currently undergoing various degrees of plant woody encroachment by ERC. Two field sites were established in adjacent grassland areas (34.983598 lat. And -97.529819 long.), based on degree of woody encroachment. The High Density (HD) site has a closed ERC canopy with signs of self-pruning in the lower branches, while the Low Density (LD) site has wide spacing between ERC individuals with full crowns. Soils in the LD site are classified as a Nash-Lucien complex with well-draining, high permeability, and medium run off (NRCS, USDA). Soils in the HD site are classified as Stephenville and Newalla soils with moderately deep, well-draining soils. Average long term (15 year) precipitation is 908.3 mm with the majority occurring during the spring and summer months (Oklahoma Mesonet). The average long term (15 year) temperature is 16.1 degrees Celsius (Oklahoma Mesonet).

4.2.2 Environmental Sensors

Micrometeorological sensors were installed in both sites to compare microclimate conditions. Two pyranometers (CS300-L, Apogee Instruments, Logan, UT, USA) were installed, with one in the open in the LD site and the other under the canopy in the HD site to measure incoming radiation (W/m^2). One temperature and humidity sensor (HC2S3-L, Campbell Scientific, Logan, UT, USA) was placed in the tree canopy at each site. Three auto tipping rain buckets were installed (TE-525MM, Texas Instruments, Dallas, TX, USA): one in the open at the LD site, one in a gap in the canopy in the HD site, and the other under the HD canopy to estimate canopy interception of precipitation. Four 30 cm soil water content reflectometers (CS650-L, Campbell Scientific, Logan, UT, USA) were installed at each site an averaged. These 8 soil moisture probes were only used in 2018 due to animal damage. In 2019 soil moisture was measured approximately once per month in 6 locations per site with the 20 cm probed Hydrosense II (HSII, Campbell Scientific, Logan, UT, USA). All environmental sensors were powered by four 85 watt solar panels (STP-085, SunTech, Xinwu District, Wuxi, China) and four 12v marine batteries wired to a multiplexer (AM16/32B, Campbell Scientific, Logan, UT, USA) and logged on a CR100 datalogger (Campbell Scientific, Logan, UT, USA). Environmental data was logged every 30 seconds and averaged every 30 minutes. Daily averages, maximums, and minimums were then calculated.

4.2.3 Sapflux Measurements

To measure sapflux, 2cm Granier style thermal dissipation probes (Granier 1985, 1987) were installed in eight trees per site (total $n=16$). Probes were installed in the main stem below the lowest live branch (0.15-0.48 m) on random sides to capture all water movement up the main trunk and to capture circumferential variability. An aluminum tin was placed over the sensors to minimize direct solar irradiance and ambient temperature fluctuations (Pangle et al. 2015).

Measurements were taken every 30 seconds and averaged at the half hour. Raw millivolt values were processed with the R software program TRACC (Ward et al. 2017) to get sapflux density (J_s ; $g/m^2/s$). TRACC parameters were set to a length of a minimum of four half-hourly readings at a maximum of 0.02 kPa for the zero-flow selection. We then used the ‘Clean’ and ‘Convert’ functions to calculate J_s . For trees with a sapwood depth less than 2 cm, sapflux density measurements were modified with the Clearwater correction to account for the proportion of the probe which was in non-conducting sapwood. The sapwood depths were all close to 2 cm (1.09-2.38 cm) so there was no need to account for possible radial variations in sapflux. The sapflux density (J_s) in the outer 2 cm of sapwood was adjusted to breast height values by multiplying measured J_s by the ratio of sapwood area at breast height to sapwood area at the measurement height. Measurements were recorded between Julian Day 165-276 in 2018 and 65-312 in 2019.

4.2.4 Tree Level Transpiration

To scale from J_s ($g H_2O m^{-2} s^{-1}$) to tree level transpiration (E_T), sapflux density was multiplied by the sapwood area to give ($g H_2O s^{-1}$) and aggregated to give ($g H_2O d^{-1}$). To estimate the sapwood area of study trees two relationships were derived, diameter² <150 cm ($Y = 0.659x + 68.833$, $r^2 = 0.9805$) and diameter² >150 cm ($Y = 0.2864 + 57.661$, $r^2 = 0.7215$). These relationships were built on a model from 49 ERC cores taken from KAEFS from a range of tree sizes (3.5-12.4 m height, 8-43.7 cm DBH) and sapwood depths (0.73-3.1 cm). Two separate fits were used due to smaller trees having a different relationship when regressed with diameter². Diameter² was used instead of diameter to linearize the relationship.

4.2.5 Stand Level Transpiration

To scale to stand/site level transpiration required the estimation of sapflux density and sapwood area in trees that did not have sapflux probes. To calculate the sapflux of trees not directly measured it was necessary to account for the fact that J_s was related to tree size (most strongly canopy size). Thus, we derived for all measured trees a ratio of the average J_s across the study period for that tree to the average J_s for all measured trees and regressed against canopy size. This formula: $(J_{s_i}/\text{average } J_s) = 0.0332x + 0.4346$, where x is the canopy size of each tree, was then multiplied by average daily J_s in each site to give daily sapflux values (g s^{-1}) for each non-directly measured tree. Canopy size was chosen as it had the best relationship with J_s ratio ($r^2=0.50$) as opposed to height, DBH, and canopy light exposure (CLE) ($r^2= 0.06, 0.08, \text{ and } 0.007$, respectively). The total ground area of the HD site was 769 m^2 while the LD site was $1,527 \text{ m}^2$ using Google Earth Pro and GPS coordinates taken in the field (Garmin eTrex Vista H, Olathe, KS, USA).

4.2.6 Statistical analysis

For air temperature, relative humidity, VPD, precipitation and soil moisture, repeated measures t-tests were utilized to examine differences between sites. Linear regressions were used to quantify relationships between tree characteristics to sapflux, tree level transpiration, and canopy level transpiration. All statistical comparisons were conducted using the software Sigmaplot Version 10.0 and IBM SPSS Statistics 27 (IBM Corp., Armonk, NY).

4.3 Results

4.3.1 Environmental Variables

Over the study period daily average temperature ranged from -2.8 - $33.6 \text{ }^\circ\text{C}$ (HD) and -2.29 - $33.46 \text{ }^\circ\text{C}$ (LD), with an average of 21.1 and 21.2 degrees $^\circ\text{C}$, respectively. There was no

difference between sites in temperature across the study period ($p=0.89$) or within year for 2018 or 2019 ($p=0.94$, 0.91 , respectively, Figure 1). Daily average relative humidity was approximately 3% higher in the LD site during the study period ($p<0.001$) and was driven by differences in 2019 ($p<0.001$), but not 2018 ($p=0.56$, Figure 1). Daily average VPD in both sites reached up to 3.64 MPa and averaged 0.88 and 0.83 for the HD and LD sites, respectively, for the study period. VPD did not differ across the study period ($p=0.16$) or in 2018 ($p=0.75$), however it was different between sites in 2019 due to higher RH in the LD site ($p=0.01$, Figure 1). Maximum Incoming solar radiation, measured at the onsite Mesonet station varied from 44-1251 W/M² and 65-1240 W/M² in 2018 and 2019, respectively (Figure 1). Precipitation was measured and analyzed in two locations: in the HD site under the canopy to measure throughfall, and in the open to capture all precipitation in the LD site. For the study period, precipitation events ranged from 0-70.8 mm (throughfall), and 0-82.9 mm for LD site. Total precipitation during the study period was 1272.4 mm and throughfall was 798 mm (Figure 2).

4.3.2 Soil Moisture

Volumetric soil water content (VWC), averaged daily, ranged from 19% (11-35 %) to 27% (18-40 %) in 2018 for the HD and LD sites, respectively. The LD site had on average 8% higher VWC than the HD site ($p<0.001$). RWC was on average of 28% higher in the LD (23-100%) than HD (0-82%) site ($p<0.001$) in 2018. Although VWC was measured only periodically during 2019 (once in Mar, Apr, May, Jul, and Sep), the difference in RWC between the sites was similar to that in 2018 (30% vs. 28%). The two sites differed (all $p<0.02$) on all dates in 2019 except July ($p=0.55$) when the soil was driest (Figure 2).

4.3.3 Sapflux

Sapflux density (J_s) trends between the two sites were different ($p < 0.05$) in 2018 versus 2019, with 37% higher sapflux in the HD site in 2018 and 59% lower sapflux in 2019 relative to the LD site. Across the study period daily averaged J_s ranged from 0.36-397.2 and 0.17-760.68 $\text{g m}^{-2} \text{s}^{-1}$ in the HD and LD sites and was on average 12% higher in the LD site (46 versus 52 $\text{g m}^{-2} \text{s}^{-1}$) (Figure 3).

4.3.4 Tree Level Transpiration

Moving from sapflux density to tree level transpiration (E_T) incorporates impacts of tree size (sapwood area) on water use and is represented here in kg d^{-1} (equivalent to liter per day). Throughout the entire study period, E_T ranged from 13-1168 and 6-1133 kg d^{-1} in the HD and LD sites, respectively (Figure 3). In 2018, E_T averaged 580 and 225 kg d^{-1} in the HD and LD sites, with 88% higher E_T in the HD site ($p < 0.001$). The average E_T in 2019 was 494 and 419 kg d^{-1} for the HD and LD sites, with E_T in the HD site not significantly higher than in the LD site (16%; $p = 0.162$). Throughout the entire study E_T was 49% higher in the HD site ($p < 0.001$) relative to the LD site (Figure 4). Although E_T was not significantly related to tree height or diameter ($p = 0.3$ and $p = 0.15$, respectively), it significantly increased with increasing canopy size ($R^2 = 0.32$, $p = 0.003$). When expressed on a canopy area basis, E_T was not different between the trees in the HD and LD sites ($p > 0.05$; Figure 5).

4.3.5 Stand Level Transpiration

Canopy transpiration (E_C) is highly influenced by stand density. In our study the HD site had 286 trees ha^{-1} while the LD site had less than half that density (111 trees ha^{-1}). During the study period E_C ranged from 0.02 – 1.5 and 0.004 – 0.74 $\text{kg m}^{-2} \text{d}^{-1}$ in the HD and LD sites, respectively. Stand transpiration was higher in HD than LD in both 2018 (0.75 versus 0.15 $\text{kg m}^{-2} \text{d}^{-1}$)

$^2 \text{ d}^{-1}$; $p < 0.005$) and 2019 (0.64 versus 0.27 $\text{kg m}^{-2} \text{ d}^{-1}$; $p < 0.005$), respectively (Figure 3). Across the two years of the study, E_C was 231% higher in HD ($p < 0.005$; Figure 4).

4.4 Discussion

This study aimed to examine how transpiration rates of encroaching woody species eastern redcedar varies among individuals across a range of sizes in two canopy types: high density with a closed canopy and low density with widely spaced individuals establishing in grassland. We found that while environmental conditions in the two sites were similar, stand transpiration (E_C) was 54% higher relative to the LD site in 2018 and 2019 with differences being driven by a higher density of larger trees in the HD site while the LD site had fewer, smaller trees. We also documented differences in soil moisture and relative humidity throughout the study period and differences in VPD between sites in 2019. The difference in relative humidity was driven primarily by conditions in 2019. Sapflux (J_s) averaged 37% higher in the HD site in 2018 (HD), but was then 59% lower than LD in 2019, with an overall difference of 12% across the study. Tree level transpiration (E_T) ranged from 5 – 60 kg d^{-1} depending on tree size and was on average 49% higher in the HD site.

We found that while air temperature between sites was the same throughout the study period, relative humidity was different driven by values in 2019 which showed RH to be higher in the LD site than in the HD site. This result was unexpected as a high-density stand typically retains moisture in the form of soil evaporation and transpiration trapped within the canopy (Caterina, 2013; De Frenne et al., 2021; Oren and Pataki, 2001). However, soil evaporation is driven in part by the amount of solar radiation at the soil surface, which would be lower in the HD site than the LD site. Additionally, soil moisture was consistently lower in the HD site relative to the LD site (Figure 2) throughout the study period, likely due to higher levels of

transpiration and precipitation interception by the closed canopy, which could lead to lower levels of soil moisture evaporation.

The results from the measured environmental variables suggest that 2019 was a more favorable year in terms of being slightly cooler, more humid, and having higher precipitation levels than 2018. However, both years were cooler and wetter than the 15-year average for the site (Mesonet.org). A possible cause for higher J_s rates in the LD site could be that younger trees generally have higher rates of transpiration than older ones (Aranda et al., 2012; Dunn and Connor, 1994; Friend, 1993). The Hydraulic Limitation Hypothesis proposed by Ryan and Yoder (1997) showed that with increased tree height there is decreased stomatal conductance due to limitations in the tree hydraulic system. Ryan et al. (2006) expanded on this theory and contributed factors such as increased distance for water transport, increased gravitational forces and maintenance of minimum water potentials to decreases in transpiration rates in taller trees.

Compared to J_s , E_T incorporates the amount of sapwood area (active xylem) of individual trees. The results show that overall tree transpiration was higher in the HD site in 2018 and similar between sites in 2019. We also scaled transpiration to the whole stand level (E_C , $\text{kg m}^{-2} \text{d}^{-1}$) by using the allometric equations described above. This analysis revealed that larger trees and a higher density of trees in the HD site resulted in higher overall higher E_C than in the LD site. Interestingly, while E_C in the HD site was significantly higher in 2018 and 2019 (both $p < 0.005$), the difference between sites in across the study period is 231%. These results could be a reflection on the more favorable conditions ERC was subject to in 2019 as opposed to 2018. Awada et al. (2013) found that in the Nebraska Sandhills, USA, ERC individuals was able to transpire up to 27.4 kg d^{-1} , much lower than Landon et al. (2008) which found daily tree level transpiration values between $48\text{-}621 \text{ kg d}^{-1}$ in a riparian area in Nebraska. In a mesic site in

Oklahoma, Caterina et al. (2013) found that tree level transpiration varied greatly by tree size, with maximum daily rates ranging from 8.2 – 161 kg d⁻¹ for trees ranging from 2 to 35cm in diameter at breast height. Not surprisingly, due to close geographic proximity and similar climates our study found E_T values closer to those of Caterina (2013). Greater soil moisture in Landon et al. (2008) no doubt led to much higher levels of transpiration by ERC than was found in other studies, emphasizing the need for these experiments in areas with different environmental pressures as climate continues to change along with increased woody species abundance.

It is possible that with favorable environmental conditions, individuals in the HD site has the advantage of a microclimate which may have mitigated increased competition for water by decreasing the amount of radiation contacting the soil and smaller trees by way of a dense canopy structure (Still et al., 2021). These results are similar to Oren and Pataki (2001) which found variation in transpiration rates mostly due to incoming solar radiation absorbed by the canopy. This is also another possible mechanism by which seedlings establish themselves more readily under the dense HD canopy as opposed to in the open within the LD site.

These studies results revealed that as ERC encroachment continues to expand its range and form dense closed canopy forests in some areas of the Great Plains region, we may start to see a decrease in available soil moisture due to increased levels of transpiration and canopy interception. However, it is still not possible to fully predict the extent at which ERC will continue to expand and encroach grasslands due to its relatively new history of such behavior (within the last 150 years). While these results can and should be expanded to help predict water use, specifically transpiration, in the Great Plains region, one should take care in expanding these results beyond that range. It is also of note that while more precipitation was seen in 2019, as

opposed to 2018, both years had above normal precipitation. leading to favorable environmental conditions. Additional studies should be conducted during dry years to examine and compare the responses of ERC in wet versus dry years.

4.5 Literature Cited

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Tables and Figures

Table 4.6: Tree height (m), diameter at breast height (cm), and crown area (m²).

Site	Avg Height (range)	Avg DBH (range)	Avg Crown Area (m ²) (range)
<i>Closed Canopy</i>	8.03 (7.3-8.9)	20.8 (18.6-23.2)	17.48 (11.75-28.44)
<i>Open Canopy</i>	5.29 (4.8-6.2)	12.03 (9.4-19.1)	13.28 (4.97-33.52)

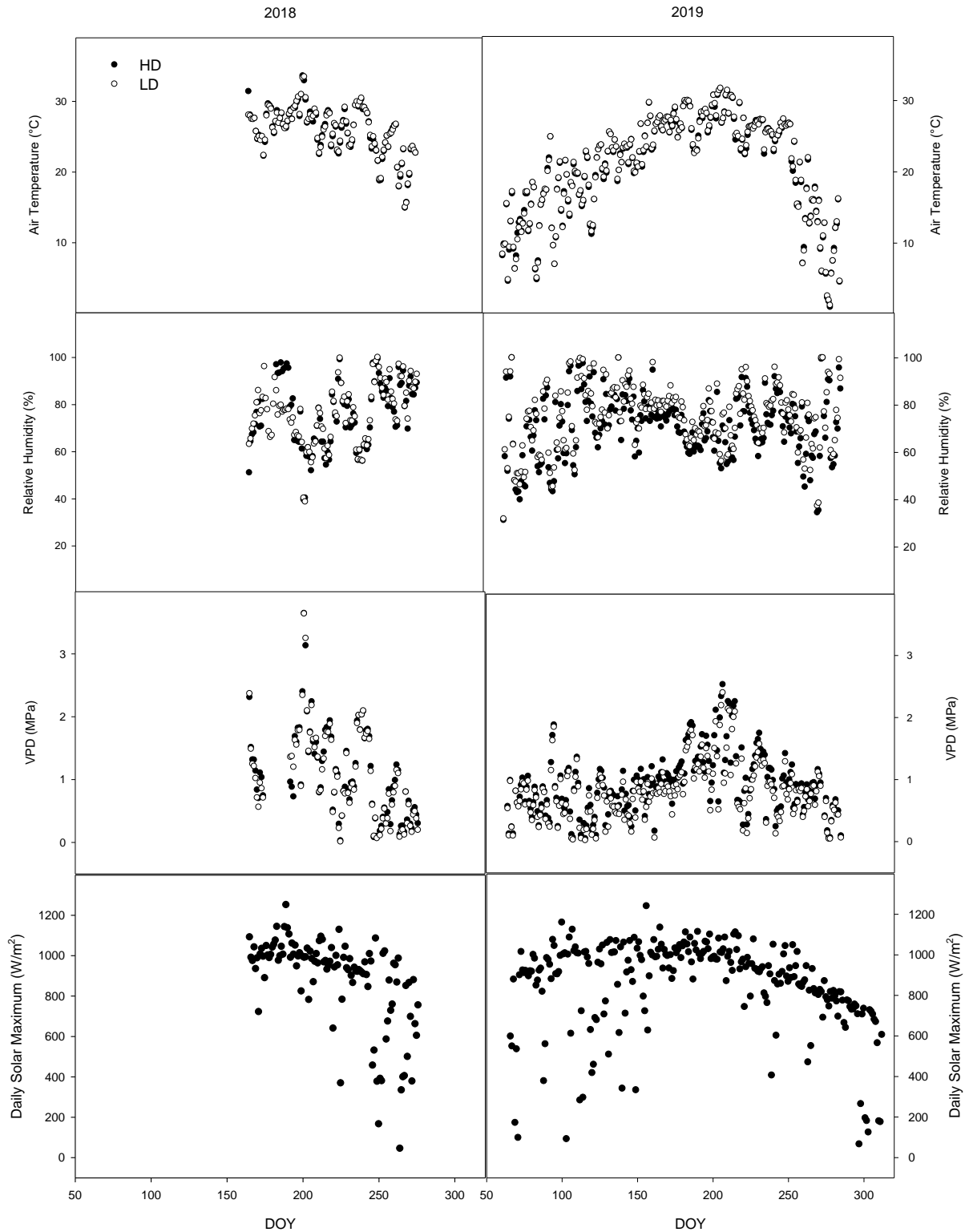


Figure 4.20. Average Daily Air Temperature ($^{\circ}\text{C}$), Relative Humidity (%), Vapor Pressure Deficit (MPa), and daily solar maximum (W/m^2) for 2018 (left panels) and 2019 (right panels) for both sites. HD sites are indicated by closed black circles and LD site is indicated by open circles.

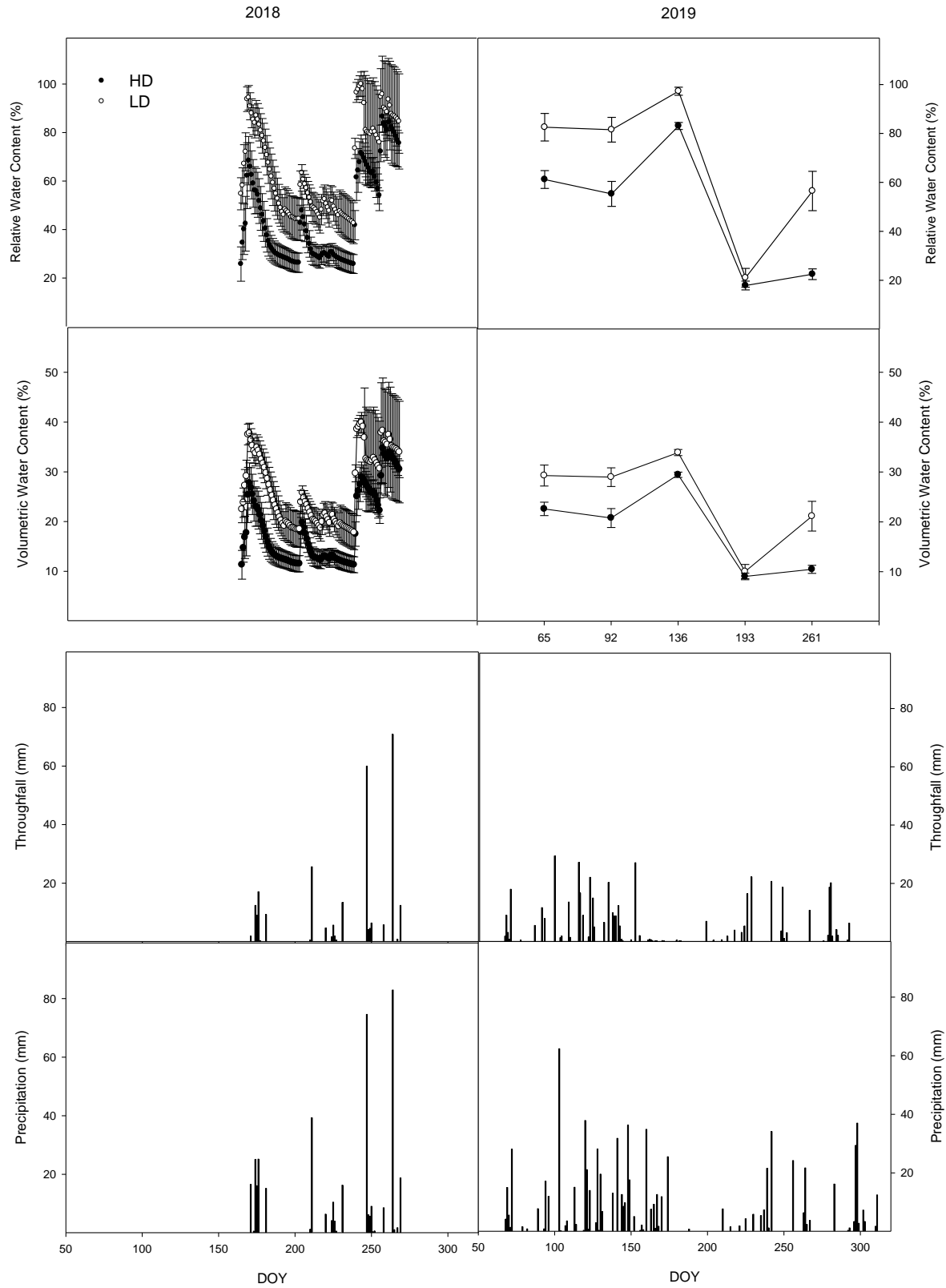


Figure 4.21. Relative Soil Water Content (%), Volumetric Soil Water Content (%), Throughfall (mm), and Precipitation (mm) for 2018 (left panels) and 2019 (right panels) for both sites. HD sites are indicated by closed black circles and LD site is indicated by open circles. Error bar are standard error.

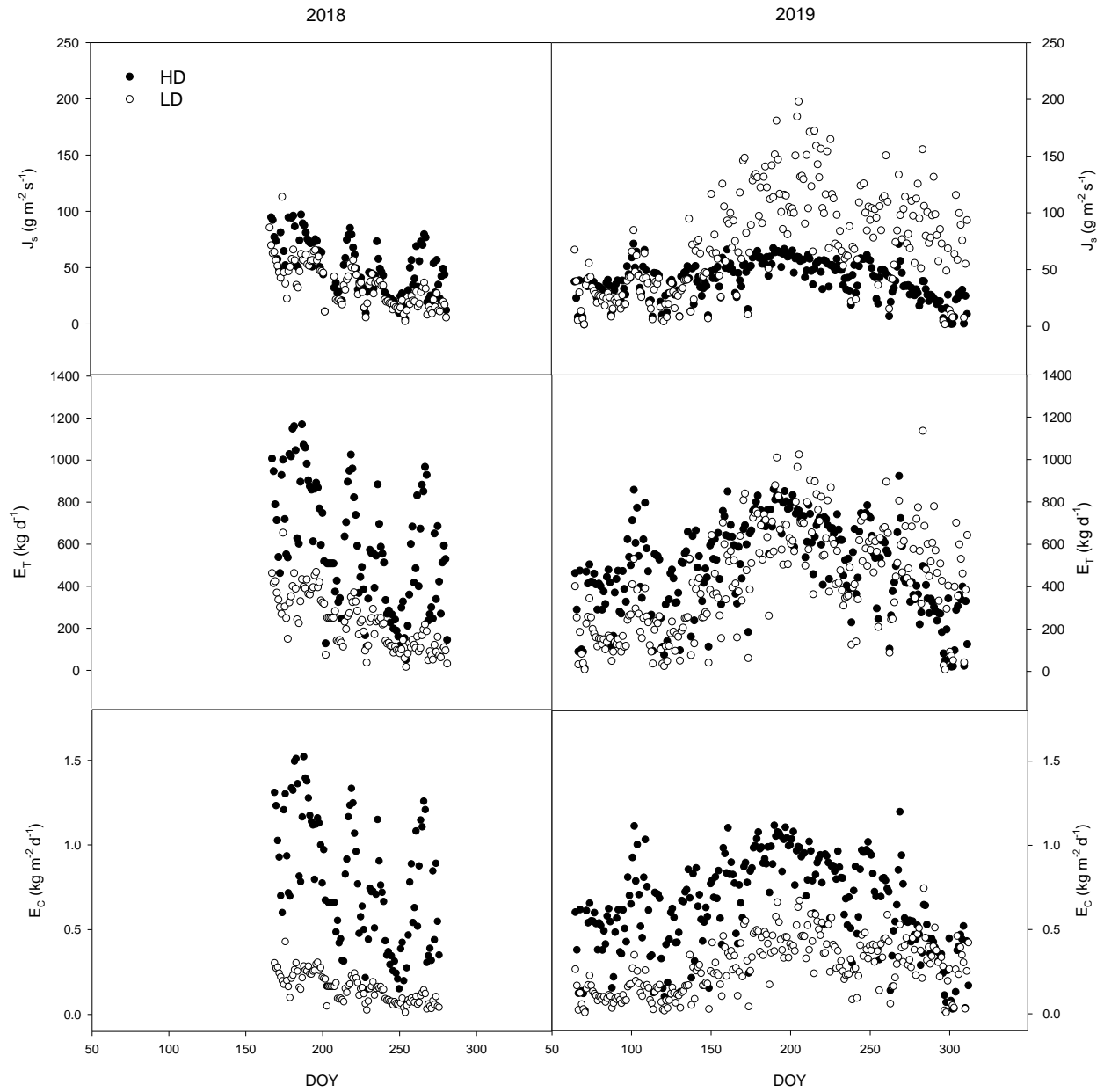


Figure 4.22. Sapflux (J_s , $\text{g m}^{-2} \text{s}^{-1}$), Tree Level Transpiration (E_T , kg d^{-1}), and Stand Level Transpiration (E_C , $\text{kg m}^{-2} \text{d}^{-1}$) for 2018 (left panels) and 2019 (right panels) for both sites. HD sites are indicated by closed black circles and LD site is indicated by open circles.

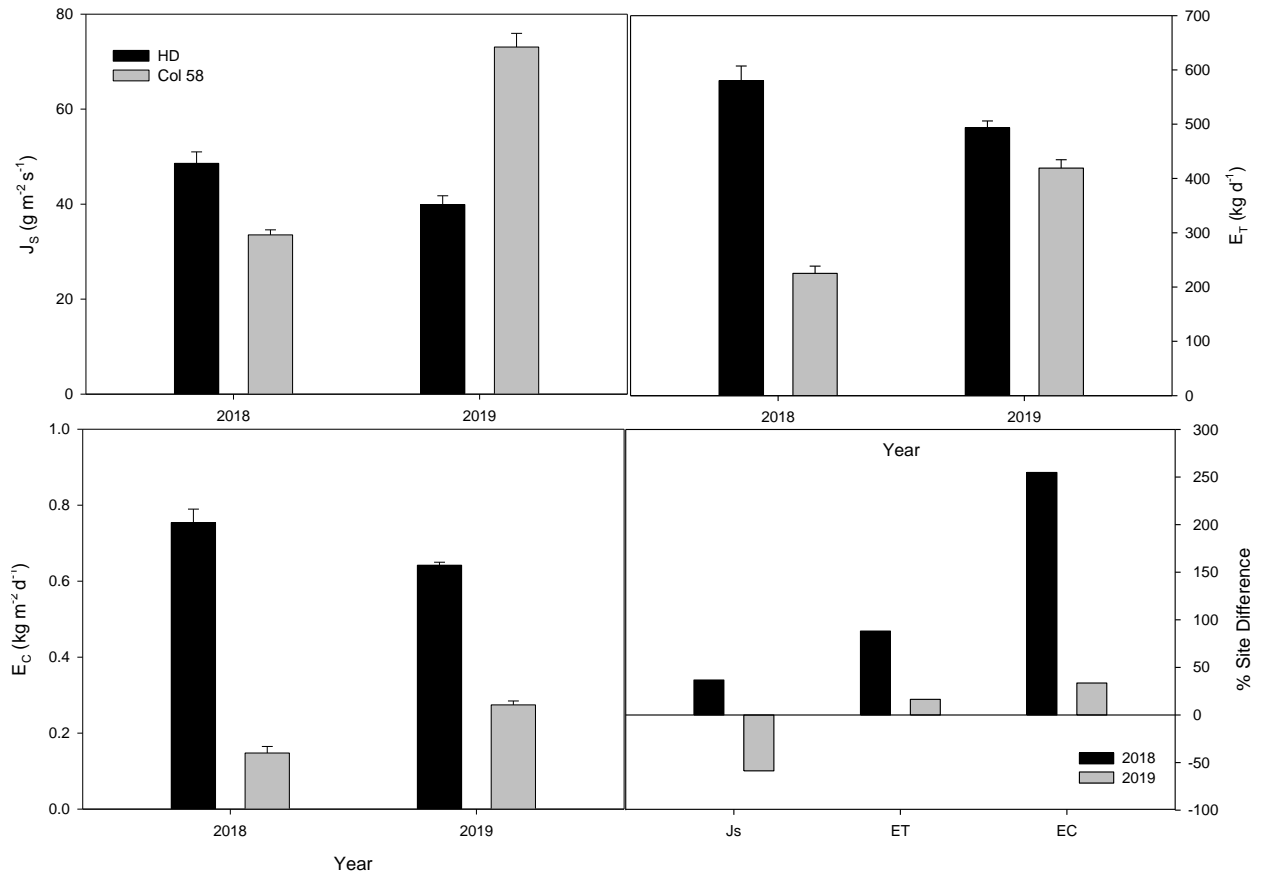


Figure 4.23. Average site level differences in Sapflux (J_s , $g\ m^{-2}\ s^{-1}$), Tree Level Transpiration (E_T , $kg\ d^{-1}$), and Stand Level Transpiration (E_C , $kg\ m^{-2}\ d^{-1}$). HD site is represented by black bars while LD site is represented by grey bars. Error bars are standard error. Site level % difference (HD/LD) in 2018 and 2019 is represented for J_s , E_T , and E_C for 2018 (black bars) and 2019 (grey bars).

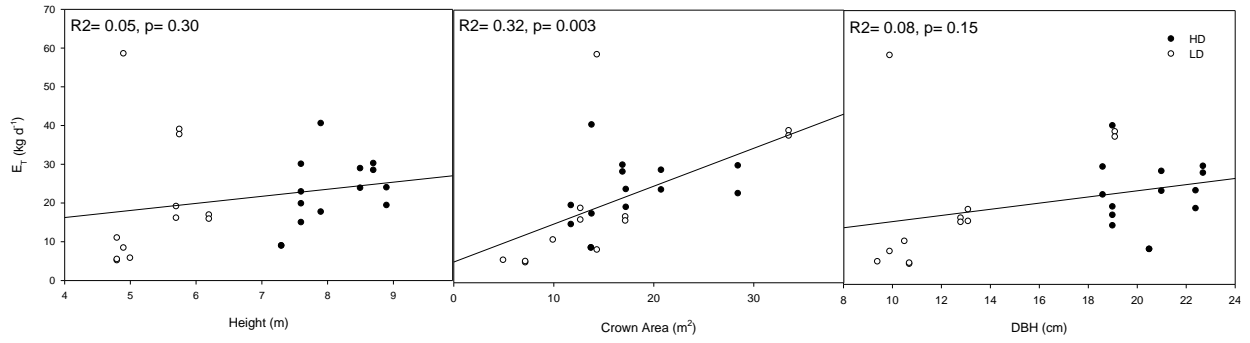


Figure 4.24. Tree Level Transpiration (E_T , kg d^{-1}) relationships with Height (m), Crown Area (m^2) and Diameter at Breast Height (DBH, MPa) for the entire experimental sampling periods of 2018 and 2019. HD site is represented by closed black circles while LD site is represented by open circles.