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CHANGES TO SOIL ORGANIC MATTER TURNOVER AND SOURCE WITH ENCROACHMENT OF EASTERN RED CEDAR INTO A CENTRAL OKLAHOMA DEGRADED GRASSLAND

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CHANGES TO SOIL ORGANIC MATTER TURNOVER AND SOURCE WITH ENCROACHMENT OF EASTERN RED CEDAR INTO A CENTRAL OKLAHOMA DEGRADED GRASSLAND

A THESIS APPROVED FOR THE

DEPARTMENT OF GEOGRAPHY AND ENVIRONMENTAL SUSTAINABILITY

BY THE COMMITTEE CONSISTING OF

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Abstract:

Woody plant encroachment (WPE) is a global land change phenomenon that is caused by a combination of drivers such as a decrease in native grazers, suppression of wildfires, land disturbance from overgrazing, and increases in atmospheric CO_2. As grasslands are an important source of biodiversity and are large terrestrial carbon sinks, there is concern on how WPE will affect global carbon cycling. In the Southern Great Plains, the impacts of WPE by trees such as honey mesquite (*Prosopis glandulosa),* ashe juniper *(Juniperus ashei),* and eastern red cedar *(Juniperus virginiana)* on grassland biodiversity, above- and below-ground litter inputs, and soil organic carbon (SOC) stocks have been studied extensively. However, the impact of WPE on degraded grassland soils is not well understood, and the impacts of WPE on SOC are not generalizable as SOC storage is affected by climate, litter quality, microbial community composition, and soil texture. This thesis utilizes stable carbon isotopes to track changes in soil physical and chemical properties, litter inputs, SOC, and SOC turnover after 25 years of eastern red cedar (ERC) encroachment in a Central Oklahoma degraded grassland ecosystem. We hypothesized that there would be a complete replacement of remnant grassland (RG) above- and below-ground litter and that there would be an increase in SOC in the upper 5 cm of the soil profile with decreasing influence with depth. Secondly, we explored how using different endmembers for a two end-member isotope mixing model influences the fraction of remaining grassland carbon that is reported. To test our hypotheses, we estalished 15 paired ERC-RG plots at the Kessler Atmospheric and Ecological Field Station (KAEFS) in McClain County, OK. Soil cores and litter samples were collected from each paired ERC-RG plot, and stable isotope methodologies were used to analyze litter and soil OC contents. A two-endmember mixing model was also used to estimate how much RG litter, roots, and SOC was remaining beneath the ERC canopies. We found a 42% increase in fine root carbon stock and a 556% increase in above-ground litter stock. Using stable carbon isotope modeling, we found that only 11-15% of the RG C_4 roots were remaining beneath the ERC canopies and 68-77% RG carbon was remaining in the upper 5 cm of the soil after ~25 years of ERC. Despite these findings, there was no significant increase in SOC stock at any depth interval as soil bulk density decreased significantly in the surface soil under ERC. Under some of the ERC trees, carbon stable isotope modeling indicated that RG carbon is lost at the same rate as ERC carbon is being incorporated with not net change in carbon content and that ERC establishment had yet to influence the accumulation or replacement of SOC below 15 cm depth. Lastly, we found that the choice of ERC soil carbon input to the RG, e.g ERC needles, root, or needle litter, could result in dramatic difference in isotope mixing model results given variations in $\delta^{13}C$ values among plant components. Overall, we identified a significant shift to greater above and below ground litter input, driving changes in SOC source and content in the near surface soil horizon during this early stage in ecosystem change.

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List of Abbreviations:

AIR = Ambient Inhalable Reservoir

ANPP = above-ground net primary productivity

BNPP = below-ground net primary productivity

C = carbon

DOC = dissolved organic carbon

EA-IRMS = elemental combustion analyzer-isotope ratio mass spectrometer

ERC = eastern red cedar

FC = fraction of remaining grassland carbon

KAEFS = Kessler Atmospheric and Ecological Field Station

MAOM = mineral-associated organic matter

N = nitrogen

OUGA = L-Glutamic acid

PDB = pee dee belemnite

POM = particulate organic matter

RG = remnant grassland

SIC = soil inorganic carbon

SOC = soil organic carbon

SOM = soil organic matter

VPBD = Vienna Peedee Belemnite

WPE = woody plant encroachment

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1. Introduction

1.1. Background and significance:

Grassland ecosystems are essential ecological, climate, and economic resources (Carlier et al., 2009; Archer et al., 2017), and more than 800 million people below the poverty line rely on grasslands for food, fiber, and fuel (GRaSS Alliance, 2023). However, it is estimated that about 49% of grasslands globally have been degraded to some extent (Gang et al., 2014). Degraded grasslands can be defined as grasslands that have lost ecosystem services with significant alterations to vegetative cover, soil carbon storage, erosion, and species diversity (Noojipady et al., 2015; Bardgett et al., 2021). Grassland degradation is a global phenomenon, with drivers such as agriculture, overgrazing, invasive species, and changes in fire regimes (reviewed in Bardgett et al., 2021). However, some authors suggest that determining the true extent of global grassland degradation is difficult because there are many different and competing definitions of grassland degradation (Gibbs and Salmon, 2015). Secondly, there is a knowledge gap in understanding how further land alterations, such as WPE, modify degraded grassland systems, and there are large differences in the sensitivity of methodologies for quantifying the extent and rate of change (Archer et al., 2017). Therefore, understanding local environmental drivers of grassland degradation and vulnerability and the history of regional management-related stress is important.

The increase in the expansion of invasive or native woody plant populations into both healthy and degraded grasslands, i.e. woody plant encroachment (WPE), is a global land change phenomenon that has become increasingly prevalent (Archer et al., 2017). Extensive woody plant encroachment has been documented in globally, including Africa (Venter et al., 2018; Mogashoa et al., 2021), Australia (Price and Morgan, 2008; Zeeman et al., 2014), Asia

(Wieczorkowski and Lehmann, 2022), Europe (Bagaria et al., 2015; Koch et al., 2018), South America (de Abreu et al., 2010; Taylor et al., 2016), and North America (Van Auken, 2009; Barger et al., 2011), with the causes and effects of WPE being highly variable. Factors contributing to WPE include complex relationships between overgrazing by non-native livestock, suppression of natural and managed fire, a decrease in native herbivores, increases in atmospheric CO_2, and changes in climate (Bond and Midgely, 2000; Limb et al., 2010; de Graff et al., 2014; Archer et al., 2017). WPE alters grassland ecosystems in many ways, including loss of biodiversity (Bennion and Ward, 2023), changes in shallow groundwater and soil hydrology (Acharya et al., 2017), changes to stock and source of soil carbon and nitrogen (McKinley and Blair, 2008), increased hot-burning, woody wildfire risk (Archer et al., 2017), and loss of grazing lands (Moleele et al., 2002; Pierce and Reich, 2010). As temperate grassland soils average $3.31x10^4$ g/m^2 of soil organic matter and store 12% of the Earth's soil organic matter (Abberton, 2010), there is great concern about how the loss of native grasslands by WPE will influence global carbon cycling (Jackson et al., 2002; Liao et al., 2006a; Eldridge et al., 2011; Li et al., 2023).

WPE in the Great Plains of the United States has been studied extensively over the past few decades. Approximately 684,000 km^2 of grassland has been lost in the United States due to WPE over the last 30 yrs (Morford et al., 2022), and this shift has deleterious effects on livelihoods of communities dependent on grassland function (Archer et al., 2017). Some of the major woody plants currently encroaching grassland ecosystems in the Great Plains of the United States include honey mesquite (*Prosopis glandulosa*) (Brown and Archer, 1989; Ansley et al., 2001; Ansley et al., 2023; Yang et al., 2024), ashe juniper (*Juniperus ashei*) (Fuhlendorf et al.,

1996; Yager and Smeins, 1999; Wang et al., 2018), and eastern red cedar (*Juniperus virginiana*) (Gehring and Bragg, 1992; Pierce and Reich, 2010; Bennion, 2023).

In particular, eastern red cedar (ERC), while native to the United States, has expanded rapidly over the past 50 years into both healthy and degraded grassland systems in Oklahoma (Engle and Kulbeth, 1992; Wang et al., 2018; Londe et al., 2022). Loss of grasslands in Oklahoma is of economic concern, as Oklahoma generated about $4.6 billion in revenue in 2022 from the cattle industry, which is dependent on grasslands for forage (USDA, 2023). Estimates of annual lost revenue because of losses associated with forage production, reduced groundwater, increased wildfires, and lower hunting opportunities could be hundreds of millions to the State of OK (Drake and Todd, 2002). Prior to the suppression of wildfires, ERC trees were present in small clusters in grassland ecosystems in the Great Plains in rocky outcrops and areas protected from wildfires (Lawson, 1986). The rapid expansion of ERC in many areas in the Southern Great Plains is the result of a complex legacy of overgrazing and intensive cropping, erosion, fire suppression, and planting of windbreaks of ERC to minimize erosion after the Dustbowl (Wilcox et al., 2018).

Undisturbed tall grass prairies in the U.S. are a major carbon sink due to deeply rooted grass species depositing carbon below ground (Sylvester and Gutmann, 2008). However, with the introduction of intensive and extensive agricultural practices, including deep tillage and grazing, tall grass prairie ecosystems have been degraded, and the once deep, organic carbon-rich Mollisols have been depleted due to wind and water erosion and microbial oxidation (Opie, 1989). Despite thorough research conducted throughout the Great Plains focusing on soil carbon dynamics with WPE (Steuter et al., 1990; Engle and Kulbeth, 1992; Smith and Johnson, 2003; Filley et al., 2008; Boutton et al., 2009; Spencer et al., 2009; Creamer et al., 2012), there is a lack

of understanding related to tree-grassland boundaries, rate and depth of carbon exchange, and impacts to both the rhizosphere and the surface litter that drive changes to soil carbon storage caused by WPE, particularly within degraded grassland ecosystems. This thesis investigates how approximately 25 years of ERC encroachment have altered soil physical and chemical properties, litter inputs, soil organic carbon, and soil carbon turnover in a degraded grassland ecosystem in Central Oklahoma using elemental C and N and stable isotope analyses.

1.1.2. A brief history of grassland degradation in the Great Plains:

The Great Plains has experienced intensive management-based land disturbance over the past 150 years. Before European settlement, the Southern Great Plains was dominated by short, mixed, and tall grass prairie ecosystems interspersed with woody plant patches dependent on geographical constraints, local climate, and frequency and intensity of environmental disturbances such as fire and native grazers (Robertson et al., 1997; Wick et al., 2016). These grassland ecosystems had deep, fertile Mollisols that formed over millennia due to high root biomass below the soil surface and favorable climate (Sylvester and Gutmann, 2008). Prior to the 1800s, the Southern Great Plains was managed by Indigenous communities using controlled burns (Fowler and Konopik, 2007). After the introduction of the Homestead Act of 1892, Indigenous farming practices were banned, wild and controlled fires were suppressed, native grazers were eliminated, and grasslands were converted to conventionally tilled row cropping systems and heavily grazed rangelands (Samson et al., 2004; Wilcox et al., 2018). About 30% of the grasslands in the Great Plains were converted to cropland, and a large portion of the remaining land was converted to grazing land by the 1930s (Cunfer, 2005). Conventional tillage methods, such as moldboard plowing, were primarily used to quickly increase agricultural

outputs, which led to the breaking of soil structure but also the loss of SOM and fine clays and silts (Opie, 1989).

Grasslands that once had deep-rooting systems and thick organic layers were converted to highly eroded systems with short rooting systems, erosive features, and compacted soils (McLeman et al., 2014). Over time, a combination of severe drought, high winds, and intensive agriculture led to severe erosion and translocation of the topsoil in regions of Texas, Oklahoma, Colorado, and Kansas, a period of time called the Dust Bowl of the 1930s (Cunfer, 2008; Porter, 2014). By 1935, 14 counties in the Dust Bowl region of the United States had less than 40% of their original grassland sod (Cunfer, 2008). Analysis of historical imagery from 1931 to 1940 found that lands west of the 100th Meridian experienced degradation by agriculture and wind deposition, which created large sand dunes and sheets, while lands east of the 100th Meridian largely saw land degradation from cultivation and fluvial deposits (Bolles and Forman, 2018). The Soil Erosion Service was formed in 1933 to address soil erosion and conservation in the Great Plains, which was later named the Soil Conservation Service (SCS) when transferred to the United States Department of Agriculture in 1935 (USDA, 2025). By the 1980s, conservation tillage was introduced in many areas of the U.S., and the SCS created grassland and wetland restoration programs to reestablish native grasses and ecosystem services (USDA, 2025; Papanicolaou et al., 2015). Soil degradation contributed to the increase in woody plant encroachment, as woody plants such as ERC were purposefully planted as wind breaks (Wilcox et al., 2018), and erosional sites are hotspots for woody plant growth (Archer 2017).

1.1.3. Soil carbon stabilization mechanisms and major conceptual models:

Historically, there have been many competing conceptual models for the formation and destabilization of soil organic matter (Lehmann and Kleber, 2015). From the 1800s to the early 2000s, the concept of soil humus guided many studies on soil carbon stabilization (Stevenson, 1994). Humus, and its constituent parts, i.e. humics, fulvics, and humin, is generally defined as complex, high-molecular-weight organic molecules that are extracted using successive alkaline and acidic laboratory procedures (Stevenson, 1994; Schmidt et al., 2011) – essentially, a chemical operational definition of soil organic matter pools. Similarly, conceptual models based on organic chemical recalcitrance (or the thermodynamics of embodied chemical energy and the difficulty with enzymes breaking it down) emphasized a chemical structure's inherent stability under idealized conditions over microbial accessibility in a complex soil environment (Dungait et al., 2012). However, a metadata analysis by Schmidt et al. (2011) found that there are no soil organic compounds that degrade faster than one another or in comparison to the soil body as a whole. With increases in modern analytical techniques such as X-ray diffraction (Liu et al., 2017), soil ^{13}C tracer studies (Jastrow and Miller, 1998), and solid-state ^{13}C NMR (Kolbl and Kogel-Knabner, 2004; Witzgall, 2021), new models based on complex interactions of organics, microbial processes, and mineral surface activation have been generally accepted as they more reflect biologically relevant physical carbon pools (Jastrow and Miller, 1996; Six et al., 2002; Kleber et al., 2010; Sollins et al., 2009; Cotrufo et al., 2013; Lehman and Kleber, 2015). For example, the Microbial Efficiency-Matrix Stabilization (MEMS) model combines microbial activity and edaphic/textural controls on plant carbon input and stabilization with measurable parameters that can be modeled to predict soil carbon change. The MEMS model proposes that microbial necromass and microbial exudates ultimately compose the majority of the mass of

stabilized SOM, similar to work by Liang et al. (2007; 2011; 2019), with microbial efficiency of assimilating and decomposition of plant carbon and nitrogen materials being dependent on substrate quality and environmental suitability (Cotrufo et al., 2013; Robertson et al., 2019). A secondary component of the model argues that the minerals making up the soil matrix control long-term stabilization of carbon and nitrogen compounds (Cotrufo et al., 2013), in keeping with past work demonstrating such a phenomenon (Krull et al., 2001; Sollins et al., 2009; Kleber et al., 2010). The soil mineral matrix can be composed of many different types of minerals, including 2:1 clays, 1:1 clays, and metal oxyhydroxides that soil organic matter can adsorb to with varying degrees of strength (Six et al., 2002; Kaiser and Guggenberger, 2003). The measurable components of soil organic carbon are dissolved organic carbon (DOC), particulate organic matter (POM), and mineral-associated organic matter (MAOM), each of which can be stabilized and destablized under the proper environmental conditions (Cotrufo et al., 2013; Lavallee et al., 2020). While ecological and soil research is greatly advancing our understanding of soil organic matter stabilization/destabilization dynamics, how soil organic matter (SOM) is stabilized across major landcover changes, such as by woody plant encroachment, is still an area of active research given its complex edaphic, plant, climate, microbial, and climate controls.

SOM is formed largely from plant litter, root tissue, and soil microbial detritus in various states of decomposition (Rumpel and Kogel-Knabner, 2011; Dungait et al., 2012; Lehmann and Kleber, 2015); however, pyrogenic organic matter, formed from wildfires, can be more than 30% of stable soil carbon (Forbes et al., 2006; Hatton et al., 2016), which is then dynamically allocated to different physical components (e.g. aggregated structure, mineral surfaces, particulate matter) (Jastrow and Miller, 1996). Initially, decomposition of plant material, whether in above or belowground litter form, is controlled by its chemical composition and nutrient

content (Schmidt et al., 2011; Klotzbucher et al., 2011, 2013). Nutrients from initial decomposition are mineralized and taken up into microbial biomass over the course of days to years (Christensen, 1996; Cotrufo et al., 2013). However, the portion of SOM that is not immediately mineralized into microbial biomass can be stabilized through physical protection by macro- and micro-aggregates (Six et al., 2004; Filley et al., 2008), sorption to clays (Lehmann and Kleber, 2015; Kleber et al., 2011), or made inaccessible to microbes in soil pores (Adu and Oades, 1978). If SOM is stabilized under the right conditions, it can remain stored in soils for thousands of years (Six et al., 2004). Overall, however, the extent and rate of decomposition and stabilization of SOM are influenced by environmental factors such as soil temperature, moisture (Parton et al., 1987), litter quality (Schmidt et al., 2011), microbial consortia (Liang et al., 2007), and soil texture (Parton et al., 1987; Krull et al., 2001). Therefore, it is not easy to predict how soil carbon will stabilize or destabilize globally.

Organic material introduced rapidly through bioturbation or root sluffing enters the soil first in a particulate organic matter (POM) form (Filley et al., 2008; Crow et al., 2009; Ma et al., 2013), which can be defined as coarse fragments of organic material >53 um that are vulnerable to decomposition (Lavallee et al., 2020; Witzgall, 2021). Its chemistry most resembles plant chemicals of low degradation (Six et al., 1999; Witzgall, 2021). With progressive decomposition and depolymerization of POM, carbon can be solubilized and associated with mineral surfaces to form mineral-associated organic matter (MAOM), often defined operationally as the fraction of soil that is <53 um or denser than 1.8 g/cc and is sorbed to minerals such as clays (Lavallee et al., 2020). Simultaneously, water introduced into the soil matrix via precipitation dissolves organic molecules from plant material, root exudates, and microbes into what is known as dissolved organic carbon (DOC) that travels through soil pores (Kalbitz et al., 2000; Kaiser and Kalbitz,

2012; Gmach et al., 2019). Molecules contained in DOC vary with plant material composition and undergo a series of depolymerization and oxidation reactions, in which the molecules are more easily adsorbed to mineral surfaces (Kleber et al., 2015). The MAOM fraction of carbon is considered the more stable fraction, as it is more protected from exoenzyme decay of soil in comparison to POM, but MAOM, when desorbed, can be decomposed more readily due to its lower C:N ratio (Tipping et al., 2016). As POM and DOC are reflective of litter composition, drastic changes to litter inputs may shift carbon pools. Therefore, major alterations to vegetative structure, such as by WPE, have the potential to fundamentally alter SOC storage in grassland ecosystems, as the introduction of woody plants alters soil temperature and moisture, litter composition, and rooting structures.

1.1.4. Documented changes to species richness and litter dynamics with eastern red cedar encroachment:

It is demonstrated that ERC alters the soil environment beneath the canopy through a number of interacting factors, including allelopathic suppression, altering light and moisture levels, and changing the amount and chemistry of above- and below-ground litter inputs. Allelopathic chemicals are phytochemicals released from decomposing leaf and root material and released as root exudates that prevent the growth and survival of surrounding organisms (Bais et al., 2006). Many tree species have been found to use phytochemicals to compete with other plants (Young and Bush, 2009; Kato-Noguchi et al., 2017; Tsombou et al., 2022; Bennion and Ward, 2023). However, studies on the allelopathic effects of ERC litter and roots on competitiveness with grasses in the Great Plains have found mixed results. For example, some studies have found that only certain species (such as *Coreopsis palmata*, *Pascopyrum smithii*,

and *Bromus inermis)* are negatively impacted by ERC litter (Stipe and Bragg, 1989; Bennion and Ward, 2022; Bennion and Ward, 2023), while another study found that ERC litter had no allelopathic effect on native grassland species such as *Schizachyrium scoparium, Sorghastrum nutans, Chasmanthium latifolium, Chamaecrista fasciculata*, and *Rudbeckia hirta* (Corbett and Lashley, 2017), indicating that ERC phytotoxins can impact species richness beneath the canopy. Bennion and Ward (2022) found that C_3, cool-season grass species were suppressed in height and biomass, while C_4 dry-season grass species were unaffected, which they suggest may mean that ERC produces phytotoxins that are used to compete directly with other C_3 plants. Therefore, the findings of these studies indicate that allelopathy can contribute to the elimination of grass species under ERC canopies, but it is not the only driving factor.

The introduction of ERC has also been shown to decrease herbaceous biomass overall under the canopy with increasing canopy area (Engle et al., 1987, Limb et al., 2010, Domeier et al., 2025). Briggs et al. (2002) found that there was over a 99% reduction in herbaceous biomass beneath an ERC forest in an undisturbed prairie in the Flint Hills of Kansas. Similarly, a study conducted in Nebraska found that herbaceous biomass production decreased by 83% beneath individual ERC trees (Smith and Stubbendieck, 1990). Individual ERC trees as young as 20 years old or less (Gehring and Bragg, 1992) and with canopy diameters as small as 2 m in length (Domeier et al., 2025) have been shown to dramatically reduce herbaceous biomass.

For example, ERC canopies alter species composition and richness. Some studies have found that herbaceous species richness declined (Briggs et al., 2002; Limb et al., 2010; Domeier et al., 2025) and species composition altered (Arnold, 1964; Gehring and Bragg, 1992; Pierce and Reich, 2010) underneath the ERC canopy while some studies reported no alteration in species composition with canopy cover ranging from 0% to 80% (Limb et al., 2010). Engle et al.

(1987) found that the changes in herbaceous biomass and richness are only occur directly beneath the canopy and not along the dripline, corroborating suggestions from other authors that these changes are due to complex interactions with changes in lighting (Smith and Stubbendieck, 1990; Pierce and Reich, 2010), temperature (Wang et al., 2021), moisture (Pierce and Reich, 2010), and moisture competition from fine roots (Smith and Stubbendieck, 1990). Alterations and elimination of herbaceous biomass under ERC canopies can lead to the buildup of ERC carbon, which has the potential to change soil carbon stability and concentration.

Lastly, the establishment of eastern red cedar causes significant alterations to litter accumulation (Norris et al., 2001a; Sauer et al., 2007), litter chemistry (Williams et al., 2013), and location of carbon storage (Norris et al., 2001b; Li et al., 2023). For example, one study in the Flint Hills of Kansas found that ERC stands annual above-ground net primary productivity (ANPP) ranged from 7,250 to 10,440 kg/ha/yr while tall grass prairie ANPP was estimated to be about 3,690 kg/ha/yr (Norris et al., 2001b), demonstrating a drastic shift from below ground carbon storage to above ground carbon storage, which could change soil carbon accumulation. Norris et al. (2001a) found that in paired grassland closed canopy sites, forest litterfall was about 500 +- 19.9 g/m^2/year while the tall grass prairie sites had a litter fall of about 142 g/m^2/yr in unburned sites. In tandem with high litter fall, ERC litter can have lower C:N ratios to grassland species (Norris et al., 2001a), higher C:N ratios in comparison to grassland species (Mellor et al., 2013), and higher lignin content (Norris et al., 2001a), which can influence litter decomposition rates. Additionally, Norris et al. (2001a) found that the decomposition rate of prairie litter was 21% higher than that of ERC forest litter when litter samples were put in both prairie and forest plots. These changes in litter accumulation, litter chemistry, and the location of carbon storage can change the understory soil environment and lead to a buildup of ERC carbon.

1.1.5. Rooting systems of eastern red cedar:

ERC outcompetes the root systems of grass species by increasing fine root mass (Norris et al., 2001a), increasing rooting depth (Zou et al., 2010), introducing root mass that takes longer to decay (Norris et al., 2001a), and by introducing root litter that may leave a legacy effect of competitive suppression of native grasses (Bennion and Ward, 2023). Globally, temperate grassland species have about 83% of their root biomass in the upper 30 cm of a soil profile, while temperate coniferous forests only have 52% (Jackson et al., 1996). ERC roots, for example, have a thick, fibrous rooting system in the upper soil profile and develop a taproot system with maturity (Walker, 1967) that can penetrate up to 7.5 m (Anderson, 2003) and spread roots laterally up to 6 m (Lawson, 1986). However, ERC roots in shallow soil profiles can penetrate the upper 1 m of the soil profile but dominate in the upper 10 cm (McKinley, 2006). Therefore, ERC roots will directly compete, or outcompete, grass species for space, water, and nutrients and alter soil structure at deeper depths. It has been noted that there may be no difference in water uptake between ERC and grass species in shallow soils, as both rooting systems occupy the top layer and may dry the top layer of soil quickly (Zou et al., 2010), an important point for shallow eroded soils above bedrock, common in many areas in Oklahoma.

The location and biomass of roots after WPE are important, as it has been demonstrated in the tallgrass prairies of Kansas that grass roots decayed at a 30% higher rate than ERC roots, which may lead to a buildup of ERC roots with increasing tree or stand age (Norris et al., 2001a). That same study found that ERC roots had 32.3% lignin in comparison to 16.4% in native big bluestem (*Andropogon gerardii*) (Norris et al., 2001a), which may contribute to initial slows in decomposition rates of ERC litter. Furthermore, increased associations between arbuscular mycorrhizae and ERC roots (Williams et al., 2013; Liang et al., 2017) may improve ERC

nutrient and water acquisition above those of grasses in degraded soils. Finally, a recent study found that grass growth was inhibited with increasing ERC root leachate addition in a laboratory setting (Bennion and Ward, 2023). Furthermore, ERC root accumulation creates a legacy effect of suppression that will support the re-establishment of ERC after removal (Bennion and Ward, 2023). The combination of these root attributes will allow ERC to successfully outcompete and suppress grass species beneath the canopy.

1.1.6. Using stable carbon isotopes to quantify changes in SOC from WPE into C_4 dominant grasslands:

Stable carbon isotopes are useful tools for identifying the sources and turnover of SOC after land cover changes (Boutton et al., 2009). C_3 plants (trees and many grasses) and C_4 plants (some grass species) utilize different photosynthetic pathways to capture CO_2 from the atmosphere. C_4 plants utilize phosphoenolpyruvate (PEP) for the first stage of photosynthesis in the mesophyll cells, which results in only a small isotopic fractionation against ^{13}C (~2.2‰) prior to final fixation using ribulose bisphophate carboxylase oxygenase (RUBISCO) (Farquhar 1983). C_3 plants, on the other hand, use RUBISCO as the first stage of photosynthetic uptake in the mesophyll cells, and this causes a large discrimination against ^{13}C (~-29 ‰) in CO_2 (Roeske and O'Leary, 1984). This distinction means that C_4 plants have relatively more ^{13}C in their tissue than C_3 plants (Hatch and Osmond, 1976; Farquhar, 1983).

On average, the $\delta^{13}C$ values of C_4 plants are relatively higher, with an average value of ~ -14‰ relative to the pee dee belemnite (PDB) international standard, while C_3 plants are relatively lower in $\delta^{13}C$ values, with an average value of ~ -27‰ relative to PDB (Hobbie and Werner, 2004; Liao et al., 2006b). However, small differences in $\delta^{13}C$ values of soils and litter,

e.g. 1-2 ‰, are difficult to attribute to changes in plant source as environmental factors such as soil moisture for C_3 water use efficiency discrimination and decomposition rate in litter could alter ^{13}C values to that degree (Ehleringer et al., 2000). The difference of ~13‰ between the two plant types allows for two end-member isotope mixing models to quantify the proportion of each plant type in soil and litter (Balesdent et al., 1988; Boutton, 1996). Tall grass prairie ecosystems in Oklahoma are composed of mostly C_4 grasses with a mixture of C_3 forbs and grasses, with C_4 and C_3 composition shifting with seasons (Waterfall, 1952). As ERC is a C_3 plant, we can leverage the different $\delta^{13}C$ values of the C_4- dominant grassland carbon and the new C_3 tree plant matter input to track changes in SOC sourcing and turnover after ERC encroachment (Boutton, 1998; Boutton et al., 2009).

Specifically related to tracking impacts of WPE in the Southern Great Plains, the large difference in the $\delta^{13}C$ values between the vegetation sources make stable isotope tracer studies a viable tool for ecosystem impact measurement (Creamer et al., 2013; 2026; Liao et al., 2006b). Many studies have utilized $\delta^{13}C$ values of plant tissue and soil to track changes in organic matter input by WPE (Smith and Johnson, 2003; Filley et al., 2008; Spencer et al., 2009; Creamer et al., 2011; Paul et al., 2013) including regional studies from Arizona (McPherson et al., 1993; Wheeler et al., 2007), South Dakota (Tieszen, 1991; Spencer et al., 2009), Nebraska (Steuter et al., 1990; Mellor et al., 2013), Texas (Jessup et al., 2003; Liao et al., 2006a; Liao et al., 2006b; Creamer et al., 2011), Kansas (Connell et al., 2021), and New Mexico (Connin et al., 1997). Generally, analyzed soil profiles from areas under WPE in the Great Plains exhibit a replacement of C_4 grassland carbon across shallow depths by C_3 woody inputs over the last 150 years, evidenced by the use of $\delta^{13}C$ values (i.e. Wheeler et al., 2007; Spencer et al., 2009). Smith and Johnson (2003) found that on silty clay loams and clay loams in the Flint Hills region of Kansas,

about 42% of carbon in soils encroached by ERC was from C_3 tree plant carbon, but, importantly, they found that there was no net increase in total carbon beneath the canopy when compared to tall grass prairie soils, suggesting a replacement of carbon source – meaning the equivalent amount of past grassland carbon was degraded as replaced by new tree carbon. Additionally, Spencer et al. (2009) documented a 5-7‰ decrease in $\delta^{13}C$ values in soil cores after WPE in the remnant grasslands of the Loess Hills of South Dakota. If a woody plant has been established for a long period of time, $\delta^{13}C$ values at deeper depths will reflect a combination of relict C_4 and C_3 carbon (Jessup et al., 2003). However, most of the experiments conducted using these methods studied the effects of long-established ERC trees or groves, leaving a large knowledge gap in how early establishment ERC trees (~20-25 years) alter grassland ecosystem carbon dynamics.

A common finding of WPE studies on soils in the Great Plains is that there is an increase in the concentration of C (wt% mass/mass) in the upper layers of the soil with WPE (Boutton et al., 2009; Springsteen et al., 2010; Creamer et al., 2011; Mureva and Ward, 2017; Xiang et al., 2019; Sauer et al., 2023). Many factors, however, control soil carbon dynamics including differences in plant input rate and chemistry among species (Jessup et al., 2003), soil texture, mineralogy, surface area, soil moisture, and stand age (Springsteen et al., 2010; Lehmann and Kleber, 2015; Bai and Cotrufo, 2022). Like other woody species in the Great Plains, ERC encroachment can result in increased concentrations (wt %) of C in the upper layer of soil in comparison to neighboring degraded grasslands (Li et al., 2023). While there may be a documented increase in C accumulation in the upper layer of the soil with ERC encroachment, studies have also found that deep soil carbon concentrations can increase (Mellor et al., 2013), stay the same (McKinley, 2008; Sauer et al., 2023) or decrease (Li et al., 2023) with ERC

encroachment, which may be indicative of soil texture and litter accumulation and other factors such a microbially-stimulated priming effects (Kuzyokov, 2002, Wang et al., 2015). Although many studies have utilized ^{13}C as a proxy for changes in vegetation cover in both remnant grasslands (Jessup et al., 2003; Liao et al., 2006b) and relatively undisturbed grasslands (Connell et al., 2021), more studies are needed to directly relate how past land use changes and ecosystem degradation may influence the accumulation of carbon under woody plant encroachment.

1.2.) Hypotheses:

The objective of this study was to quantify the changes to litter, root biomass, and soil carbon and nitrogen stocks during the early stages of establishment of eastern red cedar (about 25 years of establishment) in a degraded grassland ecosystem in Central Oklahoma. The overarching hypothesis for this study is that after ~25 years of ERC encroachment, there will be a net exchange, replacement, or increase in soil organic carbon (SOC) and that fast turnover of surface and root litter will be converted to exclusively ERC source. The following predictions were then tested in this thesis:

1.) The surface litter under the ERC canopy will only contain carbon sourced from ERC, as litter mass is much higher than RG litter mass

2.) The establishment of ERC will increase fine root input throughout the soil profile, with particular impact to the upper 0-5 cm compared to the adjacent RG plots. By 25 years, all fine root tissue under the ERC canopy will be sourced from the ERC as original grassland root tissue will have degraded

3.) Soils under the ERC canopy will exhibit increased soil organic carbon stock and concentration with the greatest accumulations in the upper 5 cm and with diminished to non-

measurable impact with increasing depth in comparison to remnant grassland plots. The increase in ERC-derived C will be from both litter and fine root input.

4.) The proportion of ERC-derived SOC will increase faster than the proportion of RG carbon decreases in the top 5 cm of the soil profile. As the soil reaches the bedrock, the proportion of ERC-derived carbon will be negligible, with SOC $\delta^{13}C$ values similar to the soils in the RG plots as there is insufficient time in 25 years to alter the deeper carbon pools through root direct deposition.

1.3.) Thesis Overview and Organization:

In this thesis, chapter 2 describes the study site and methods for each study in the following chapters, including field and laboratory methods. Chapter 3 documents the results from an experiment that analyzed the differences in above-ground litter mass, C, and $\delta^{13}C$ values between remnant grassland plots and ~25-year-old eastern red cedar trees. Chapter 3 also reports the results from an experiment that analyzed the differences between fine root mass, C, and $\delta^{13}C$ values between remnant grassland plots and 25-year-old eastern red cedar plots. Chapter 4 investigates the changes in soil properties, such as soil texture, pH, and bulk density, and SOC storage and distribution along soil depths after the establishment of young eastern red cedar trees in comparison to remnant grassland plots. Lastly, chapter 5 describes the final conclusions and future directions for this research.

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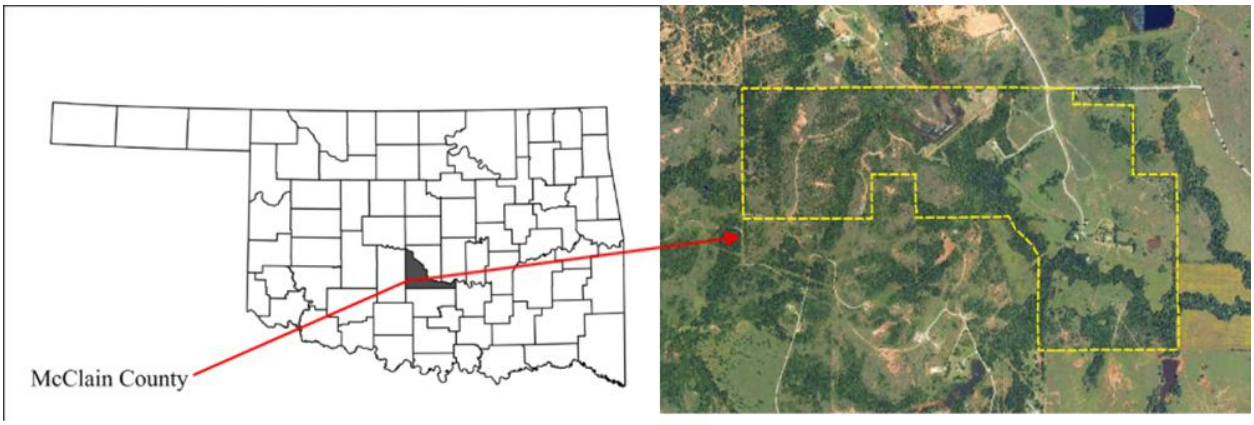
2.0. Methods:

2.1. Site Description:

This study was conducted at the University of Oklahoma Kessler Atmospheric and Ecological Field Station (KAEFS) in McClain County, Oklahoma (34°58'53.7" N 97°31'18.1" W) (Figure 1). Kessler Atmospheric and Ecological Field Station is located in the subtropical humid (Cf) climate zone (Trewartha, 1968). The climate at this site is temperate humid subtropical, and the mean annual temperature at this site is 16.3 °C, with an average of 28.2 °C in July and 4.3 °C in January (Oklahoma Climatological Survey, Norman, OK from 1991-2000). The mean annual precipitation is 922 mm, with a majority of precipitation occurring between April and June (Oklahoma Climatological Survey, Norman, OK). Currently, KAEFS is home to many long-term ecological experiments exploring the impact of climate change on plant and microbial biodiversity, connectivity and function (Shi et al., 2015; Xu et al., 2015; Guo et al., 2018; Prather et al., 2020; Wu et al., 2022).

Figure 1: Location of Kessler Atmospheric and Ecological Field Station (KAEFS). KAEFS is located in McClain County, OK, in the city of Washington. Aerial imagery is from LANDSAT satellite imagery of KAFES with the yellow dashes indicating the border of the site

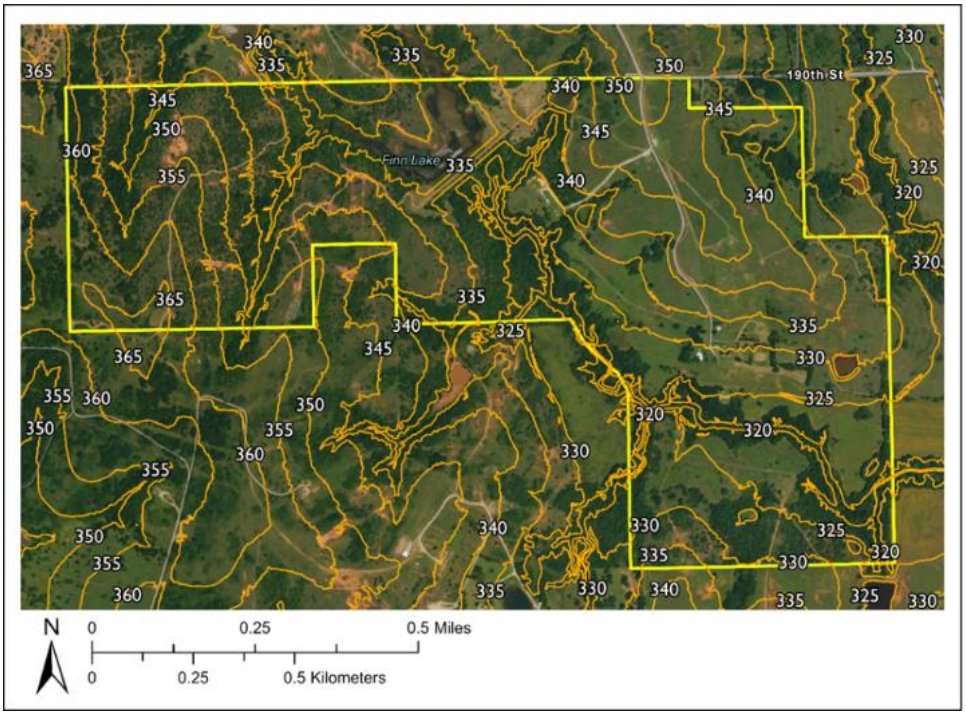


The recent, post European settlement, history of the land within KAFES was management for agriculture, including sorghum, cotton, and wheat. Land was also set aside for cattle grazing from 1904 to the 1970s (Buthod and Hoagland, 2016). A portion of the land was donated to the University of Oklahoma by Ed Kessler for research purposes in 1988, and the remaining sections of land were donated in 2004 (KAEFS, n.d.). Much of the original ecology of the landscape, prior to land clearing and intensive agricultural management, was tallgrass prairie (Robertson et al., 1997; Wick et al., 2016). Grassland vegetation at KAEFS is currently dominated by C_4 grasses with a mixture of C_3 perennial forbs, grasses, and some legume species (Xu et al., 2015). In the open grass regions, C_3 scrub woody plants, C_3 forbs, and C_3 and C_4 grasses dominate. The remnant grasslands and pastures at KAEFS are predominated by the perennial C_4 grasses little bluestem *(S. scoparium),* prairie dropseed (*Sporobolus heterolepsi*s), and Indian grass *(S. nutans).* The site also has many scattered C_3 forbs such as grey goldenrod (*Solidago nemoralis)* and western ragweed (*Ambrosia psilostachya),* and has eastern cottonwood (*Populus deltoides),* American elm (*Ulmus americana*), and sugarberry (*Celtis laevigata*) along the riverbeds and bottomlands (Buthod and Hoagland, 2016). Extensive areas of the bottomland were converted to bermudagrass (*Cynodon dactylon*) for grazing and crop production. Like many areas in this region (Jessup et al., 2003; Mellor et al., 2013; Archer, 2017; Sauer et al., 2023), eastern red cedar (ERC) has been expanding at KAEFS for a few decades and is scattered in both sparse and closed canopy configurations (reviewed in Chapter 1). Evidence from historical land use imagery has demonstrated that KAEFS has a major erosional feature in the top northwest corner and has experienced erosion in most other areas of the site (Anwar, unpublished). A combination of severe erosion and intensive land management has allowed for the encroachment

of ERC at this site. Historical expansion of ERC makes KAEFS an optimal site to study WPE dynamics.

Kessler Atmospheric and Ecological Field Station is located geologically on the Hennessey Group of the Bison Formation, which consists of 50-90 ft thick, calcareous, blocky shales that are gray to red-brown in color (Oklahoma Geological Survey, 1974, Map HA-3). Web Soil Survey indicates the soils to be dominated by highly eroded Nash-Lucien complex soils (coarse-silty, mixed, superactive, thermic Udic Haplustolls and loamy, mixed, superactive, thermic shallow Udic Haplustolls, respectively). This is only a coarse characterization of the soil, and no soil pits were established at KAEFS to perform a detailed soil profile. The site has undergone severe wind and water erosion due to intensive tillage agriculture and cattle grazing, removing or burying the A soil horizon. During soil collection, soils were found to be ~30-40 cm thick. Finn Creek crosses KAEFS from the northwest to the southeast, creating an elevation gradient from the flat uplands to the stream. The elevation of Finn Creek is 335 m, while the flat uplands on each side of the creek are 345-365 m in elevation on the western side and 340-350 m on the eastern side (Fig. 2).

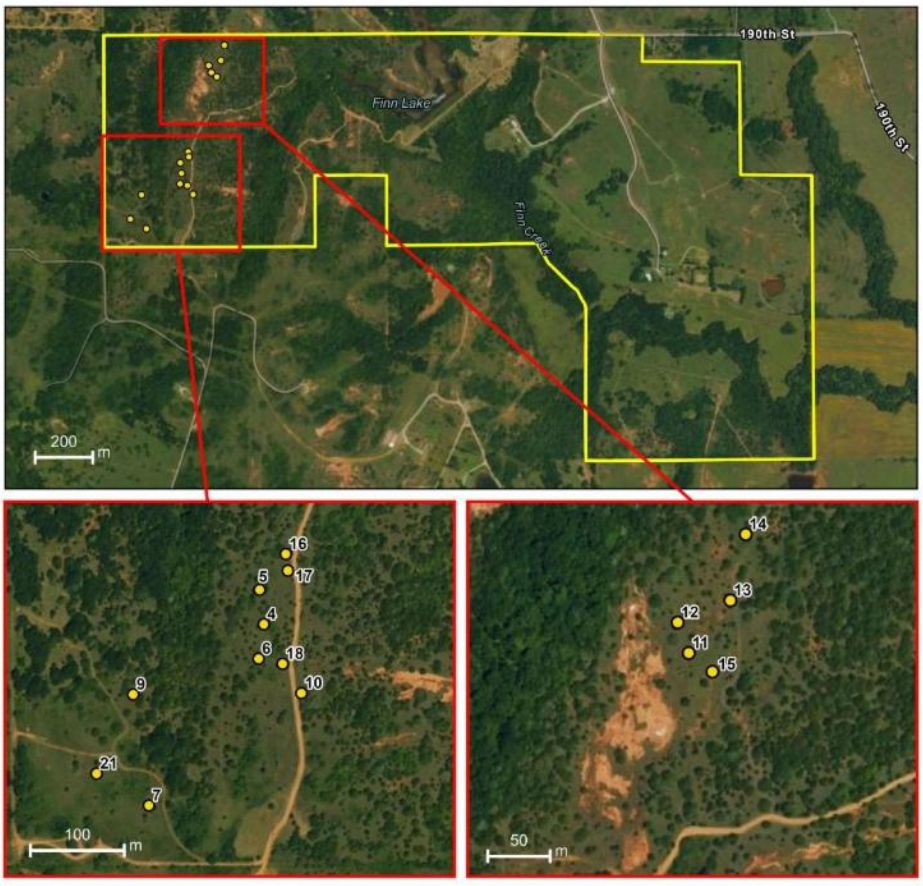
Figure 2: Topographic map of KAEFS using LANDSAT imagery. The yellow border represents the border of the research station.



2.2. Identification and sampling of tree-grass plots:

In this experiment, a plot is defined as an isolated, individual eastern red cedar tree paired with an adjacent grassland patch 5 m distance from the tree canopy. Plots were of slope values between 1.21 and 6.09 m and had similar land use histories and edaphic conditions as identified using Web Soil Survey (Soil Survey Staff b, n.d.) and historical land use images (Anwar, unpublished). Five plots were selected in the northern portion of the site, and ten plots were selected in the southern portion of the site to get a representative sample of trees across the site (Fig 3).

Figure 3: Aerial imagery of Kessler Atmospheric and Ecological Field Station (KAEFS) in 2023 from the LANDSAT satellite. Paired grassland and tree field plots marked by yellow dots and with their designated plot number (Credits: Anwar, unpublished)



Northern plots are situated to the east of a deep erosional feature and have an elevation range of 347-351 m. Southern plots are not situated near a major erosional feature and have an elevation range of 360-365 m. Fifteen ERC trees and fifteen nearby (within 5 meters of a tree canopy height of the tree pair) remnant grassland (RG) plots were selected in pairs according to methods modified from Boutton et al. (1998). For this study, only trees within the range of 20-30 years old were used, which represents a stage for ERC where there is suppression of grasses underneath the canopy (Gehring and Bragg, 1992). Visual inspection indicated complete suppression of grassland species beneath the ERC canopies at this site. The sex of the tree was

determined by looking for the production of pollen vs. cones within our sampled trees (see Chapter 3, Table 3.1.). Five of the 14 trees were female. Plant litter and soil samples were collected in May 2024 over the course of two weeks. Appendices A and B give more detail on plot soil texture, elevation, slope, and aspect.

2.3. Field Methods:

2.3.1. Tree Age Dating:

To control for differences in tree maturity and to find trees that have complete suppression of herbaceous plants and graminoids in the understory, which we defined as 25-30 years old (Gehring and Bragg, 1992), using an allometric equation developed by Giddens and McCarthy (unpublished) (Eq. 1). The allometric equation was developed by measuring 102 trees at the KAEFS spread over a larger area than the trees sampled for this study (Giddens and McCarthy, unpublished data).

Each of the trees were aged using the allometric equation in early May 2024 (Eq. 1), and those estimated to be 24-28 years old, were single standing and not in a grove, and had an open patch of grassland at least 5 m from the tree were selected as field plots. Trees in this age range were selected because suppression of grass species was evident, which was necessary to adequately compare an encroached soil environment to the grassland soil environment (our control plots). Trees with two main stems were avoided.

$$Tree\ Age\ (yrs) = 12.776 + \big((0.535 \cdot DBH) - (1.219 \cdot CLE)\big) + (0.073 \cdot CA) \qquad (Eq. 1)$$

Where:

- *DBH (cm) = tree diameter at breast height*
- *CLE = canopy light exposure*
- *CA (m^2) = canopy area*

Diameter breast height (diameter of the tree trunk at 1.37 m from the ground) was measured. Canopy area was calculated based on two measures of diameter underneath the canopy. The diameter values were averaged, and the area of the canopy was calculated as the area of a circle: $A = 1/4\pi d^2$. Canopy light exposure was scored on a scale of 1, 2, 3, 4, and 5 based on the tree's exposure to sunlight, where 1 is defined as having only one side of the ERC unobstructed in any cardinal direction and 5 is no obstruction. 2, 3, and 4 are intermediate values indicating 2 sides of obstruction (2), 3 sides of obstruction (3), and 4 sides of obstruction (4). Figure 4 captures a representative sample of what the vegetation looked like at the paired tree-grassland plots.

Figure 4: Example images of a.) eastern red cedar (ERC) plot and b.) remnant grassland (RG) plot. The RG plots represent a mixture of C_3 forbs and C_3 and C_4 grasses with patches of exposed soil.

a. *b.*

2.3.2. Soil and litter sampling:

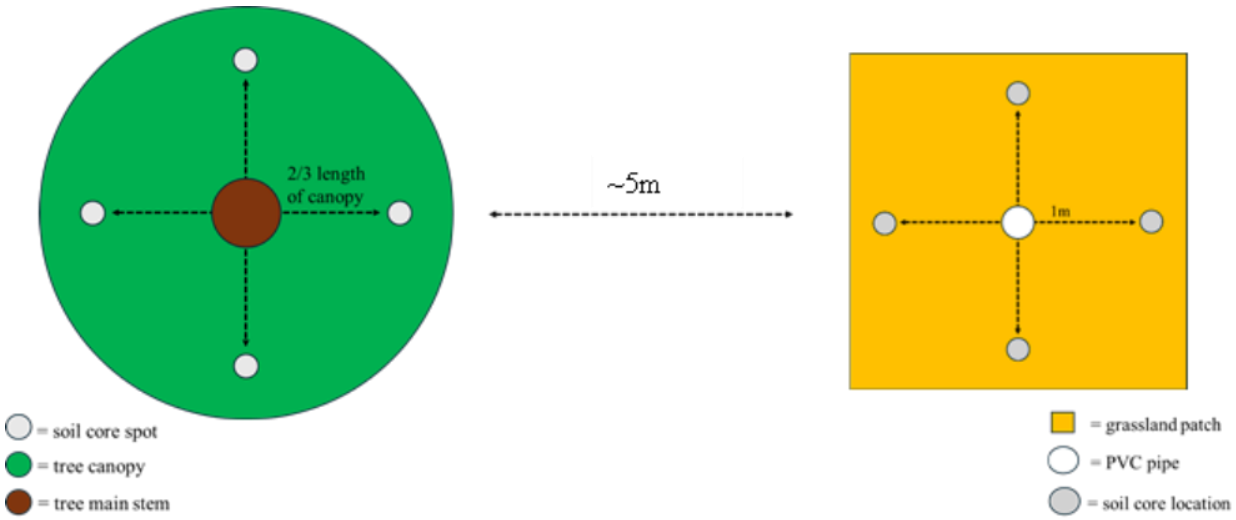
Plant litter and soil samples were collected over the course of two weeks in May 2024. Within the plots containing trees, four sampling locations were marked at ⅔ the distance from the main stem to the canopy edge (roughly 120 cm) in each cardinal direction (Fig. 5). Although there exists the potential for the presence of herbaceous plant roots when sampling further from the bole (Throop and Archer, 2008), sampling away from the bole avoids large woody roots. For each grassland plot, four sampling locations were marked 1 m from a central point in each cardinal direction (Figure 5).

A 15.24 cm diameter PVC ring was used to collect ERC and RG litter. Litter beneath ERC trees was defined as fallen tree debris (e.g. needles and cones) at the surface of the soil (mineral or organic), while RG litter was defined as all shoots (alive or dead) and fallen grass

blades. The ring was placed at the designated location, and all litter within the ring's border was collected down to the organic horizon or mineral soil surface. Collected litter was placed into plastic freezer bags and temporarily stored in coolers on ice and then moved to a laboratory freezer at –20 °C until processed.

Subsequently, four soil cores were taken at each ERC and RG plot, totaling 8 soil cores for each ERC-RG pair. As these soils were very shallow, only ~30 cm soil cores could be sampled before bedrock was hit. Soil cores were extracted using a 30 cm long and 5 cm diameter AMS Split Corer (Art's Manufacturing and Supply, Inc., Idaho, United States of America). Each split core had six 5 cm (~98 cm^3 per ring) long aluminum rings. The four soil cores at each plot were segmented into 0-5 cm, 5-15 cm, and 15-30 cm increments and composited in the field to create a representative soil sample depth per tree or grassland plot. Soils were placed in a cooler with ice for transport and stored in a 2°C refrigerator until sieving.

Figure 5: The soil and above-ground litter sampling scheme at each paired tree-grassland plot. Under the tree canopy, above-ground litter and a soil core was taken at ⅔ the distance from the main stem to the canopy edge (roughly 120 cm) in each cardinal direction. At about 5 m away from the canopy edge, a PVC pipe was placed in an adjacent grassland plot. The above-ground litter and a soil core were collected 1 m from the central point (PVC pipe) in each cardinal direction.



2.4. Physical Sample Processing and Analysis:

2.4.1. Soil and Litter Preparation

In the lab, bulk soils were sieved at field moist conditions to 8 mm and allowed to air dry for four days. All roots and rocks > 2 mm were removed and weighed. Clay-rich soils developed hardened clumps upon drying, and these were gently broken using a marble rolling pin. Soils were then sieved to 2 mm. After sieving to 2 mm, roots < 2 mm in diameter were hand-picked out using tweezers, rinsed thoroughly with deionized water, and dried in a forced air oven until constant weight. Soils were then placed in a forced air oven at 40 °C and dried until a constant weight, with verification every 24 hours. The cone and quartering method was used to subsample

soils for analysis (Campos-M and Campos-C, 2017). Soil texture was determined on a 50-100 gram subsample of soil (Ward Labs, Nebraska, United States of America). Subsamples of soils were powdered using a Mixer Mill 400 (Verder Scientific, Hann, Germany) and stored for chemical and isotope analysis.

The litter from both ERC and RG plots were sieved with a 2 mm sieve to remove soil particulates and were dried in a forced air oven at 40 °C until constant weight. Cones were kept in all ERC litter samples. To avoid compaction during grinding, litter samples were frozen using liquid nitrogen. Specifically, subsamples of litter were placed in a steel grinding jar, and the jar was immersed in liquid nitrogen for 2 minutes, ground to a fine powder on a Mixer Mill 400 for 3 minutes and stored for chemical and isotope analysis in ashed scintillation vials. Fine roots (< 2 mm roots) were hand-ground in liquid nitrogen to get a coarse grind. The roots were then placed in steel grinding jars and immersed in liquid nitrogen for 2 minutes. The roots were ground to a fine powder using a Mixer Mill 400 (Verder Scientific, Hann, Germany) for 3 minutes and stored for chemical and isotope analysis in ashed glass scintillation vials.

2.4.2. Bulk Density

Soil cores were processed for bulk density using the 5 cm diameter aluminum rings of known volume (~98 cm^{-3}) within the AMS Split Corer. Bulk density was determined by drying the separate < 2 mm soil, root, and rock fractions of each sampling depth in a forced air convection oven at 40 °C until constant weight. The bulk density was then calculated by subtracting the volume of the rocks and roots, and was determined for each ERC and RG plot for the 0-5 cm, 5-15 cm, and 15-30 cm depths using Equations 2-4:

$$Bulk\ Density\ = \frac{mass\ of\ dry\ soil}{\left(\left(V_{rings} \cdot number\ of\ rings\ collected\right) - \left(V_{roots} + V_{rocks}\right)\right)} \qquad (Eq.\ 2)$$

Where the bulk density is in units of g/cm^3, mass of dry soil is in units of g, V_{rings} is the total volume of the aluminum rings in one soil section in units of cm^3, V_{roots} is the total volume of the roots in one soil section in units of cm^3, and V_{rocks} is the volume of the rocks in one soil section in units of cm^3

The volume of the roots and rocks was determined using the following equations, where 2.65 g/cm3 is the density of rock (Eq. 3), and 0.6 g/cm3 is the estimated density of roots (*Gray and Sotir 1996*) (Eq. 4):

$$Volume\ of\ Rocks\ (cm^3)\ =\ \frac{mass\ of\ dry\ rocks\ (g)}{2.65\ \frac{g}{cm^3}} \qquad (Eq.\ 3)$$

$$Volume\ of\ Roots\ (cm^3)\ =\ \frac{mass\ of\ dry\ roots\ (g)}{0.5\ \frac{g}{cm^3}} \qquad (Eq.\ 4)$$

(root density value obtained from Korkut and Hiziroglu, 2013)

2.4.3. Soil pH:

A 1:1 soil-to-water (w/v) method was used to assess soil pH. 5 mL of deionized water and 5 g of soil were placed in a Falcon tube and hand shaken to homogenize the soil and water solution for each land cover type (tree and grassland plots). pH was measured at each soil depth increment (0-5 cm, 5-15 cm, and 15-30 cm) (Subcommittee D19.03, 2018) using a Mettler Toledo SevenCompact S220 Benchtop pH Meter.

2.4.4. Bulk Carbon and Nitrogen Elemental and Stable Isotope Analysis of Litter, Root, and Soil:

Carbon (C) and nitrogen (N) elemental concentration and stable isotope values were measured at the Stable Isotope Measurement Facility (SIM Facility) at the University of Oklahoma. Carbon and nitrogen isotopes are reported as per mil (‰) relative to the international reference standard Vienna Peedee Belemnite (VPDB) and air (atmospheric N_2), respectively (Eq. 5). Ground soil, roots, and litter were analyzed for weight percent C and N as well as $\delta^{13}C$ and $\delta^{15}N$ values using a combined flash elemental combustion analyzer isotope ratio mass spectrometer (EA-IRMS) operating on continuous flow – a Sercon Integra 2 EA-IRMS (Sercon Ltd., Crewe, United Kingdom). A dual analysis collected %C, %N, $\delta^{13}C$, and $\delta^{15}N$, but $\delta^{15}N$ values were not reported.

$$\delta X(‰) = \left(\frac{R_{sample} - R_{STD}}{R_{STD}}\right) \cdot 1000 \qquad (Eq. 5)$$

Where δX‰ is the $\delta^{13}C$ of a sample in units of ‰, R_{sample} is the ratio $^{13}C/^{12}C$ of the unknown sample, and R_{STD} is the $^{13}C/^{12}C$ ratio of the VPDB referenced standard (Coplen 1996).

For root and litter samples, a lab standard of maize ($\delta^{13}C$= -11.99‰) was used for calculating weight percent concentrations and linearity. For soils, a LECO soil (502-697, $\delta^{13}C$= -17.25‰) standard was used for calculating weight percent concentrations and linearity. L-Glutamic Acid (OUGA) was used for all sample types as an internal standard for drift correction at 1 mg ± 0.025 mg. Finally, a two-point normalization linear equation was calculated using USGS-40 ($\delta^{13}C$= -26.4‰) and USGS-41 ($\delta^{13}C$= +36.5‰). Overall, 10% of the soil, litter, and

root samples were randomly chosen for analysis replication. Instrument mean precision for standards and replicates was ±0.3% for C concentration, ±0.1% N concentration, and ±0.2‰ for $\delta^{13}C$. To obtain the C and N elemental and stable isotope values, powdered samples were weighed into 9x5 mm tin capsules to a weight appropriate for approximately 0.20 moles of C and 0.14 moles of N.

2.4.5. Determination of Soil Organic and Inorganic Carbon:

As Nash-Lucien complex soils are known to contain both pedogenic and geogenic carbonates, soil organic carbon (SOC) was measured directly after acid fumigation and soil inorganic carbon (SIC) was calculated by difference from total C. To remove soil carbonates, soils were acidified using the procedure of Harris et al. (2001). Specifically, powdered soils were weighed into 9x5 mm silver capsules to a mass of ~30 mg and moistened with 50 µL of deionized water. The capsules were then loaded onto Teflon 96-well plates and acidified by HCl (12.1 molar) fumigation within a glass chamber to remove carbonates. Samples were fumigated overnight and then allowed to dry at 60°C for 4-5 hours before enclosing the silver capsules in outer tin capsules. Soil organic carbon was determined directly as the carbon mass values measured from soils post acid fumigation by EA-IRMS, and the percent organic carbon and the $\delta^{13}C$ of the organic carbon were obtained. Overall, 10% of the acidified samples (9 samples) were randomly chosen for analysis replication. A separate full soil sample analysis was done for each soil to obtain the percent total soil carbon and the $\delta^{13}C$ of the total carbon (inorganic plus organic). The amount of inorganic carbon was determined by difference using equations 6 and 7:

$$wt\%SIC = wt\%TC - wt\%SOC \quad (Eq. 6)$$

Where wt%SIC is the calculated percent inorganic carbon of the soil sample in units of percent, wt%TC is the weight percent soil total carbon in units of percent, and wt%SOC is the weight percent soil organic carbon of the soil sample in units of percent

$$\delta^{13}C_{SIC} = \frac{(C_{CT} \cdot \delta^{13}C_{CT}) - (C_{SOC} \cdot \delta^{13}C_{SOC})}{C_{CT} - C_{SOC}} \quad (Eq. 7)$$

Where $\delta^{13}C_{SIC}$ is the $\delta^{13}C$ of the inorganic carbon in units of ‰, C_{CT} is the soil total carbon in units of percent, $\delta^{13}C_{CT}$ is the $\delta^{13}C$ of the soil total carbon in units of ‰, C_{SOC} is the soil organic carbon in units of percent, and $\delta^{13}C_{SOC}$ is the $\delta^{13}C$ value of the soil organic carbon in units of ‰.

2.4.6. Soil carbon stock:

For each soil depth increment, soil carbon stock was calculated using the following equation (Eq. 8):

$$SOC\ Stock = \rho_b \cdot \left(\frac{wt\%SOC}{100}\right) \cdot h \cdot 10000 \quad (Eq. 8)$$

where SOC stock is the soil organic carbon stock in units of tonne/ha, ρ_b is bulk density in units of tonne/m^3, wt%SOC is the weight percent soil organic carbon in units of percent, and h is the soil layer thickness in units of m.

2.4.7. Root and litter carbon stock:

For each depth increment, root carbon stock was calculated for total root mass and fine root mass using the following equation (Eq. 9):

$$Root\ Carbon\ Stock\ =\ root\ mass\ \cdot \left(\frac{wt\%R_{OC}}{100}\right) \qquad (Eq. 9)$$

where root carbon stock is in units of gC/m^2, root mass is the dry total or fine root biomass in units of g/m^2, and $wt\%R_{OC}$ is the weight percent organic carbon of the fine roots in units of percent

Litter carbon stock was calculated in the same manner (Eq. 10):

$$Litter\ Carbon\ Stock\ =\ litter\ mass\ \cdot \left(\frac{wt\%L_{OC}}{100}\right) \qquad (Eq. 10)$$

Where litter carbon stock is in units of $gC/m^{2,}$ litter mass is the dry litter mass in units of g/m^2 , and $wt\%L_{OC}$ is the weight percent organic carbon of the litter in units of percent

2.4.8. Estimating Relative Contributions of Tree and Grassland C in Soil Beneath ERC Canopy:

A two-end-member mixing model was used to calculate the remaining fraction of grassland carbon (FCgrass) beneath the ERC canopy (Boutton, 1998; Liao et al., 2006b; Boutton et al., 2009). The FCgrass was calculated as a pairwise analysis, where the soils of each of the

paired tree-grass plots were used. The end members were calculated using the average stable isotope values of the field ERC and RG roots as the primary source of plant inputs (Eq. 11). The average ERC carbon end member was -26.6‰, and the RG carbon end member was -19.5‰:

$$FC_{grass} = \frac{(\delta^{13}C_{TS} - \delta^{13}C_{tree})}{(\delta^{13}C_{GS} - \delta^{13}C_{tree})} \quad (Eq. 11)$$

where FC_{grass} is the remaining proportion of grassland carbon in the tree soil C, $\delta^{13}C_{TS}$ is the $\delta^{13}C$ value of the ERC soil C at a specific depth, $\delta^{13}C_{tree}$ is the average $\delta^{13}C$ value of the tree root, above-ground litter, or average of the two material in that same fraction, and $\delta^{13}C_{GS}$ is the $\delta^{13}C$ value of the RG soil organic C at a specific depth.

The next equation was utilized to estimate the fraction of C_3 roots in the roots beneath ERC trees in a similar fashion:

$$FC_{roots} = \frac{(\delta^{13}C_{TR} - \delta^{13}C_{IGR})}{(\delta^{13}C_{ITR} - \delta^{13}C_{IGR})} \quad (Eq. 12)$$

where $FC_{C3roots}$ is the proportion of C_3 roots found beneath the ERC canopy, $\delta^{13}C_{TR}$ is the $\delta^{13}C$ value of the root samples beneath the ERC canopy, $\delta^{13}C_{ITR}$ is the idealized $\delta^{13}C$ value of grassland roots (Eq. 13), and $\delta^{13}C_{IGR}$ is the idealized $\delta^{13}C$ value of tree roots (Eq. 14).

To estimate the proportion of C_3 roots beneath the ERC canopies, two endmembers are necessary: the $\delta^{13}C$ value of grassland species roots and the $\delta^{13}C$ values of the ERC roots. However, because we cannot identify and separate the roots beneath either the ERC or the RG plots, we must estimate the values of the end members. The following equations were used to estimate the values of the grassland and tree endmembers (Eq. 13 and 14):

$$\delta^{13}C_{ITR} = \delta^{13}C_{tree} + \Delta C_3\ above - to - below - ground\ biomass \qquad (Eq. 13)$$

$$\delta^{13}C_{IGR} = \delta^{13}C_{grass} + \Delta C_4\ above - to - below - ground\ biomass \qquad (Eq. 14)$$

Where $\delta^{13}C_{ITR}$ is the idealized $\delta^{13}C$ value of the ERC roots, $\delta^{13}C_{tree}$ is the $\delta^{13}C$ value of the ERC litter, $\delta^{13}C_{IGR}$ is the idealized $\delta^{13}C$ value of C_4 grasses, and $\delta^{13}C_{grass}$ is the $\delta^{13}C$ value of the RG litter. The $\delta^{13}C$ discrimination value for C_3 plants is +1.2‰ and +0.3‰ for C_4 plants (Werth and Kuzyakov 2010). By adding the discrimination value of the above- and below-ground biomass to the litter value, we can estimate the root $\delta^{13}C$ values for the ERC and the RG species at this site.

2.5. Data Analysis:

Data were tested for normality by using a Shapiro-Wilks test and results displayed as Q-Q plots. Distributional results were non-normal. Therefore, log and square transformations were attempted. Homogeneity of variance was tested by using the Levene's test. Normality and homogeneity results did not meet the assumptions of parametric tests, so non-parametric analysis was conducted. A Mann-Whitney U test was used for each depth interval separately (i.e. 0-5 cm, 5-15 cm, and 15-30 cm) for the following data analysis (Mann & Whitney, 1947). Above-ground

and root litter mass, wt%C, wt%N, OC stock, and $\delta^{13}C$ values between ERC and RG plots were compared with significance set to $p < 0.5$. In addition, soil bulk density, texture elements, pH, wt%SOC, wt%SIC, wt%N, SOC stock, and $\delta^{13}C$ values between ERC and RG plots were compared, with significance indicated at $p < 0.5$. A Kruskal-Wallis test was utilized to see if there were significant differences across depth intervals between the ERC and RG plots for all the above-ground litter, root, and soil data sets (Kruskal & Wallis, 1952). Data was analyzed using R version 4.4.1 (R Core Team 2024), and graphs were produced using the package ggplot (Wickham, 2016).

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3. LITTER QUANTITY AND ISOTOPE VALUES ARE ALTERED AFTER 25 YEARS OF WOODY PLANT ENCROACHMENT

3.1. Introduction

Plant material is a primary component of soil organic matter and the principal nutrient source for all microbial processes entering the soil via above-ground (i.e., leaf litter) and below-ground inputs (i.e., roots) (Trumbore 1997). Plant material can differ in relative quality, which is often defined by its C:N ratio, lignin content, and aliphatic compounds (Couteaux et al., 1995; Kogel-Knabner, 2002; Dungait et al., 2012). Litter quality and abundance control initial decomposition rates and the nature of decay through targeted decomposition processes (Schmidt et al., 2011), but long-term decomposition rates and carbon storage are primarily controlled by climate, the soil matrix, and microbial community composition (Adu and Oades, 1978; Parton et al., 1987; Six et al., 2004; Lehmann and Kleber, 2015; Liang et al., 2017). Therefore, changes in local ecosystem litter input type, rate as well as plant chemistry, strategy, and physiology will influence ecosystem function and soil carbon dynamics.

Woody plant encroachment (WPE) into grasslands is a major land cover change process that alters the rate and chemical composition of litter inputs as well as interactions with soil microbial communities (Norris et al., 2001a, 2001b; Filley et al., 2008; Creamer et al., 2012; Archer et al., 2017). However, the wide variation in plant communities involved in WPE globally provides for numerous possibilities in encroachment rate, plant -microbe-soil interactions, and litter input dynamics, all of which can impact short and long-term soil carbon storage within different ecosystem types and edaphic properties. In general, woody plant encroachment into grasslands significantly alters above- and below-ground litter inputs (Hibbard et al., 2001; Norris et al., 2001b; McKinley, 2006; Bennion and Ward, 2022). Woody plants increase both surface litter mass and potentially root litter input in comparison to grasslands

(Norris et al., 2001a; Sauer et al., 2007; Li et al., 2023). Woody plants tend to have much higher above-ground net primary productivity (ANPP) and standing litter stock in comparison to grassland species and can even have higher below-ground NPP (BNPP) than grass species (Barger et al., 2011; Boutton et al., 2009). The introduced litter mass, however, is often of poorer quality (Norris et al., 2001a; Filley et al., 2008; Creamer et al., 2012) as defined by its chemical composition, and thereof decrease the efficiency of decomposition of woody materials by microbial communities (Norris et al., 2001a; Mason et al., 2010; Creamer et al., 2012; Klotzbucher et al., 2011). For example, ERC roots contain significantly higher lignin content (~32.3%) compared to the C_4 grass big bluestem (~16.4%), the roots of which decay at a rate 30% higher than ERC roots (Norris et al., 2001a). Similar molecular differences were found between mesquite and local grasses in a mesquite parkland in Texas (Filley et al., 2008). In a lab incubation experiment comparing particulate organic matter under mesquite and adjacent grasslands, organic material of mesquite origin decayed slower than grassland POM, indicating suppressed decomposition due to chemical composition (Creamer et al., 2011). These and similar findings (Creamer et al., 2013, 2016) indicate that WPE has many biogeochemical impacts on soil carbon dynamics. Therefore, a combination of increased rates of above- and below-ground litter inputs, litter quality, and regional environmental conditions can impact grassland biogeochemical processes after WPE.

WPE also alters root architecture and the rooting zones in grassland ecosystems. Woody plant roots tend to be of greater diameter that increase preferential water flow and soil aeration (Archarya et al., 2018). Rooting structure for both grass species and woody species decreases exponentially with depth (Nippert et al., 2012), but woody plants increase rooting depth and have higher root biomass at deeper depths than grass species (Boutton et al., 1999; Archer et al., 2001;

Hibbard et al., 2001; Jackson et al., 2002). For example, woody plants adjust to soil depths, with shorter, more fibrous rooting structures in shallow soils and deeper tap rooting systems in deep soils (Lawson, 1990; Acharya et al., 2018), creating direct competition with grass species at deeper depths. Lastly, the rooting systems of woody plants alter soil microbial processes by exuding allelopathic chemicals. Allelopathic chemicals can be released into a soil ecosystem via root exudates or leachates from fresh and decomposing surface litter or roots (Bais et al., 2006). Chemicals found in ERC, such as tannins, quinines, falvonoids, and phenolics, can be allelopathic to surrounding herbaceous species (Li et al., 2010). Eastern red cedar allelochemicals have been shown to alter species richness and biomass beneath the canopy in grassland ecosystems (Stipe and Bragg, 1989; Bennion and Ward, 2023), specifically targeting other C_3 species (Bennion and Ward, 2022) and creating a legacy effect of root build-up and grass suppression (Bennion and Ward, 2023).

Changes in plant rooting structures can alter soil carbon storage and stability (Miller and Jastrow, 1990; Six et al., 2004; Srivastava and Yetgin 2024) as rooting systems influence soil water flow, soil aggregation (Tisdall and Oades, 1982; Miller and Jastrow, 1990), and soil microbial activity via root priming, associating with fungal communities, and allelopathy (Ma et al., 2002; Bais et al., 2006). Within soil pores, microsites of water and air regulate microbial activity and decomposition of organic matter (Ma et al., 2022). Fine roots, fungal hyphae, and microbial byproducts contribute to the formation of microaggregates within macroaggregates, creating clay-encrusted pockets of organic matter that are protected from microbial decomposition (Six et al., 2004). Fine roots also exude sugars to stimulate microbial activity for nutrient acquisition (Ma et al., 2022). Zones of rooting are responsible for the majority of soil carbon storage, so changes to fine root biomass, depth, and architecture have the potential to alter

organic matter decomposition and respiration. However, the effects of rooting system alterations after WPE on soil carbon storage are hard to quantify, as it is difficult to accurately sample rooting structures- particularly true for documenting the interactions of ERC interactions in the degraded grasslands of Oklahoma. This limitation has led to a gap in knowledge related to the interaction between changes in root density and soil organic matter dynamics in ERC encroachment studies.

The purpose of this chapter is to quantify the changes in carbon stock, plant source, and litter quality (e.g. C:N) of root tissue and surface litter in plots of 25-year-old ERC and adjacent grasslands at the Kessler Atmospheric and Ecological Field Station KAEFS in McClain County, OK. The objective is to understand any changes to soil organic and inorganic carbon explored in Chapter 4 of this thesis. As articulated in Chapter 2, we used stable isotope and elemental carbon and nitrogen analyses to assess the dynamics of litter change with WPE.

Predictions:

1.) The surface litter under the ERC canopy will only contain carbon sourced from ERC, as litter mass is much higher than RG litter mass

2.) The establishment of ERC will increase fine root input throughout the soil profile, with particular impact to the upper 0-5 cm compared to the adjacent RG plots. By 25 years, all fine root tissue under the ERC canopy will be sourced from the ERC as original grassland root tissue will have degraded

3.2. Methods

Site characterization, sampling, analysis, and data treatment protocols used to test hypotheses in this chapter are presented in Chapter 2.

3.3 Results:

3.3.1. Eastern red cedar/grassland plot location, biophysical characteristics, and tree age:

Using the allometric relationships provided by H. McCarthy, University of Oklahoma-unpublished (Chapter 2, Equation 1), the average ERC tree age among the 15 plots in this study was 26 (±1) years, ranging from 24 to 28 years (Table 1). Ten trees were male, and 5 trees were female (identified by visible cones). Thirteen of the ERC trees had no obstruction from sunlight, i.e., they were isolated trees, while trees in plots 10 and 15 (Table 1) had one side partially blocked by an adjacent tree. All trees had a single trunk, and the average diameter was 12.7 (±2.4) m, with a range of 8.6 m-14.6 m. The average dripline diameter, based on 2 measurements per tree, was 4.2 (±1.2) m. Trees in plots 11-15 were located at the Northwest side end of KAEFS near a historical erosional site dating back to the mid-1930s (Anwar, 2025).

Table 1: Tree age, stem diameter, dripline distance, canopy light exposure, and sex at each tree plot location.

Tree Plot	Latitude	Longitude	Sex	Stem Diameter (cm)	Average Dripline Diameter (m)	Canopy Light Exposure	Age (yrs)
4	34.98256	-97.53615	F	13.1	3.8	5	27
5	34.98283	-97.53619	M	8.6	3.4	5	24
6	34.98230	-97.53620	M	14.0	7.8	5	26
7	34.98114	-97.53725	M	11.8	4.1	5	26
9	34.98201	-97.53740	M	8.6	4.0	5	24
10	34.98202	-97.53579	M	14.0	4.6	4	26
11	34.98514	-97.53522	M	14.6	4.3	5	28
12	34.98532	-97.53530	M	16.6	4.3	5	29
13	34.98545	-97.53492	M	14.0	2.2	5	27
14	34.98584	-97.53481	M	10.5	4.3	5	26
15	34.98503	-97.53505	F	13.1	5.0	5	27
16	34.98311	-97.53594	F	12.4	3.6	5	26
17	34.98298	-97.53592	F	9.9	2.9	4	24
18	34.98225	-97.53597	M	14.6	3.6	5	27
21	34.98140	-97.53780	F	14.3	5.0	5	28

A map of tree locations within KAEFS is found in Chapter 2, Fig. 3

3.3.2 Eastern red cedar and remnant grassland plot root biomass and depth distribution:

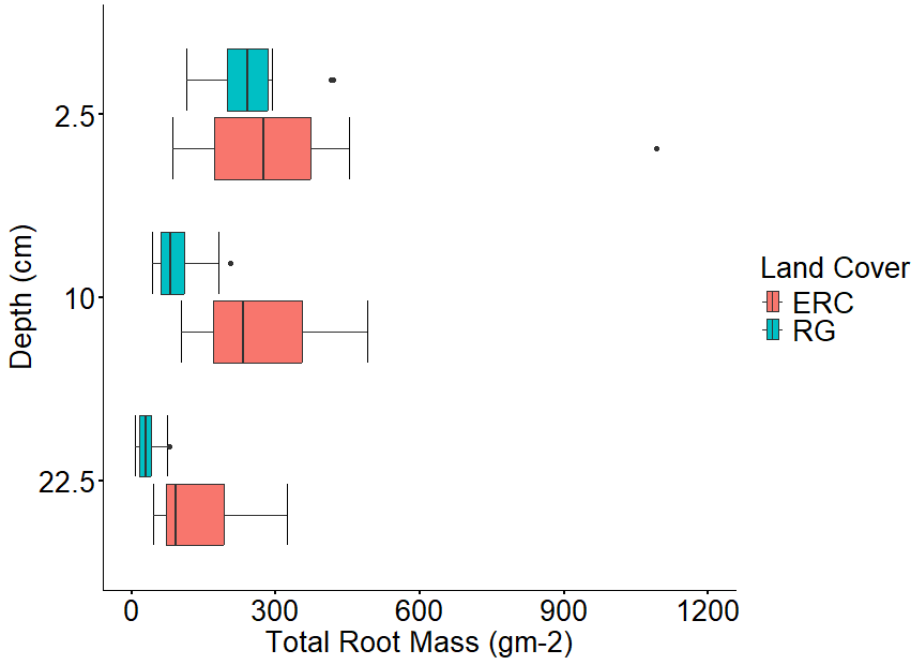
Root Biomass Distribution: The mean fine root (<2mm diameter) dry biomass was the highest at the 0-5 cm depth interval for both ERC (205.77 ±75.1 gm-2) and remnant grassland (RG) (165.27 ±50.4 gm-2) (Fig. 6). Mean fine root dry biomass under all ERC plots was significantly higher than under all RG plots for both the 5-15 cm depth increment (p=0.01) and 15-30 cm depth increment (p=0.01) (Table 2). The ERC fine dry root biomass significantly decreased with

soil depth between the 0-5 cm and 5-15 cm depth intervals (p=0.03) but did not change between 5-15 cm and 15-30 cm depth intervals (p=0.05). Beneath RG plots, mean fine dry root biomass among all plots significantly decreased between the 0-5 cm and 5-15 cm depth intervals (p=0.001) but did not change between the 5-15 cm and 15-30 cm depth intervals (p=0.07).

Overall, total dry root biomass (fine plus coarse roots) was the highest for mean ERC plots at the 0-5 cm depth interval with an average of 323.23 (± 242.88) gm-2 compared to RG plots at 247.28 (± 86.66) gm-2. Because total root biomass sampling was biased against large roots (see Chapter 2), no further discussion of total root biomass will follow.

Fig. 6: Box plots of (a) total (>2mm and <2mm) root biomass and (b) fine (<2mm) root biomass with depth for eastern red cedar (ERC) and remnant grasslands (RG) at KAEFS for cores from 0-5 cm (avg 2.5 cm), 5-15 cm (avg 10 cm), and 15-30 cm (avg 22.5 cm)

a.)



b.)

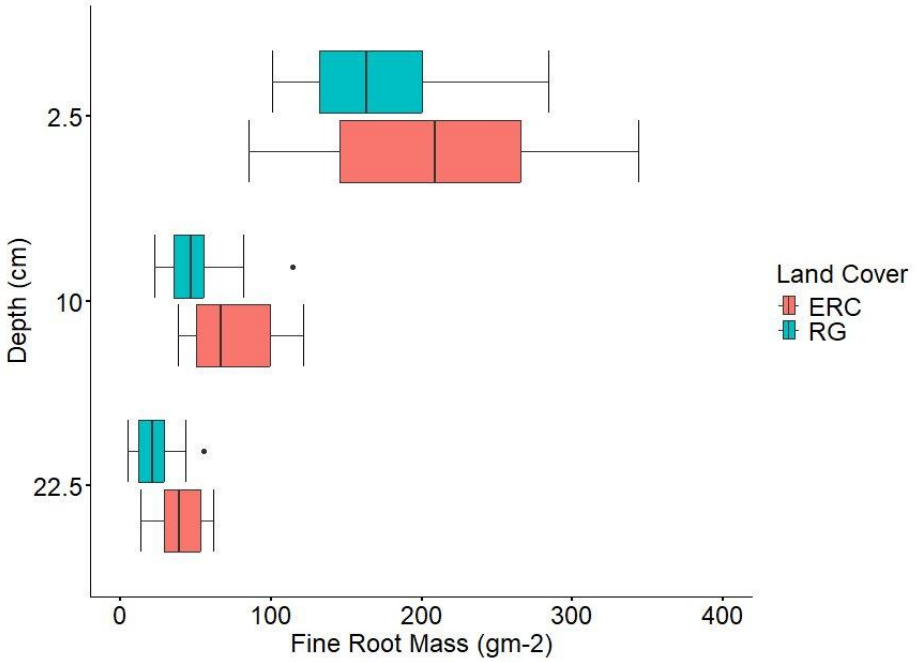


Table 2: Root biomass and chemistry beneath eastern red cedar (ERC) and remnant grassland (RG) plots at three different soil depth increments

Depth	Land Cover	Total Root Biomass (gm-2)	Fine Root Biomass (gm-2)	Fine Root Wt %C (%)	Total Root Carbon Stock (gCm-2)	Fine Root Carbon Stock (gCm-2)	Fine Root $\delta^{13}C$ (‰)
0-5 cm	RG	247.28 (±86.66)	165.27 (±50.39)	41.3* (±1.8)	101.5 (±75.4)	67.7 (±19.1)	-19.5 * (±2.8)
	ERC	323.23 (±242.88)	205.77 (±75.08)	43.9* (±1.5)	139.8 (±98.8)	89.8 (±31.9)	-26.6 * (±1.1)
5-15 cm	RG	93.36 * (±47.80)	50.05 * (±24.19)	43.5 * (±1.3)	40.6 *** (±20.7)	21.7* (±10.3)	-20.6 * (±2.4)
	ERC	272.44 * (±133.30)	74.99 * (±28.53)	45.1* (±1.5)	123.0 *** (±60.1)	33.8 * (±12.9)	-27.0 * (±0.8)
15-30 cm	RG	32.10* (±21.53)	23.44 * (±13.84)	43.1* (±1.1)	13.7 *** (±9.0)	10.1 * (±5.8)	-22.4 * (±2.6)
	ERC	132.17 * (±83.61)	39.38 * (±16.37)	43.9 * (±1.4)	58.0 *** (±37.1)	17.2 * (±7.1)	-27.1 * (±0.5)

*The reported values above show the means of fifteen field replicates (standard deviation of the mean). Significance values were determined using a Wilcoxon Rank-Sum test with the significance value set at $p < 0.05$, with *= $p<0.05$, **=$p<0.001$, and ***=$p<0.0001$. Significance values should be read by each sampling depth within its column. Significance values comparing tree to tree and grass to grass values are not represented in this table.*

3.3.3. Root chemistry, stock, and stable C isotope composition:

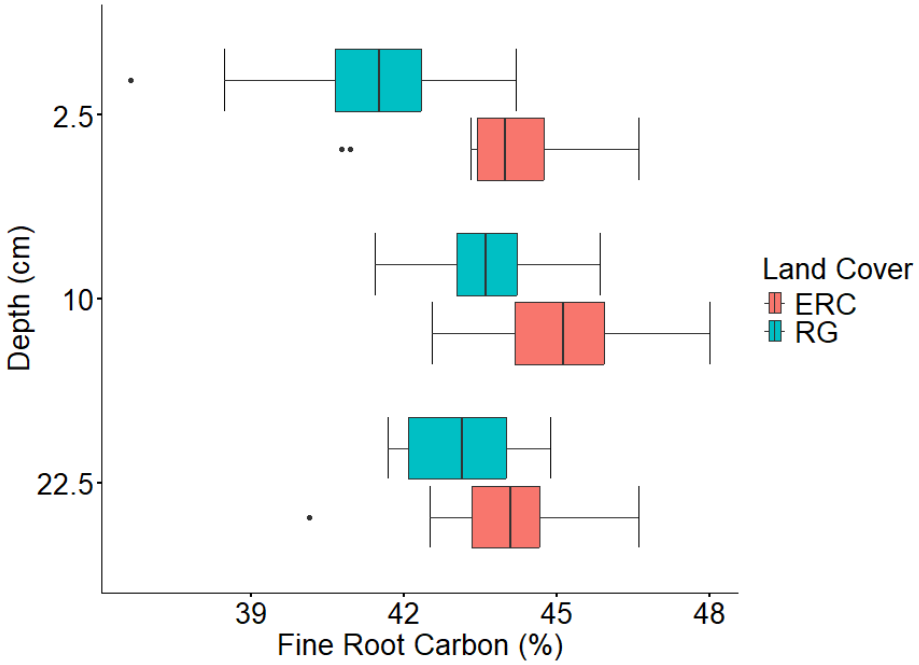
Across all soil depth intervals, mean ERC fine root wt % C (average 0-5 cm = 43.9± 1.5 %, 5-15 cm =45.1± 1.5%, and 15-30 cm =43.9 ±1.4%) exceeded those of RG plots (average 0-5 cm = 41.3 ± 1.8%, 5-15 cm = 43.5 ± 1.3%, and 15-30 cm = 43.1 ± 1.1%) ($p < 0.05$) (Table 2, Fig. 7). Also, across all plots, the wt % C of mean ERC fine roots did not significantly change with depth. However, grassland plots exhibited a significant increase in wt %C between the 0-5 and 5-15 cm depth intervals ($p= 0.0015$) but not between the 5-15 and 15-30 cm depth intervals. Fine root carbon stock exhibited no difference between ERC (89.90 ± 31.88%) and RG (67.70 ±

19.10%) plots at 0-5 cm, while ERC plots had comparatively higher fine root carbon stock at the 5-15 cm and 15-30 cm depth intervals ($p < 0.001$), increasing from 21.7 to 33.8 g/m^2 and from 10.1 to 17.2 g/m^2, respectively. Along the soil profile for RG plots, there was a decrease in fine root carbon stock with depth ($p < 0.001$), while there was no significant difference with depth beneath ERC plots.

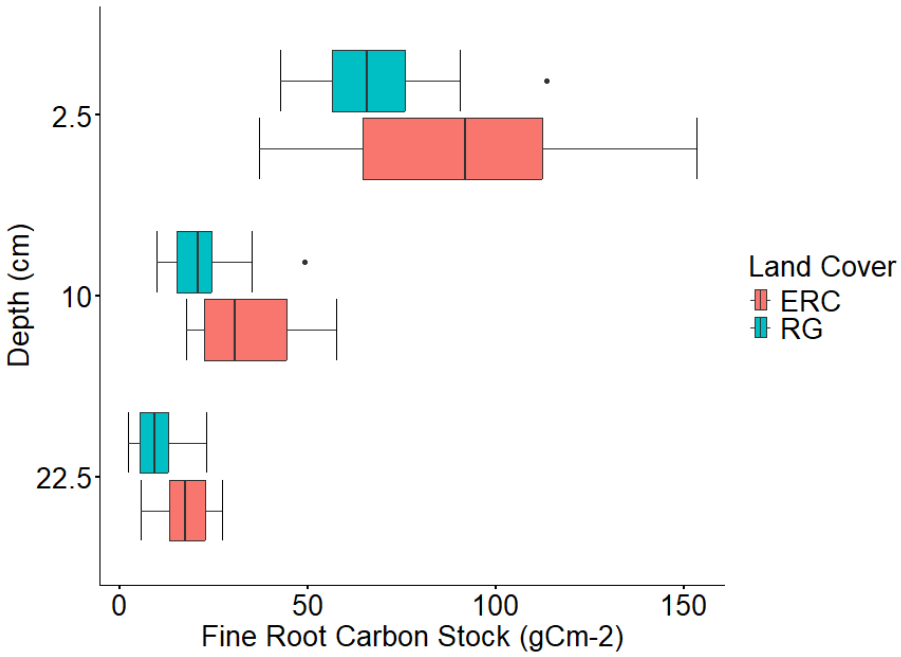
Across all depth intervals, root $\delta^{13}C$ values for ERC plots were significantly more negative in comparison to RG plots ($p < 0.001$) (Table 2, Fig. 7). The ERC fine root $\delta^{13}C$ values ranged from –24.5‰ to –28.1‰ across all depths. Fine roots under RG plots, however, exhibited a much larger range in values from –15.0‰ to –25.7‰, illustrating a mixture of C_3 and C_4 plants growing in the RG. There was no significant difference in $\delta^{13}C$ values with depth beneath ERC or beneath RG plots.

Fig. 7: Box plots of (a) fine root weight percent carbon, (b) fine root carbon stock, and (c) fine root $\delta^{13}C$ values for RG and ERC plots by soil depth. Mean values are provided in Table 2, columns 3, 5, and 6

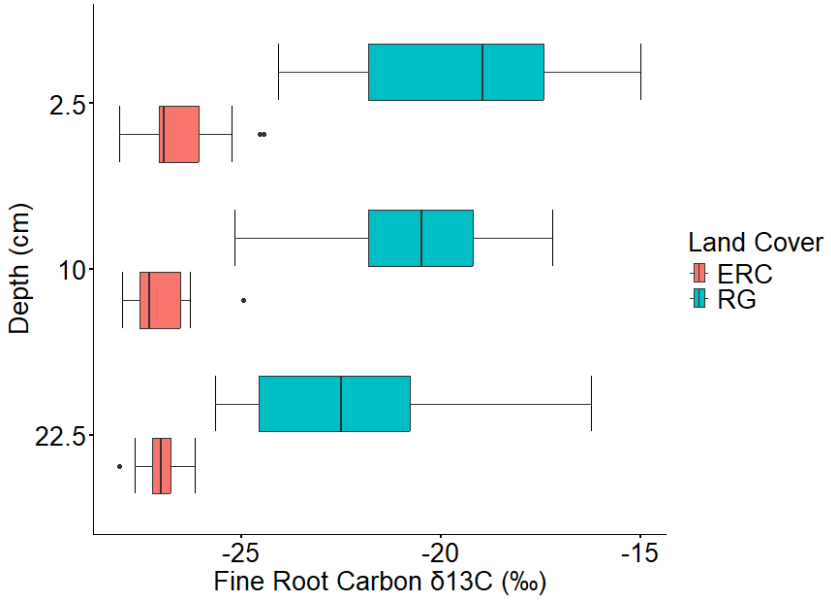
a.)



b.)



c.)



3.3.4. Eastern red cedar and remnant grassland plot living needle, shoot, and surface litter:

Surface litter mass and chemistry: The ERC plots had significantly higher surface litter mass ($p < 0.001$) (Table 4). There was no significant difference between ERC and RG plots in terms of wt %C. However, ERC plots had a significantly higher surface litter carbon stock than RG plots ($p = <0.001$). Lastly, there was no significant difference between the C:N ratios in the ERC and RG surface litter.

Surface litter $\delta^{13}C$ values: Surface litter under ERC exhibited significantly lower $\delta^{13}C$ values than RG ($p<0.5$) (Table 3). Specifically, RG surface litter $\delta^{13}C$ values, which included live and dead shoots, ranged from –13.1 ‰ to –15.6‰ with a mean value of –15.3 ± 1.5 ‰ (Table 3). The ERC litter $\delta^{13}C$ values ranged from –26.7‰ to –28.9‰ with a mean value of –27.4 ± 0.5‰.

A sampling of fresh ERC needles from 5 trees scattered among the plots gave an average value of –30.0‰ (Table 3). Five samples of little bluestem (*Schizachyrium scoparium*) (C_4) were sampled from nearby plots and showed an average value of –13.7‰ (Table 3). Four samples of Heller's rosette grass (*Dichanthelium oligosanthes*) (C_3) were sampled from nearby plots and showed an average value of –28.5‰ (Table 3).

Table 3: Above-ground litter biomass, carbon stock, C:N, and $\delta^{13}C$ values of eastern red cedar (ERC) and remnant grassland (RG) plots

Land Cover	Litter Biomass (gm-2)	Litter Weight Percent Carbon (%)	Litter Carbon Stock (gCm-2)	Litter C:N Ratio	Litter $\delta^{13}C$ (‰)	C_4 & C_3 Grass and C_3 Needle $\delta^{13}C$ (‰)
RG	466.36 * (±193.13)	38.0 (±2.0)	176.24 *** (±71.25)	46.8 (±11.4)	-15.3 * (±-1.5)	C_4: -13.7 (±0.29) C_3: -28.5 (±0.36)
ERC	3060.86 * (±21.20)	35.9 (±3.0)	1099.72 *** (±90.11)	41.2 (±4.9)	-27.4 * (±-0.5)	-30.0 (±0.58)

*The reported values above show the means of fifteen field replicates (standard deviation of the mean). Significance values were determined using a Wilcoxon Rank-Sum test with the significance value set at $p < 0.05$, with *= $p<0.05$, **=$p<0.001$, and ***=$p<0.0001$. Significance values should be read by each sampling depth within its column. Significance values comparing tree to tree and grass to grass values are not represented in this table.*

3.3.5. Using $\delta^{13}C$ values of living needles and grass shoots to estimate $\delta^{13}C$ values in roots:

Because root tissue in our system could be a combination of C_3 and C_4 plants, we could not assume the measured bulk root tissue under the ERC reflected endmember ERC inputs, even after 25 yrs. As the root $\delta^{13}C$ isotope values for RG plots range from -15.0‰ to -25.4‰, there was a clear mixture of C_3 and C_4 inputs (Table 2). Likewise, the ERC roots ranged from -24.5‰

to -28.1‰, suggesting there was some small proportion of C_4 still remaining or newly added (Table 2). We calculated "idealized" ERC and C_4 root isotope endmember values by taking the C_3 fresh needle and C_4 grass values (Table 4) and adding the +1.2‰ discrimination value for the C_3 fresh needle and +0.3‰ discrimination value for the C_4 grass (Werth and Kuzyakov, 2010) in an attempt to estimate the proportion of C_4 roots remaining under the ERC plots. We used these calculated root values to drive a two-end-member mixing model to estimate the proportion of C_4 fine roots beneath the ERC (Eq. 9 and 10). By estimating these root isotope values, we can make better predictions about changes to soil carbon storage later on.

Using equation 12 (see Chapter 2), the fraction of C_4 root carbon (FC_{root}) was estimated for RG and ERC plots. FC_{root} values were calculated using a pairwise analysis approach (i.e. ERC and the paired RG isotope values were used for each calculation). The tree C_3 $\delta^{13}C$ end member was estimated as –27.3‰ based on the root-to-shoot fractionation of ERC as described in Chapter 2, equations 13 and 14. The C_4 $\delta^{13}C$ root endmember was estimated to be –13.4 ‰. The FC_{root} ranged from 11-15% beneath ERC plots, while RG plots ranged from 35-56% (Table 4). With depth, the FC_{root} did not differ for ERC plots (Table 4). However, for RG, the FCroot decreased with depth (Table 5).

The fraction of C_4 litter (FC_{litter}) beneath ERC trees and in RG plots was estimated using equation 12 (Chapter 2). For ERC plots, the C_3 endmember was from fresh ERC needles (–30 ‰), and the C_4 endmember was from the average of 5 little bluestem (*Schizachyrium*

scoparium) samples (-13.7‰). To more accurately assess the fraction of C_4 plants in RG plots, a C_3 grass endmember was used rather than the ERC fresh needle endmember. For RG plots, the C_4 litter endmember was the same as for the ERC plots (-13.7‰), while the C_3 endmember was from the average of 4 Heller's rosette grass (*Dichanthelium oligosanthes*) samples (-28.5‰). The average FC_{litter} litter under the ERC canopies was 16 ±0.03 % (Table 5) with a range of 7-20%. Remnant grassland above-ground litter was found to have an average FC_{litter} of 89 ±0.10% with a much wider range of 64-104% (Table 4).

Table 4: Average fraction of C_4 roots and litter in eastern red cedar (ERC) canopies and remnant grassland (RG) plots

Depth (cm)	Fraction of C_4 roots beneath ERC canopies using idealized root values (%)	Fraction of C_4 roots in RG plots using idealized root values (%)	Fraction of C_4 litter beneath ERC canopy (%)	Fraction of C_4 litter in RG litter (%)
0-5	15 (±0.07)	56 (±0.20)	16 (±0.03)	89 (±0.10)
5-15	12 (±0.05)	48 (±0.17)		
15-30	11 (±0.03)	35 (±0.19)		

3.4. Discussion:

3.4.1. Root input chemistry, stock, and depth distribution are significantly altered under ERC growth:

Encroachment of ERC into the degraded grassland at KAEFS was accompanied by significant changes to fine root carbon stock, depth distribution, and chemistry after ~25 years of tree growth. These changes in biomass C stock (overall 42% increase) were most evident in the 5-15 and 15-30 depth increments, demonstrating that ERC shifts more root residue to lower

depth intervals than what is found in the RG (a 55% increase in the 5-15 cm depth increment and a 70% increase in 15-30 cm depth increment). Our study demonstrated that there was a significant increase in the average fine root biomass for all 30 cm of soil after encroachment by ERC plots (320 ± 105 gm-2) in comparison to remnant grassland plots (239 ± 65.2 gm-2) (Table 3). Our findings match those of research in the region that analyzed the impact of other encroaching trees (Boutton et al., 1999; Archer et al., 2001; Hibbard et al., 2001; Jackson et al., 2002). For example, Jackson et al. (2002) found that at paired grassland and invaded tree sites with trees such as *Prosopis* (mesquite), *Larrea* (creosote), and *Juniperus* (juniper) spp., mean 95% fine root depth increased on average by 2 m or more across a precipitation gradient in grasslands in New Mexico, Colorado, and Texas. Jackson et al. (2002) may have shown a larger difference in rooting depth because the soil profile reached a depth of at least 10 m, while our soil profile is only ~30 cm in depth above bedrock. Hibbard et al. (2001) compared monthly fine root biomass growth at grassland and *Prosopis grandulosa* (honey mesquite) sites in the top 10 cm of a soil profile in a subtropical savanna at the La Copita Research Area (LCRA) in south Texas. They found that monthly fine root biomass increased across all grove, woodland, and discrete mesquite cluster sites (Hibbard et al., 2001). Discussion of rooting depth after WPE is limited in that study, as only the top 10 cm of the soil were sampled.

Our work is one of a few studies reporting data on changes in chemistry, stock, and distribution of roots with ERC encroachment into grasslands. McKinley (2006), exploring ERC encroachment into the northern Flint Hills region of Kansas, found no statistical difference in total fine root biomass in the upper 10 cm of soil beneath ERC plots (562 ± 167 gm-2) in comparison to adjacent native grassland plots (482 ± 80 gm-2). The study differed from our work in that the ERC trees averaged 35-55-year-old and occurred in contiguous forest cover. The

grasslands in Kansas were relatively high in carbon from the Haplustol and Argiustoll soil series and might promote better rooting overall. Our lower root biomass values for the grassland plots in comparison to the values found by McKinley may also be the result of different methods of sampling. McKinley (2006) extracted 25 x 25 cm and 10 cm deep soil monoliths for the grassland plots and estimated ERC root biomass by calculating 25%-40% of above-ground biomass, while we took soil cores and hand collected fine roots. Additionally, our plots at KAEFS were located in a highly degraded grassland system that has undergone significant erosion because of overgrazing and intensive tillage (McDonald, 1938; Buthod and Hoagland, 2016), so the soils were very shallow. Grasslands impacted by grazing and agriculture have been found to have shorter rooting systems than those of relatively undisturbed grassland systems (Hauser et al. 2022), like those investigated in the McKinley et al. study.

We found that grassland soils at KAEFS exhibit decreasing root biomass with depth is consistent with the literature and known root physiology of grasses. Globally, grass and tree root mass decrease exponentially with depth (Jackson et al., 1996). Nippert et al. (2012), studying C_4-dominant grasslands in the tall grass prairie in eastern Kansas at the Konza Prairie Biological Station found that most of their fine root biomass was concentrated in the upper 20 cm of the soil and decreased exponentially with depth to 150+ cm with both grazed and ungrazed treatments. Eastern red cedar trees are known to have a shallow, fibrous rooting system with large tap roots at deeper depths with maturity (Walker, 1967), and in shallow soils tend to have a more shallow, fibrous rooting system than in deep soil profiles (Lawson, 1990). The results from this study suggest that the ERCs at our site have large, fine-rooting systems at shallow depths and outcompete grassland fine root biomass at deeper depths. However, the sampling methods for fine roots in this study are just a coarse estimate of fine root biomass and are biased against very

fine roots, which are difficult to hand-pick. Studies utilizing root ingrowth cores and rhizotrons may be a better option to fully characterize rooting profiles (Makkonen and Helmisaari, 1999; Madji et al., 2005).

Our findings that the fine root carbon content was significantly higher beneath ERC plots (average 44.3 wt %C) in comparison to RG plots (average 42.6 wt %C) is consistent with root physiology as ERC roots have higher lignin content, a C-rich biomacromolecule (Kogel-Knabner, 2002), than grass roots (Norris et al. 2001a). This distinction in chemistry has implications for root decay rates and chemical persistence as ERC roots have been found to persist longer in soils in comparison to grass roots – a feature attributed to higher lignin content (Norris et al., 2001a). Therefore, the increase in fine root biomass observed in our study may have implications for below-ground biomass turnover and conversion to microbial biomass and soil organic matter. However, an assessment of live versus dead roots was not conducted in this study, so we cannot directly differentiate whether the increase in fine root biomass was due to increases in live fine roots or if the roots from ERC decay more slowly than grassland roots. Certainly, the degradation of fine roots by soil microbes contributes to the formation of mineral-associated and particulate soil organic matter (Six et al., 2004; Liang et al., 2017; Bai and Cotrufo 2022), so changes in the relative decay rates and quality of rooting structures may alter the relative stability of the carbon in the system.

3.4.2. Eastern red cedar increases surface litter accumulation above remnant grasslands:

Our study found that after ~25 years of woody plant encroachment by ERC, there was a 556% increase in surface litter biomass (3060.86 ±21.20 gm-2) in comparison to RG plots (466.36 ±193.13 gm-2), respectively, an increase on average of 923.48 gC/m^2. Our findings are consistent with other regional studies on ERC encroachment that explored litter standing stock

and litter fall. A study conducted in the Flint Hills region of Kansas found that litterfall annually beneath ERC canopies averaged 500 gm-2yr-1 with a seasonal high litterfall in July, while grassland plots averaged 52gm-2yr-1 (Norris et al., 2001a). In a mixed scotch pine and eastern red cedar shelterbelt, Sauer et al. (2007) found that the litter biomass beneath the trees ranged from ~1000 gm-2 to 8000 gm-2, with lower masses of litter being at the edges of the canopy line. However, litter mass can be highly variable. For example, among 9 sites across a precipitation gradient in Oklahoma, litter mass beneath ERC was found to be 7.60 g m-2 and 1.86 g m-2 for adjacent grassland sites in tree stands that ranged in age from 10 to 100 years old (Sams, 2025). The litter mass found by Sams (2025) is much lower than other estimates in the region, but we don't know the state of the vegetation production of the trees or if there were erosional losses. Increases in above-ground litter biomass by ERC may be attributed to ERC's higher annual above-ground net primary productivity and slower needle decomposition rates in comparison to grass shoots in grasslands (Norris et al., 2001a; Norris et al., 2001b).

Our study also found no difference in the median C:N ratio of ERC litter (average= 41.2 (±4.9)) and RG litter (average= 46.8 (±11.4)), although the range of values found for the grassland was much larger. As the density and species distribution and diversity of grasses in RG vary by season, geography, and edaphic properties and timing of sampling (Epstein et al., 1998; Balasko and Nelson 2003), one might expect that C/N ratios found among grasslands will vary widely. Therefore, it is not surprising that our comparison of ERC and grassland C/N ratios at KAEFS may differ from other studies. Other regional studies have found that ERC litter layers had significantly lower C:N ratios in comparison to grassland species (Norris et al., 2001a; Mellor et al., 2013). The Norris et al. (2001a) study in the Flint Hills region of Kansas found that the C:N ratio of ERC foliage was significantly lower (51.9 +- 0.98) than big bluestem foliage

(69.9 +- 0.81). Similarly, our findings differ from a study conducted in a sand prairie in Nebraska, where the authors found that the C:N ratios of 55 and 72-year-old eastern red cedar litter were 25.7 and 33.8, respectively, while the prairie litter had a C:N ratio of 36.8 (Mellor et al., 2013). Potentially, our results may differ from Norris et al. (2001a) as our grassland litter samples were a mixture of C_3 and C_4 grasses and forbs and included live and dead samples, while Norris et al. (2001a) only analyzed one species of grass (big bluestem). As we found no change in the C:N ratios of ERC in comparison to RG, initial decomposition rates should not be impacted by litter nutrient content (Schmidt et al., 2011). However, although not expressly measured in this study, other components of ERC litter, such as tannins and aliphatic compounds, may slow decomposition rates (Kogel-Knabner, 2002; Filley et al., 2008).

3.4.3. Stable carbon isotopes of litter and roots track changes in landscape cover:

Upon collection of fresh needles and surface litter from ERC plots, this study found a range of -26.7 to -30.0‰. Previous studies on ERC in the Southern Great Plains (Smith and Johnson, 2003; Mellor et al., 2013) found that the $\delta^{13}C$ values for ERC litter and needles in the range of -26.6‰ to -27.9‰, which closely matches our values and is what is expected for a C_3 plant. Smith and Johnson (2003) also found that the $\delta^{13}C$ values of ERC roots averaged at –26.0 (± 0.1) ‰. The range in the $\delta^{13}C$ values of ERC needles demonstrated here can be due to the variation that occurs seasonally with wet and dry periods. For C_3 plants, there is a linear relationship between water use efficiency (WUE) and isotope discrimination at the leaf (Condon, 2004). During short-term, hot, dry periods, C_3 plants close their stomata, which decreases discrimination against ^{13}C and increases the $\delta^{13}C$ value of the plant tissue (Obidiegwu et al., 2015; Domergue et al., 2022). On the other hand, when C_3 stomata are open, the $\delta^{13}C$ value will

be more negative as there is higher water exchange with the environment (Condon, 2004). With this in mind, we can interpret the findings of stable isotope analysis at KAEFS.

3.4.4. $\delta^{13}C$ values of grassland litter and roots exhibit a complex C_3 and C_4 assemblage across KAEFS:

The root $\delta^{13}C$ isotope values for grassland plots range from -15.0‰ to -25.4‰, showing a larger range in values for RG plots than ERC (-24.5‰ to -28.1‰) (Table 3). The grassland above-ground litter $\delta^{13}C$ values were closer to end member C_4 values (-13.7‰), but the roots represented a mixture of C_3 and C_4 (Table 3). There are many possible explanations for this, but two of the most likely are: 1.) seasonal shifts in the proportions of C_3 and C_4 grasses and forbs produce different amounts of root tissue, or 2.) ERC root intrusion into the RG plots. Surface litter values are biased towards C_4 because of the time we sampled, while root samples are representative of an annual accumulation of grassland material. In the Southern Great Plains, grassland species composition shifts in proportions of cool-season grasses (C_3) and warm-season grasses (C_4) based on seasonality, where cool-season grasses dominate in the spring and cool months of fall, while warm-season grasses dominate in the summer months (Balasko and Nelson 2003). In May 2024, when we took our litter and root samples, C_4 grasses were at their growing peak in the Southern Great Plains (Balasko and Nelson 2003). The time between the transition from cool to warm season grasses and the time of our sampling is much shorter than the estimated fine root turnover times. Estimates of fine root turnover in temperate grasslands vary, with one study estimating about 53% of fine roots in a grassland ecosystem decompose annually (Gill and Jackson 2000), a second estimating ~24% decomposing annually (Solly et al. 2014),

and another estimating that fine roots in grasslands take about 2.9 years to decompose (Wang et al. 2019). On the other hand, a global metadata analysis found that above-ground litter takes between 1.4 to 3.4 years to turnover (Matthews, 1997). Therefore, the roots collected in the RG site include a representative mixture of the C_3 and C_4 grasses present throughout the year while the surface litter is only a snapshot of the plant assemblage at the time of sampling and is subject to grazing pressures by deer and other herbivores occupying KAEFS. Sampling of surface litter should happen monthly to track variation in C_3 and C_4 plant assemblage. A second explanation could be that our RG plots were too close to the ERC fine rooting system. ERC roots can grow laterally up to 6 m (Lawson 1986), and our RG plots were placed at ~5m away from the ERC plots. There is a possibility that some ERC fine roots have intruded into our RG plots. Additional inspection of root morphology would be needed to quantify woody plant ERC roots from grasses.

3.4.5. ERC encroachment replaces remnant grassland roots and surface litter by 25 years:

The $\delta^{13}C$ values of the fine roots beneath the ERC plots were found to be similar to ERC roots in the region (-26.0‰) (Smith and Johnson, 2003), indicating that the fine roots beneath the tree canopies are mostly C_3 in origin at all soil depths after ERC encroachment (Table 3). Similarly, surface litter beneath $\delta^{13}C$ values are similar to ERC needle $\delta^{13}C$ values in the region (-26.6 to –30.0‰) (Table 4), supporting the finding that ERC reduces grassland species biomass beneath the canopy (Engle et al., 1987; Smith and Stubbendieck, 1990; Briggs et al., 2002; Limb et al., 2010; Domeier et al., 2025). Our results are not surprising, as ERC have been shown to significantly increase ANPP, with 7,250 to 10,440 kg/ha/yr of ANPP in comparison to adjacent grassland plots, which had about 3,690 kg/ha/yr (Norris et al., 2001b). In tandem with increased

ANPP, ERC litter has been shown to decompose at a slower rate than big bluestem in the Flint Hills region of Kansas (Norris et al., 2001a). Increased ANPP, allelopathy, and slower decomposition rates likely explain why there is little isotopic evidence of C_4 grasses after ~25 years of WPE by ERC.

Using the approach described in section 3.2.5., we estimated that by 25-30 years of growth, C_3 roots, likely of ERC origin, have replaced 85-89% of all C_4 roots (Table 5). Similarly, on average, ERC surface litter replaced ~84% of RG surface litter (Table 5). Replacement of C_4 roots beneath ERC can be caused by a variety of reasons such as root mean residence time (Wang et al., 2019), allelopathic suppression of grassland species by ERC root and litter leachates (Stipe and Bragg 1989; Corbett and Lashley, 2017; Bennion and Ward 2022; Bennion and Ward 2023), and shading from the canopy (Smith and Stubbendieck 1990; Pierce and Reich 2010). Fine root mean residence time of grassland species is estimated to be around 3.1 years globally using structural equation modelling (Wang et al., 2019). By 25-30 years of ERC encroachment, any remaining grassland roots should have been decomposed and turned over, assuming that no grass species are continuing to grow beneath the canopy and that there is no lateral intrusion of grassland roots beneath the canopy.

Secondly, ERC roots and litter have been shown to have allelopathic effects on grassland species (Stipe and Bragg 1989; Bennion and Ward 2022; Bennion and Ward 2023), although results have been mixed. For example, Corbett and Lashley (2017) found that there was no evidence of allelopathic suppression on species such as *Schizachyrium scoparium, Sorghastrum nutans, Chasmanthium latifolium, Chamaecrista fasciculata*, and *Rudbeckia hirta,* while another study found that only C_3 grasses were affected by phytotoxins (Bennion and Ward 2022). As this

grassland is largely composed of C_4 grasses, allelopathic suppression may not be the main driving factor for the reduction of grassland biomass beneath the canopy, but ERC phytotoxins may still impact microbial species composition and efficiency. Lastly, changes to the soil microclimate by ERC canopies likely strongly influence grass species suppression. Studies in the region have found that changes in lighting (Smith and Stubbendieck 1990; Pierce and Reich 2010), soil moisture (Pierce and Reich 2010), and temperature (Wang et al. 2021) under ERC canopies reduce species richness and composition. A combination of the above drivers likely suppressed grassland species growth beneath the canopy and limited the input of new grassland roots, allowing ERC roots to replace C_4 roots.

Visual inspection, root isotope data, and litter isotope data all confirm that there was little to no grass species growth beneath the ERC tree. However, our mass-balance equation estimated that only about 84% of the surface litter beneath ERC canopies was from ERC. Although we used the same approach to calculate the replacement of surface litter as we did for the roots, the surface litter equation better demonstrates the fractionation associated with litter and decomposition and the leaching of isotopically depleted tannins. Caution must be taken when assessing isotope effects related to decomposition as it can cause both ^{13}C enrichment and depletion with respect to the original tissue. For example, decomposing litter material can have a 2-6‰ depletion in $\delta^{13}C$ values compared to fresh plant material (Preston et al., 2006; Osono et al., 2008) due to a build-up of lignin and reduction of carbohydrates. However, leaching of sluff of waxes can selectively remove those same 13C depleted chemicals, causing an enrichment in ^{13}C of the litter (Conte et al., 2003; Bi et al., 2005). For this reason, using equation 9 to assess C_4

litter in the needles is a misapplication of that equation because we are actually measuring the shift in fresh needles to litter rather than the amount of C_4 grasses.

The replacement of grassland organic matter by ERC organic matter may have implications for soil carbon storage in this region. KAEFS is located in Central Oklahoma, where the climate is humid subtropical. Ecosystems located in the Great Plains have been shown to have the highest carbon storage potential after woody plant encroachment due to proper precipitation conditions (Barger et al. 2011). With such an increase in organic material input mass and with the right climatic conditions, soil carbon storage may increase at this site after ERC encroachment. However, the long-term stability of new carbon inputs should be further investigated with tree age in this region using methods such as MAOM and POM separation and aggregate stability studies before more conclusions can be made about the carbon storage potential at this site, especially since there are major shifts from below-ground inputs to above-ground inputs.

3.5. Conclusions:

The introduction of ERC into a remnant grassland has shown to increase above-ground litter mass and fine root litter mass inputs. ERC roots may contribute to total organic carbon input into the soil and may be a more stable source of carbon as it decays at a slower rate than grassland inputs. However, the shift to primarily above-ground litter inputs may decrease the overall stability of the carbon in this region. Stable carbon isotope values have shown that after 25-30 years of WPE by ERC that about 90-98% of the fine root biomass beneath the canopy is of C_3 origin. Replacement of grassland roots beneath the canopy may be due to suppression of grass species via allelopathy and changes in soil climate, microbiome, or faster turnover of grassland

roots. Dynamic changes to the soil microclimate, microbiome, and litter inputs may have implications for decomposition rates and carbon cycling, so further studies are needed to make better predictions of carbon storage in this region.

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4. CHANGES IN SOIL CARBON DYNAMICS IN A CENTRAL OKLAHOMA DEGRADED GRASSLAND ECOSYSTEM AFTER WOODY PLANT ENCROACHMENT BY EASTERN RED CEDAR (*Juniperus virginiana*)

4.1. Introduction:

Woody plant encroachment is an issue that is affecting grasslands across the globe (see review in chapter 1). There has been much work done to quantify the effects of WPE on SOC (Boutton et al., 1998; Archer et al., 2017; Li et al., 2023). However, because SOC dynamics are dependent on many factors, such as climate (Parton et al., 1987; Barger et al., 2011), microbial community activity and function (Liang et al., 2007), input chemistry (Schmidt et al., 2011; Filley et al., 2008a), and soil texture and mineralogy (Parton et al., 1987; Lehmann and Kleber, 2015), the effects of WPE on SOC storage are not readily generalizable (Barger et al., 2011). Studies have found that more arid soils, for example, accumulate more carbon after WPE in comparison to wetter soils (Jackson et al., 2002), while another work found areas of higher precipitation are more likely to be areas of higher carbon storage (Barger et al., 2011). More high-resolution studies are needed to understand the range of impacts to SOC by WPE across environments and land use histories.

Significant work has been done to document woody plant alterations to soil structure and partitioning of SOC within soil particles and with depth. For example, soil bulk density has been found to decrease after encroachment by *Prosopis grandulosa* (Hibbard et al., 2001; Boutton and Liao, 2010; Zhou et al., 2017) and *Juniperus virginiana* (Sauer et al., 2007, 2023; Zou et al., 2014). Liao et al. (2006b) found that soil aggregation increased after WPE by *Prosopis grandulosa*, causing a decrease in bulk density and indicating a potential shift from more stable mineral and microaggregated carbon forms to a less stable particulate, or non-mineral associated, form of carbon storage. Soils high in bulk density are also found more likely to lose more SOC

with WPE than those with lower bulk density (Barger et al., 2011). However, in a recent global analysis, Sha et al. (2025) found that WPE had no effect on bulk density, while some have found an increase in bulk density with WPE (Throop et al., 2013). Other studies have found an increase in soil aggregation by WPE (Sauer et al., 2023), which can increase water flow and increase carbon cycling dynamics, leading to more aggregate-based protection (Six et al., 2004). Despite work being conducted on the factors contributing to changes in SOC storage, it is still not widely understood how the early stages of woody plant establishment influences soil carbon dynamics.

Due to differences in photosynthetic and water efficiency strategies, C_4 and C_3 plants differ in $\delta^{13}C$ values. C_3 plants, in general, range from –20‰ to –35‰, while C_4 plants range from –9‰ to –17‰ depending on climate of growth (Smith and Epstein, 1971). Fractionation of stable carbon isotopes in photosynthesis starts with CO_2 diffusion into the stomates, which causes a –4.4‰ discrimination in comparison to the atmosphere (-8‰), meaning that CO_2 internal to the leaf can have an instantaneous $\delta^{13}C$ value of around –12‰ (Hubick and Farquhar, 1989). However, in C_4 plants there is little additional discrimination against ^{13}C because C_4 plants isolate the Calvin Cycle into the bundle sheath cell, which prevents constant diffusion of CO_2 from in and out of the stomates (Farquhar 1983). Because of this, C_4 plant ^{13}C values do not change much from the initial –4.4‰ discrimination, hence their mean value of around –12‰. C_3 plants use Rubisco to fix carbon in the mesophyll cells during the first step of carbon fixation, which causes up to a –29 ‰ fractionation based on kinetic isotope effect of Rubisco on top of the –4.4 ‰ (Roeske and O'Leary, 1984). Actual variations in the $\delta^{13}C$ of plants are impacted by water stress, changes in atmospheric CO_2 (known as the Seuss Effect), and light availability (Hubick and Farquhar, 1989), and other limitations of photosynthetic rate. Importantly, with

respect to the study of C_3 woody plants (e.g. from *Prosopis Juniperus* species) and encroachment into predominantly C_4 grasslands of the southern great plains, this difference in the $\delta^{13}C$ value of the plant input can be harnessed to track shifts in SOC allocation and quantify change in litter (Chapter 3 of this theses) and SOC stability and source.

The principal source of annual input of carbon for soil organic matter (SOM), other than eroded or weathered geogenic carbon such as shales, is recent plant material, so $\delta^{13}C$ values of soil fractions are representative of those local vegetative inputs over the timeframe of soil evolution (Boutton et al., 2009). This is true also for carbon that has been converted to microbial residues and stored in the soil (Creamer et al., 2016). Therefore, land cover changes from primarily C_4 inputs to C_3 inputs, such as by WPE into the Central and Southern Great Plains, can be tracked by measuring the $\delta^{13}C$ values of inputs and soil fractions. There are also minor fractionations in ^{13}C with microbial decomposition (generally on the order of 1-2‰), so with increasing soil depth, as the plant contribution progressively shifts to microbial tissue and high processed plant residues, SOC generally show a shift to higher values (Ehleringer et al., 2000). Importantly, the difference in $\delta^{13}C$ value between C_3 and C_4 plants (up to ~ 14‰) is large enough to be able to track changes in vegetation cover and SOC storage (Boutton et al., 2009). This difference in the $\delta^{13}C$ values of plant inputs also allows for an assessment of depth-related influence of WPE into C_4-dominant grasslands (Boutton et al., 1998). With increasing depth, there is less influence by recent vegetation, so there is a depth horizon where no shifts in ^{13}C between remnant grasslands and those influenced by WPE, as was demonstrated by Mellor et al. (2013) in a study of *J. virginiana* encroachment in grasslands in Nebraska. A two-endmember mixing model using the $\delta^{13}C$ values of source plants can be utilized to estimate the fraction of

remaining C_4-dominant grassland carbon (Boutton et al., 2009) still under the woody plant, as has been demonstrated at field sites across the Southern Great Plains (e.g. Jessup et al., 2003; Smith and Johnson, 2003; Liao et al., 2006a,b; Wheeler et al., 2007; Spencer et al., 2009; Mellor et al., 2013; Throop et al., 2013). Many such studies often find an increase in total SOC, derived from C_3 woody plants, and a concomitant decrease in grassland-derived carbon as grass plant input ceases at some point during the WPE due to chemical or biological suppression of the grasses in the woody plant canopy (Jessup et al., 2003; Liao et al., 2006a,b; Wheeler et al., 2007; Spencer et al., 2009; Throop et al., 2013). However, isotope modeling has revealed other potential scenarios, including no net increase in SOC but a shift to more negative $\delta^{13}C$ (Smith and Johnson, 2003) or a gain in SOC with no loss of prairie C. Such isotope modeling can be approached in a number of ways, considering potential differences in woody plant source (e.g. root or surface litter) or difference in ways of parameterizing the RG soil carbon (i.e. Smith and Johnson 2003; Liao et al., 2006b; Creamer et al., 2011). For example, Smith and Johnson (2003) used woody plant C_3 input to the soil as a calculated average of the combination of fresh foliar needles and roots in contrast to others studies that used a mixture of surface litter (partially decayed leaves under the tree) and roots isolated from soil (Liao et al., 2006b). Depending on what input terms are used in the isotope mixing model, it is not always clear if published estimates of remaining grassland carbon are being over or underestimated. There are currently no studies analyzing the differences between distinct endmember methods.

Soils not only contain organic carbon but also inorganic soil carbonates that can confound isotope studies if they are not removed from the total carbon in the analysis of the stable carbon isotope composition of soils (Harris et al., 2001). Soil carbonates can have origins from direct input from limestone in bedrock or from dust or bedrock, formed by the interactions

of soil water, calcium and magnesium in soil and soil CO_2 derived from root and microbial soil respiration (Mook et al., 1974). Carbonic acid (H_2CO_3) is formed when CO_2 is introduced to the soil matrix from the atmosphere and microbial or root respiration mixes with water, and this acid can react with soil or bedrock, forming soil carbonates ($CaCO_3$) (Richter and Markewitz, 1995). Continuous interactions at the soil-water interface can cause the dissolution of existing inorganic carbon, movement of dissolved ions, and re-precipitation of carbonates in a different horizon, which is directly influenced by soil organisms and plants, precipitation, pH, soil texture, and parent material (Zamanian et al., 2016). Specifically, WPE can influence the formation of soil carbonates via enhanced weathering, increases in rooting depth, and by associations with mycorrhizal fungi (Thorley et al., 2015; Sullivan et al., 2019; Wen et al., 2020; Leite et al. 2023). Additionally, grasses are not known to build carbonates at their rooting structures (Zamanian et al., 2016). Therefore, conducting studies that track changes in inorganic carbonates after WPE is important because WPE can be an important source or sink of inorganic carbon.

The origins of soil inorganic carbon (SIC) can be estimated using $\delta^{13}C$ values. Lithogenic carbonates are defined as carbonates that originate from primary calcareous parent material, and their $\delta^{13}C$ values range from –2 to +2‰ (Ramnarine et al., 2011), reflecting limestone origins and equilibrium fractionation effects on CO_2 derived from air in water (Mook et al., 1974). Pedogenic carbonates, also known as secondary carbonates, can be formed from the dissolution of lithogenic carbonates and reaction with soil Ca^{2+}or through equilibrium between CO_2 from root and microbial respiration and water with subsequent precipitation with soil (Mook et al., 1974; Zamanian et al., 2016). The isotopic range of pedogenic carbonates is directly related to the isotopic composition of the CO_2 from the plant source and a fractionation based on equilibrium fractionation (Pendall et al., 1994). C_4 plant respiration (CO_2) averages –13‰

globally while C_3 plant respiration CO_2 averages –27‰ (Cerling et al., 1997), so pedogenic carbonates formed by plant respiration will reflect this range along with alterations made by soil fractionation processes. Carbonates can also be recycled through reprecipitation, which is associated with a temperature-dependent fractionation of ~9‰ (Mook et al., 1974). Additionally, a –4.4‰ fractionation from CO_2 gas diffusion in soil also should be considered in the overall calculation (Cerling et al., 1991). However, it is hard to predict how soil carbonates will change with a land cover change like WPE because the system is dynamic. Our primary goal is to quantify SOC source dynamics in a shallow degraded grassland ecosystem being encroached by eastern red cedar (*Juniperus virginiana*) using stable carbon isotopes. A secondary effort of this work is focused on estimating SIC changes and source.

Predictions:

3.) Soils under the ERC canopy will exhibit increased soil organic carbon stock and concentration with the greatest accumulations in the upper 5 cm and with diminished to non-measurable impact with increasing depth in comparison to remnant grassland plots. The increase in ERC-derived C will be from both litter and fine root input.

4.) The proportion of ERC-derived SOC will increase faster than the proportion of RG carbon decreases in the top 5 cm of the soil profile. As the soil reaches the bedrock, the proportion of ERC-derived carbon will be negligible, with SOC $\delta^{13}C$ values similar to the soils in the RG plots as there is insufficient time in 25 years to alter the deeper carbon pools through root direct deposition.

4.2. Methods

Site characterization, sampling, analysis, and data treatment protocols used to test hypotheses in this chapter are presented in Chapter 2.

4.3. Results

4.3.1. Soil Characterization:

Soils beneath the 15 eastern red cedar (ERC) and 15 remnant grassland (RG) plots at KAEFS did not significantly differ in percent sand, percent silt, or percent clay at any depth increment ($p > 0.05$) (Table 5), nor with depth within or between ERC and RG plots. For all depths, soil pH beneath ERC and RG plots did not differ significantly. Bulk density of the 0-5 cm depth under ERC was significantly lower compared to mean RG values ($p < 0.001$), but there was no significant difference in the 5-15 cm and 15-30 cm depth increments ($p > 0.05$). Bulk density for both ERC and RG plots increased with depth.

Textural properties are consistent with the SSURGO designation of the soils within this region which are characterized as Nash-Lucien complex (Soil Survey Staff a, n.d.). Nash-Lucien complex soils are defined as coarse-silty, mixed, superactive, thermic Udic Haplustolls and loamy, mixed, superactive, thermic, shallow Udic Haplustolls (Soil Survey Staff a, n.d.). Nash series soils are silt loams from the surface to the bedrock that can have calcareous deposits, which can range from 20-50% sand, ~75 to 95% silt, and ~0-75% clay. Lucien series soils are sandy loams from the surface to the bedrock, which can range from 50-70% sand, 0-50% silt, and 15-20% clay. Our soil texture matches closely with the expected soil texture (Table 5).

Table 5: Soil characterization beneath eastern red cedar (ERC) and remnant grassland (ERC) plots at three different soil depth increments

Depth	Land Cover	pH	Sand (%)	Silt (%)	Clay (%)	Bulk Density (gm-3)
0-5 cm	RG	7.04 (±0.85)	49 (±16)	31 (±10)	19 (±7)	1.03 ** (±0.11)
	ERC	7.21* (±0.49)	50 (±13)	30 (±8)	19 (±8)	0.90 ** (±0.76)
5-15 cm	RG	7.29 (±0.96)	46 (±18)	32 (±11)	22 (±9)	1.29 (±0.07)
	ERC	6.99 (±1.22)	49 (±16)	30 (±10)	21 (±9)	1.28 (±0.08)
15-30 cm	RG	7.71 (±0.78)	41 (±21)	34 (±14)	25 (±9)	1.45 (±0.15)
	RG	7.63 * (±1.02)	46 (±17)	31 (±13)	23 (±8)	1.49 (±0.09)

*The reported values above show the means of fifteen field replicates (standard deviation of the mean). Significance values were determined using a Wilcoxon Rank-Sum test with the significance value set at $p \leq 0.05$ with *= $p<0.05$, **=$p<0.001$, and ***=$p<0.0001$. Significance values should be read by each sampling depth within its column.*

4.3.2. Soil organic carbon content and stock

Across all soils sampled, average soil organic carbon (SOC) ranged from 0.67 wt % to 2.3 wt % and exhibited a decrease in concentration with depth ($p<0.5$) beneath both ERC and RG plots (Table 6). Under ERC, wt % SOC in the 0-5 cm depth interval was significantly higher than the equivalent depth in the RG plots ($p<0.05$) but indistinguishable between the two landcovers beneath 5 cm (Fig. 9). Soil organic carbon stock (Equation 8, Chapter 2) exhibited no significant difference between ERC and RG for any depth interval ($p>0.05$) (Table 6).

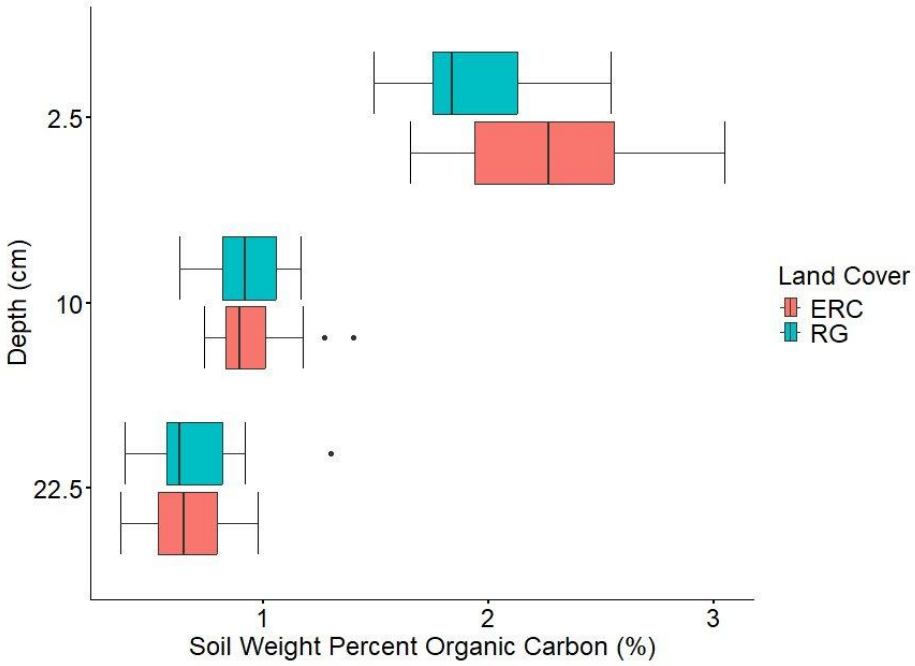
Table 6: Soil weight percent organic carbon, soil organic carbon stock, calculated soil inorganic carbon, and soil organic carbon $\delta^{13}C$ values at each sampling depth and land cover type

Depth (cm)	Land Cover	Soil Weight Percent Organic Carbon (%)	Soil Organic Carbon Stock (ton/ha)	Soil Organic $\delta^{13}C$ (‰)	Calculated Soil Inorganic Carbon (%)	Calculated Soil Inorganic $\delta^{13}C$ (‰)
0-5	RG	1.9 ** (±0.30)	9.7 (±1.7)	-18.3 *** (±1.5)	0.7 (±0.2)	-0.3 (±5.5)
	ERC	2.3 ** (±0.5)	10.2 (±1.6)	-21.0 *** (±1.3)	0.9	-2.9
5-15	RG	0.92 (±0.16)	11.6 (±2.4)	-15.9 *** (±0.8)	1.08 (±0.8)	-1.1 (±0.7)
	ERC	0.95 (±0.20)	12.2 (±2.8)	-17.1 *** (±0.9)	0.7 (±0.6)	-1.2 (±1.1)
15-30	RG	0.63 (±0.29)	14.5 (±3.5)	-14.9 (±0.6)	1.3 (±0.7)	-0.2 (±0.7)
	ERC	0.67 (±0.20)	15.0 (±4.1)	-15.1 (±0.6)	1.1 (±0.6)	-0.4 (±1.1)
Total	RG	n/a	35.9	n/a	n/a	
	ERC	n/a	37.3	n/a	n/a	

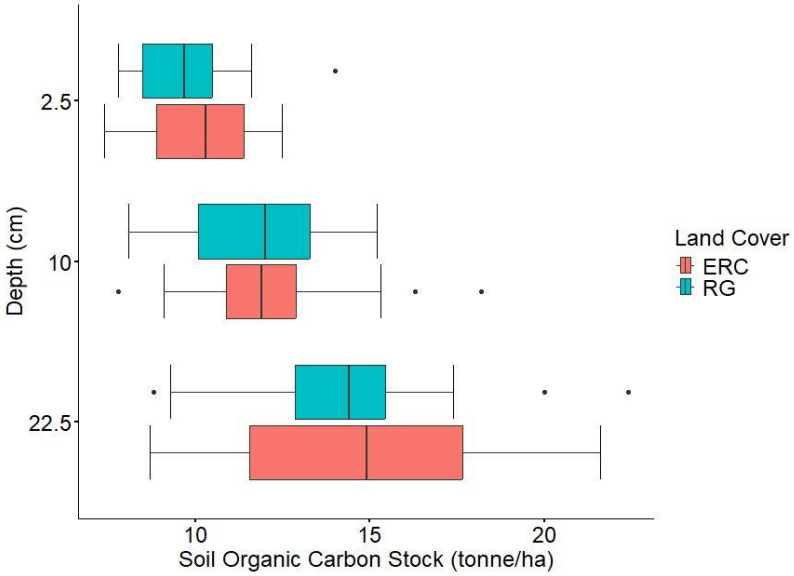
*The reported values above show the means of fifteen field replicates (standard deviation of the mean). Significance values were determined using a Wilcoxon Rank-Sum test with the significance value set at $p \leq 0.05$ with *= $p<0.05$, **=$p<0.001$, and ***=$p<0.0001$. Significance values should be read by each sampling depth within its column. BCL = below calculatable level for stable carbon isotopes.*

Fig. 8: Average weight percent SOC (a) and average SOC stock (b) with depth for eastern red cedar (ERC) and remnant grasslands (RG) at KAEFS for cores from 0-5 cm (avg 2.5 cm), 5-15 cm (avg 10 cm), and 15-30 cm (avg 22.5 cm)

a.)



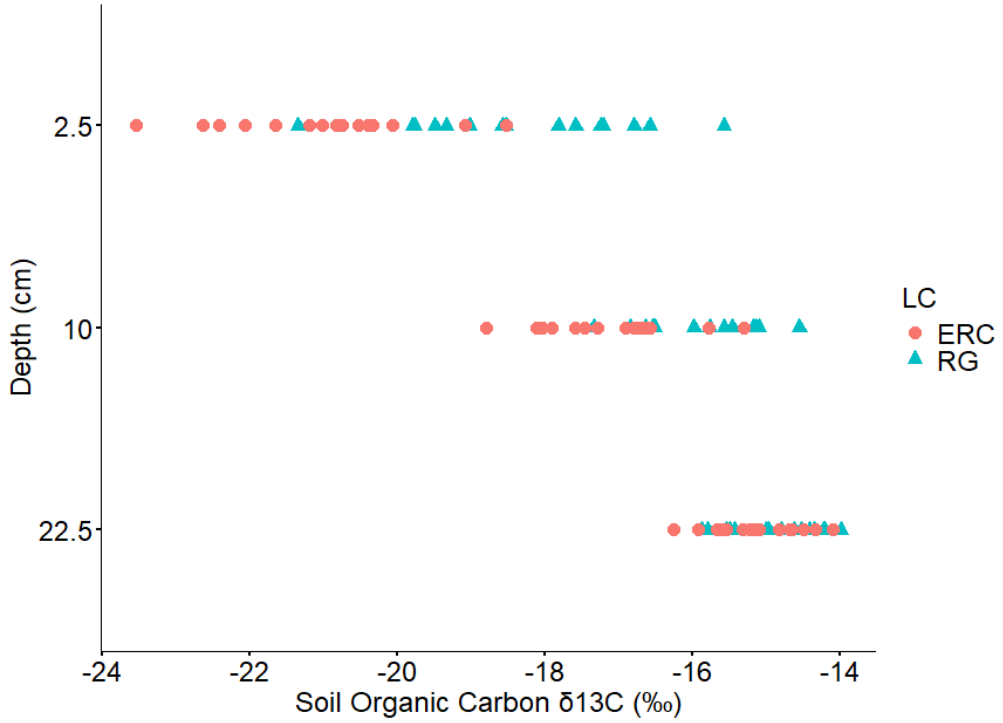
b.)



4.3.3. Soil Organic Carbon $\delta^{13}C$ Values:

Soil organic carbon $\delta^{13}C$ values under ERC ranged from –14.5 ‰ to –23.5 ‰ over the three depth intervals. The SOC $\delta^{13}C$ values of soil in the RG plots exhibited a smaller range in values from –14.0 ‰ to –21.3 ‰. The SOC $\delta^{13}C$ values of soil at 0-5 and 5-15 cm depths were significantly more negative in ERC than RG plots ($p < 0.05$) (Table 6) by as much as 2.7 ‰ While the $\delta^{13}C$ values in the 15-30 cm depth were not significantly different, the ERC exhibited a small shift to lower values, indicating a minor influence at depth by as much as –1.3 ‰ (Table 6). Soils in both ERC and RG plots exhibited a trend of increasing $\delta^{13}C$ values with depth ($p < 0.05$) (Fig. 10).

Fig. 9: Soil organic carbon $\delta^{13}C$ values for all samples with depth for eastern red cedar (ERC) and remnant grasslands (RG) at KAEFS for cores from 0-5 cm (avg 2.5 cm), 5-15 cm (avg 10 cm), and 15-30 cm (avg 22.5 cm)



4.3.4. Soil texture controls on soil organic carbon:

As soil texture is known to influence SOC storage and aggregation potential (Anderson 1984; Six et al, 2002; Liao et al., 2006a), a linear regression model was used to correlate percent

clay, silt, and sand with percent total organic carbon for each soil depth with each land cover. For ERC plots, percent clay explains 29-49% of the variation in the organic carbon stored along the entire profile (Table 7), with clay showing the highest correlation in the 5-15 cm increment, explaining about 49% of the variation in SOC. Remnant grassland SOC was not, however, seemingly dependent on clay content at any depth increment ($p > 0.05$). Silt content significantly explained 42% of the variation in SOC in the top 5 cm of the ERC plots ($p<0.01$), while it only explained 25% of the variation in percent organic carbon in the RG plots ($p<0.5$). Lastly, percent sand at the 0-5 and 5-15 cm increments beneath ERC plots explained 51% and 50% of the variation in percent organic carbon although sand has little control on SOC binding (Six et al, 2002), respectively ($p<0.01$). Percent sand explains 28% of the variation in percent organic carbon in the 15-30 cm increment of the RG plots ($p<0.05$). When considering the full 30 cm profile, there was no relationship overall for any soil texture element and percent organic carbon (Table 7). The relationships analyzed in this section should be interpreted with caution, as the residuals for some of these models are not normally distributed (Table 8).

Table 7: Adjusted r^2 values and p-values for linear regression of soil texture and percent SOC at each soil depth and the total soil profile from cores under eastern red cedar (ERC) and remnant grassland (RG) plots

Land Cover	Sand	p-val	Silt	p-val	Clay	p-val
ERC						
0-5 cm	0.5123	**0.00162**	0.4216	**0.00525**	0.2934	**0.02158**
5-15 cm	0.5037	**0.00183**	0.1937	0.05697	0.4892	**0.00223**
15-30 cm	0.1862	0.06109	-0.0088	0.3659	0.4023	**0.00659**
Total	-0.0088	0.4361	-0.0061	0.3962	-0.0197	0.702
RG						
0-5 cm	0.1183	0.1135	0.1912	0.0583	-0.0312	0.4611
5-15 cm	0.174	0.06841	0.08806	0.1491	0.1587	0.07873
15-30 cm	0.278	**0.02523**	0.2462	**0.03452**	0.1659	0.07366
Total	-0.0224	0.8499	-0.0131	0.5144	-0.0178	0.6339

The "Total" row is the results for plotting all of the depths on one linear regression. The reported adjusted r^2 values are under each soil texture column. Significance values were set at $p \leq 0.05$.

Table 8: Distribution of the residuals of the linear regression models for soil texture and percent total organic carbon

Land Cover	Sand	Silt	Clay
ERC			
0-5 cm	non-normal	non-normal	normal
5-15 cm	non-normal	normal	non-normal
15-30 cm	normal	normal	non-normal
Total	non-normal	non-normal	non-normal
RG			
0-5 cm	normal	normal	normal
5-15 cm	non-normal	non-normal	non-normal
15-30 cm	non-normal	non-normal	non-normal
Total	non-normal	non-normal	non-normal

The distribution of the residuals was evaluated using a histogram

4.3.5. Soil inorganic carbon:

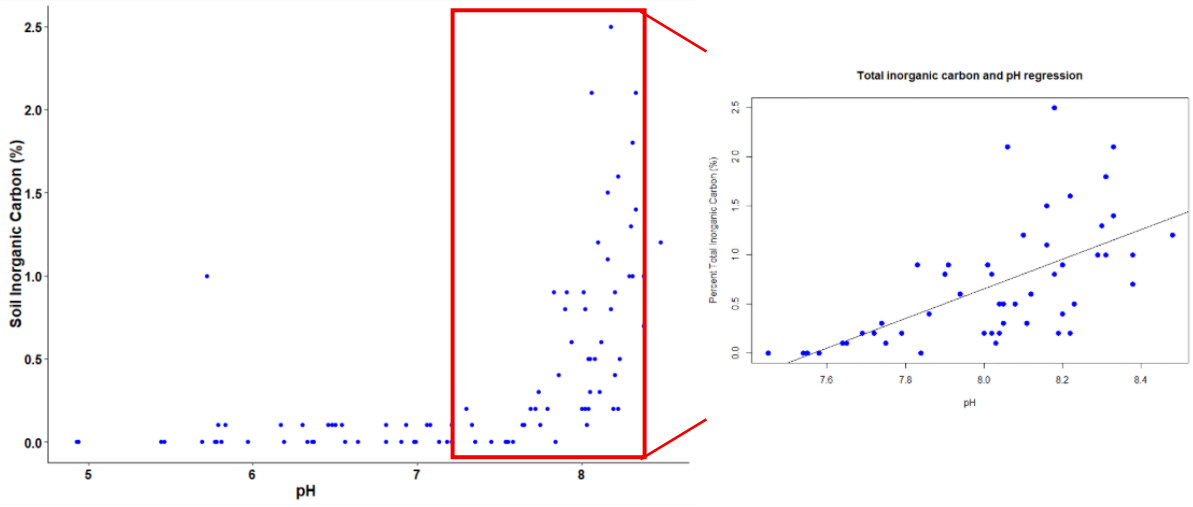
Soil inorganic carbon (SIC) data were evaluated with a limit of detection-based filter to include samples that are greater than 0.3 wt% SIC by difference (i.e. SIC = Total Cabon – Organic Carbon) before calculating the $\delta^{13}C$ value. Statistics were not run on these values because there are uneven groupings. To estimate the $\delta^{13}C$ value of the SIC, Equation 7, Chapter 2 was used. With the filter, 63 out of 90 samples had SIC values too low to estimate the SIC $\delta^{13}C$ values. For the RG plots, the 0-5 cm depth had a n=3, the 5-15 cm depth had a n=5, and the 15-30 cm depth had a n=7. For the ERC plots, the 0-5 cm depth had a n=1, the 5-15 cm depth had a n=4, and the 15-30 cm depth had a n=7. Overall, the 15-30 cm depth for both RG and ERC had

the most samples that could be estimated. Soil inorganic carbon $\delta^{13}C$ values for ERC ranged from –2.9 to +0.4‰ across all soil depths (Table 6). Remnant grassland plots showed a larger range from –6.4 to 4.1‰ (Table 6).

4.3.6. Soil inorganic carbon trends with landcover and pH:

Across all soils and depths, 27 of 90 soils analyzed exhibited measurable quantities of the calculated SIC values. No significant trend, however, was evident with depth, nor was there a difference in SIC content between ERC and RG soils (Table 6). Relationships between SIC and soil pH (Gozukara et al. 2025) were explored by applying a linear regression model. For soil samples that had no presence of inorganic carbon, a SIC concentration value of 0.0000001 wt % was used in the model. While a linear model did not fit the overall data well ($r^2 = 0.32$) (Fig. 11), values with a pH ≥ 7.5 exhibited a closer correlation with 37% of the variation in SIC explained by pH ($p<0.05$).

Fig. 10: Linear regression model of total soil inorganic carbon and soil pH



4.3.7. Stable carbon isotope modelling of residual grassland carbon under ERC:

As the carbon accumulated under the ERC will exhibit progressive loss of original grassland C due to a combined decomposition of the original carbon (Mellor et al., 2013) and replacement/addition of ERC, we can model the fractional abundance of grassland carbon (Fc) of each under the ERC with a two-endmember stable isotope mixing model (Boutton et al., 2009) - see Chapter 2, equation 8. Because plant input may vary between roots and needles/shoots, we can model the changing source of carbon pools by using these different input endmembers (i.e. needle vs litter vs root) assuming they differ in $\delta^{13}C$ values. Our model output estimates the fraction of grassland carbon (Fc) remaining in soil (in an identical calculation to what was employed in Chapter 3 to quantify C_4 plant carbon contributions to root and litter in the KAEFS research plots). In the present study, we considered 4 scenarios for a carbon input model: (see Table 10 for $\delta^{13}C$ values used): 1) average $\delta^{13}C$ values of isolated root for a particular depth interval under ERC as the main input of carbon, 2) average $\delta^{13}C$ values of ERC understory needle litter, 3) average $\delta^{13}C$ values of fresh ERC needles collected at KAEFS plots, and 4) a 50/50 mass combination of surface litter and roots (Chapter 2, Equation 11). As the $\delta^{13}C$ values for mean root values and surface litter are very close in value, there was no difference in Fc in these scenarios at any depth between these two inputs. Because ERC fresh needles are significantly depleted in ^{13}C compared to litter or roots, there is a ~9% difference in Fc between using fresh ERC needles vs. ERC roots, but only for the 0-5 cm interval of soil (Table 9). At the 0-5 cm depth, the largest replacement of RG carbon by ERC is observed, with only 68 to 77% of the RG carbon remaining, depending on the choice of end members (Table 10). With depth, the amount of RG carbon remaining under the ERC increases (Figure 8) to ~90% (5-15 cm) and ~98% (15-30 cm).

Table 9: Average fraction of remaining grassland carbon (Fc) using different endmembers

Depth	Fc Using ERC Roots	Fc Using Surface ERC Litter	Fc Using Fresh ERC Needles	Fc Using a 50/50 Mixture of ERC Roots and Surface Litter
0-5	0.68(±0.16)	0.71(±0.14)	0.77 (±0.10)	0.70(±0.14)
5-15	0.89(±0.06)	0.90(±0.06)	0.92 (±0.5)	0.90(±0.06)
15-30	0.98(±0.05)	0.98(±0.05)	0.98 (±0.4)	0.98(±0.05)
$\delta^{13}C$ endmembers (‰)	**0-5 cm:** -26.6 (±1.1) **5-15 cm:** -27.0 (±0.8) **15-30 cm:** -27.1(±0.5)	-27.4 (±0.5)	-30.0 (±0.58)	**0-5 cm**: -27.0 (±0.64) **5-15 cm:** -27.2 (±0.52) **15-30 cm:** -28.5 (±0.24)

The reported values above show the means of fifteen field replicates (standard deviation of the mean).

4.4. Discussion:

4.4.1. Eastern red cedar root and litter driven alteration of shallow soil physical properties:

Similar studies to that presented herein have shown that pine and juniper tree encroachment can result in lower soil bulk density in the top 10 cm of the soil profile (Hou et al., 2019; Sams, 2025; Sauer et al., 2023). Similar findings are also found with invasive leguminous species (i.e., *Prosopis spp.*), as has been observed in the American South and Southwest (Filley et al., 2008a; Boutton et al., 2009). The decrease in bulk density can be principally explained by the increase in fine and coarse root biomass beneath the ERC plots and the increase in surface litter mass, promoting larger aggregate development and greater void space (Ilek et al., 2017; Zhang et al., 2020). In the present study, soil bulk density decreased by 12.6% in the 0-5 cm depth after 25 years of ERC encroachment at KAEFS, but with no significant changes in the 5-15 cm and 15-30 cm depths (Table 5). Eastern red cedars have large tap-rooting systems with lateral roots that can extend up to 6 m and a taproot that can extend down to 7.5 m, but in shallow soils tend to have an expansive fine root system (Walker, 1967; Lawson, 1986; Anderson, 2003). The soil profile

in the area of KAEFS we explored was very shallow, only ~30-40 cm thick, and so the rooting system of the ERC trees may have been more concentrated at the upper layers in comparison to deeper soil profiles (Lawson 1990), leading to increased soil mixing and increased opportunity for soil modification in shallow zones. Secondly, increases in surface litter mass, needles, and berries can reduce soil bulk density at the soil surface if mixed into the soil by bioturbators (Filley et al., 2008b; Ilek et al., 2017; Macleod et al., 2025). We found that surface litter mass under ERC increased on average by 556% after 25 years of growth (Chapter 3, Table 3). In the present study, fine root litter was also observed to increase under ERC in the 0-5 cm depth by 41% (Chapter 3, Table 2) as has been observed in other plant community shifts similar to this study (Jackson et al., 2002; Archer 2017; Jastrow et al., 1998). Additionally, plant litter (needle and root) is less dense than mineral soil and promotes aeration during decay. Litter incorporation decreases with depth, so it would not affect bulk density at deeper depths. Lastly, there was evidence of bioturbation by insects and animals at our site. During the sieving process, animal frass was removed, and in the field, there was also evidence of animal tunneling under the ERC plots that may have contributed to a lower bulk density in the upper 5 cm of soil.

Changes in soil bulk density at this site could not be attributed to differences in soil texture, as there was no significant difference in any soil texture elements across the plots (Table 5). While we did not investigate changes in aggregation as a result of woody encroachment, changes from predominately micro-aggregate structures in degraded grasslands to macro-aggregate structures may reduce overall carbon stability in a soil ecosystem because carbon stored in macro-aggregates is more vulnerable to soil decomposition by soil microbes because of larger pores and larger spaces for water (Six et al. 2004; Liao et al., 2006a; Tian et al., 2015). Sauer et al. (2023) found that soils beneath ERC trees across 5 states had a 10% increase in non-

erodible aggregates and larger wet mean and dry mean weight diameters in comparison to adjacent cropping fields. In contrast, Busch and Presley (2014) found that ERC trees between the ages of 17-56 years in the Flint Hills region of Kansas had no statistical difference in mean aggregate diameter in comparison to adjacent grassland plots. Increases in soil aggregation can improve soil water flow (Boyle et al., 1989), reduce soil erosion (Thomaz et al., 2022), and improve carbon storage (Six et al., 2004). Aggregate separation of our sites at KAEFS might offer additional insights into soil restructuring, and it is recommended as a follow-up study.

4.4.2. Soil inorganic carbon is related to soil pH and is of lithogenic origin:

Most of the soils investigated in the plots had measurable, albeit low, SIC contents that ranged from 0.0- 2.5% (Table 6). Soil carbonates buffered soil pH, where at a pH >8, SIC increased with increasing pH, which is consistent with expectations of carbonate equilibrium chemistry. Gozukara et al. (2025) demonstrated that globally, soil carbonates were highest in soils with a pH of 8 but did not accumulate in highly alkaline soils (pH>8.5). We also found that there was no difference in percent inorganic carbon between ERC and RG plots (Table 6), which is consistent with a lack of change in pH between ERC and RG plots.

Based on stable carbon isotope modeling of SIC greater than 0.3 wt %, carbonates in the plots investigated were of lithogenic origin as they fit in the range of -2‰ to 2‰ (Ramnarine et al., 2011), indicating that there was little to no influence of root respiration on carbonate formation. One of the 90 samples investigated under ERC (0-5 cm) exhibited an outlier calculated $\delta^{13}C$ value of –6.4‰, suggesting carbon sourced from C_3 respiration, but this sample would need to be investigated further analyzing the soil carbonate directly for stable carbon isotopes. Based on potential plant CO_2 input carbon source, which we define as isolated roots, (Grassland root = -19.5‰, ERC root = -27.1, Chapter 3, Table 2), pedogenic carbonates could

theoretically fall between –16.9‰ to –9.3‰ (Ortiz et al., 2022). When pedogenic carbonates are formed from organic carbon weathered by carbonic acid, there is potentially up to a +10.2‰ shift in $\delta^{13}C$ that includes a temperature-dependent equilibrium fractionation (Mook 1974).

Because most of our samples suggest soil carbonates of lithogenic origin, we can say that root respiration is not driving, to any great extent, soil carbonates in this study. The carbonates being lithogenic in origin is consistent with the Nash-Lucien complex description (Soil Survey Staff a, n.d.). Likewise, we see no difference in SIC concentration or $\delta^{13}C$ between ERC and RG plots. Woody plant encroachment, however, does have the capacity to enhance carbonate weathering. For example, Leite et al. (2023) found that a site at the Edwards Plateau in Texas under *Quercus fusiformis* and *Juniperis ashei* encroachment had a 24-44% greater limestone matrix porosity due to dissolution. Work conducted in Kansas found an increase in stream solutes and an increase in carbonate dissolution after WPE, implying increased weathering of bedrock by woody plant roots (Sullivan et al., 2019; Wen et al., 2020). Lastly, trees associated with ectomycorrhizal fungi were found to also enhance carbonate bedrock weathering (Thorley et al., 2015). As the trees in this current study have only been established for a short time, there may not have been ample time to influence carbonate weathering.

4.4.3. Evidence of depth and time-dependent replacement of and addition to the original grassland SOC under ERC encroachment

At our site, percent clay can account for 29% of the percent organic carbon storage in the 5 cm depth, 49% in the 15 cm depth, and 40% at the 30 cm depth beneath tree plots, while there was only one significant effect of clay on percent carbon storage in the grassland plots (28% at the 15-30 cm depth) (Table 7). Secondly, there was a negative relationship with sand and percent organic carbon in the 0-5 cm and 5-15 cm depths for ERC plots and in the 15-30 cm for RG plots

(Table 7). Our results are unsurprising, as clays are known to store carbon efficiently because of their small size and high surface areas (Anderson, 1984; Ransom et al., 1998; Fomina and Skorochod, 2020; Schwiezer et al., 2021), and sands are found to have a negative relationship with percent organic carbon (Augustin and Cihacek, 2016). However, the differences in correlation between ERC and RG plots may be explained by differences in soil climate and litter chemistry. Carbon storage capacity of fine soil particles is dependent on temperature and moisture (Parton et al., 1987). Other studies have found changes to soil moisture (Pierce and Reich, 2010) and soil temperature (Wang et al., 2021) after ERC encroachment. Optimal moisture and temperature conditions beneath ERC canopies may explain why SOC is associated with clays while the remnant grasslands are not. Secondly, ERC had increased fine root biomass in the 5-15 cm and 15-30 cm depths (Chapter 3, Table 2). Increased root biomass allows for more opportunities for root priming, which stimulates microbial activity and may increase interactions with clays (Kuzyakov and Cheng, 2001; Ma et al., 2022). However, it should be noted that the residuals of the linear regression models were not always normally distributed (Table 8), so the results of these models should be interpreted cautiously. The data from the linear regression models show that carbon association with clays can only partially account for the variation found in soil organic carbon. Our carbon isotope data demonstrate that vegetation also influences it.

We hypothesized that after 25 years of WPE by ERC, we would see SOC isotopic signatures that reflected the more negative $\delta^{13}C$ values of C_3 vegetation (~ -26.0 ‰) in the upper 5 cm of the soil, with the $\delta^{13}C$ values increasing with soil depth to reflect C_4-dominated grassland vegetation values (-12.5‰ to –13.4‰). On average, RG plots had a SOC isotopic signature that represented a mixture of C_3 and C_4 plants in the upper 5 cm (-18.3‰) that

increased with depth (-15.9‰ at the 5-15 cm depth and –14.9‰ at the 15-30 cm depth) (Table 3). In general, with depth, soils are found to have about a + 1-3‰ isotopic discrimination due to changes in soil moisture use efficiency in C_3 plants, differences in decomposition rates, and changes in atmospheric CO_2 (Ehleringer et al. 2000; Kruger et al., 2024). Beneath the RG sites, the soil organic carbon $\delta^{13}C$ value increased by 3.4‰ with depth. Therefore, the depletion of ^{13}C under the RG plots can be attributed to these environmental factors rather than to a vegetation change.

Alternatively, as detailed in Chapter 3, the RG surface litter $\delta^{13}C$ values at the time of sampling closely matched C_4 values, but the roots represented a mixture of C_3 and C_4 (Chapter 3, Tables 2 and 3). The $\delta^{13}C$ signature we see in the upper 5 cm of RG plots reflects this mixture of C_3 and C_4 vegetation that shifts with seasons and is within the rooting depth (Balasko and Nelson 2003). Differential proportions of C_4 and C_3 grasses throughout the seasons may show as a change in isotopic signature, and the shift to values more similar to C_4 values may indicate that C_4 organic matter is more persistent in this ecosystem. As rooting depth decreased significantly in the 5-15 cm and 15-30 cm depths (Chapter 3), and there were no surface litter inputs at these depths, we can infer that the $\delta^{13}C$ signature is from relict grassland carbon.

After ERC encroachment, our results showed that in comparison to RG on average, there was a -2.7‰ difference in SOC $\delta^{13}C$ values in the top 5 cm, a -1.2‰ difference in the 5-15 cm depth, and no significant change in the δ13C values in the 15-30 cm depth (Table 3). This decrease in the $\delta^{13}C$ value compared to RG plots indicates that ERC litter is being incorporated into the upper 5 cm of the soil profile and is being gradually incorporated into the 5-15 cm

increment. The $\delta^{13}C$ value of the 15-30 cm depth increment reflects the remainder of the remnant grassland carbon, as there was no significant difference between the ERC and RG plots at this depth (Fig. 5). This finding matches other studies using $\delta^{13}C$ values as a proxy for tracking land cover change (i.e. McPherson et al., 1993; Jessup et al., 2003; Smith and Johnson 2003; Spencer et al., 2009). As detailed in Chapter 3, there was a large increase in surface litter inputs on the ERC plots in comparison to RG plots due to the larger ANPP of ERC. Likewise, there was a replacement of remnant grassland carbon below-ground inputs with C_3 below-ground inputs (Chapter 3). Therefore, the increase in C_3 above- and below-ground inputs has shifted the $\delta^{13}C$ signature to more negative values beneath the ERC plots. However, despite the large increase in litter inputs from ERC and the decrease in $\delta^{13}C$ values in the upper 15 cm, there was no change in soil carbon stock at any depth (Figure 4, Table 6), indicating an exchange of ERC and RG carbon.

Our results differ from some other findings in the Great Plains region that show an increase in SOC stock in the upper layers of soil after WPE (Sauer et al. 2007; McKinley and Blair 2008; Spencer et al. 2009; Pierce and Reich 2010; Wine et al. 2012; Mellor et al. 2013; Li et al. 2023; Sams 2025). Many studies have shown that SOC increases with a concurrent decrease in $\delta^{13}C$ after WPE (Jessup et al., 2003; Liao et al., 2006b; Wheeler et al., 2007; Spencer et al., 2009; Throop et al., 2013), indicating either the retaining of grassland carbon and an addition of C_3 carbon or that C_3 carbon is replacing grassland carbon while adding a higher amount. In this study, the decrease in the $\delta^{13}C$ SOC values with no change in SOC indicates that the ERC are replacing the RG carbon at the same rate that RG carbon is being decomposed. This finding is similar to another ERC encroachment study in a shallow soil in the Flint Hills region of Kansas that found that there was no increase in SOC despite a ~-5‰ difference in $\delta^{13}C$ SOC

values after 40-60 years of ERC encroachment (Smith and Johnson 2003). They suggested that the trees may be too recently established to show an increase in SOC (Smith and Johnson 2003). The observation of SOC replacement could not be made without the use of stable $\delta^{13}C$ measurements.

In keeping with the findings of Smith and Johnson (2003), trees in our study may have been too young to appreciably change SOC stock. For example, in a native sand prairie in the Nebraska National Forest, young ERC stands (55yr) were found to have 3.70 Mg ha-1 of C in the top 5 cm of soil and older ERC stands (72yr) were found to have 6.43 Mg ha-1 of C with both having the same Mg ha-1 in the 5-30 cm depths (Mellor et al. 2013), indicating that relative time of encroachment influences litter mass and soil carbon accumulation in the upper depths. Perhaps 25-30 years is not enough time for soil microbes to decompose and incorporate ERC litter at our site. In this system, no changes in SOC may be a function of differences in litter decomposition rates and the stand age.

4.4.4. Using fresh ERC needles over-estimates FC_{grass} values

In this study, we calculated the fraction of remaining grassland carbon using three different strategies: 1.) using the tree root $\delta^{13}C$ values, 2.) using the tree above-ground litter $\delta^{13}C$ values, and 3.) using the average of the tree root and above-ground litter $\delta^{13}C$ values (Chapter 2, Eq. 8). Other studies have used differing end members when calculating the proportion of remaining grassland carbon (i.e. Smith and Johnson 2003; Liao et al., 2006). For example, Smith and Johnson (2003) used a mixture of live ERC foliar inputs and roots for their two-endmember mixing equation, while Liao et al. (2006) used a mixture of surface litter and roots. As mentioned in Chapter 2, decaying litter can have a 2-6‰ lowering in $\delta^{13}C$ values in comparison to fresh

plant material (Preston et al., 2006; Osono et al., 2008). Therefore, the value for the proportion of remaining grassland carbon can change depending on whether fresh or surface litter is used. However, there are no studies comparing this potential difference in calculating residual or mixed carbon input in WPE scenarios.

When comparing methods, we found ~7% difference when using live ERC foliar matter as an endmember vs. ERC surface litter or roots in the upper 5 cm of the soil profile (Table 10). In our study, ERC surface litter was on average -27.4‰, while the fresh foliar litter we collected was ~30.0‰ (Chapter 3, Table 4), illustrating the 2-6‰ depletion mentioned above. If we use the litter endmember, we see that there is less remaining grassland carbon than if we use fresh foliar litter (Table 10). However, in the 5-15 cm and 15-30 cm depths, there was no difference between using surface litter or fresh foliar litter, and we see the same increase in remaining grassland carbon with depth. This is likely due to the fact that ERC litter and root biomass has not yet been incorporated into those layers, so there was little difference between the RG soil and the ERC soil when the ERC litter subtracted out. Lastly, we found no difference in the Fc value between using an average of ERC litter and roots for either fresh foliar litter or surface litter values in comparison to just using litter or just using roots (Table 10). This is likely because the root $\delta^{13}C$ values beneath the tree plots were not much different from the $\delta^{13}C$ values of the tree surface litter at all depths. After about 25 years of ERC encroachment at this site, the C_4 roots and surface litter have been completely replaced by C_3 roots (Chapter 3). Therefore, the $\delta^{13}C$ values of the roots and the surface litter under the ERC closely match each other. If another study found that there was a mixture of C_4 and C_3 roots beneath the tree canopy, they would likely see a difference in using the averages vs just the roots or just the litter. Using fresh litter results in an

overestimation in the remaining grassland C. Although we found differences in using fresh versus surface litter in the upper 5 cm, the two-endmember mixing model is still a good tool to estimate the fraction of remaining grassland carbon as the profile trends are the same. When using this equation, scientists should be aware that there will be a difference in their values and report both in the future.

4.5. Conclusions

The encroachment of ERC trees into a degraded grassland resulted in altered soil chemical and physical properties after ~ 25 years but primarily in the surface 5 cm where root and litter input would be greatest. While bulk density decreased and SOC increased in the upper 5 cm of the soil profile, there was no change in soil inorganic carbon, soil pH, or soil texture. There was also no change in mean value of soil organic carbon stock despite the large increase in above-ground litter mass and wt% carbon after ERC encroachment. Possible explanations for this discrepancy could be difficulty with consistency in bulk density calculations in soils with large roots, resulting in a large range of bulk density values under ERC. Such an error in bulk density would then translate to errors in stock calculations.-Lastly, we were able to track the changes in the storage and input of carbon to soil with ERC encroachment using stable carbon isotope methods and found that 70-98% of grassland carbon was still present beneath ERC plots. Although we did not find many changes to soil carbon stock, there is the potential for soil carbon dynamics to change with increasing stand age. Future work in this area should include analysis of soil carbon stability, such as by using MAOM and POM separations and aggregate stability tests. Additionally, the measurement of soil CO_2 flux and $\delta^{13}C$ values under these plots and along a chronosequence of WPE to better understand soil carbon dynamics in this ecosystem.

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5. SCOPE, LIMITATIONS, AND FUTURE DIRECTIONS

5.1 Scope and Limitations:

Our study focused on how early stages of woody plant encroachment into grasslands by *Juniperus virginiana* influenced litter and soil carbon stocks and source. With this, there are many limitations to consider when interpreting our data. Firstly, our ERC-RG plots were located in a highly eroded and degraded site. Soils eroded to this extent are shallow and can therefore affect rooting depth and biomass. The soils across KAEFS are also not uniformly eroded and degraded: the soils in the northeast of the park have higher vegetation cover and less evidence of erosion, for example. Because of this, more variety in sample sites is needed to scale our data to the entire field station and certainly the region. We may be able to use our data to make generalizations about highly eroded sites in Oklahoma, but more studies need to be conducted to confirm this and to compare to less degraded sites.

Secondly, due to time constraints of the thesis, we only sampled once during the spring of 2024. Grassland species composition changes with seasons and climate variability, meaning that the relative proportions of C_4 above-ground litter in the RG plot can fluctuate throughout the year. Surface litter beneath the ERC canopies, however, should not change seasonally because there is only input from the ERC litterfall, and needles degrade slowly. Therefore, the litter findings in the RG plots of our study should not be generalized for the whole year and more sampling times are necessary to accurately assess grassland input changes in this ecosystem while ERC surface litter estimates can be generalized.

As root turnover is longer than a year (Joslin et al., 2006), our root data for both ERC and RG plots can be seen as a representative sample of annual to multi-year root production. Soil organic carbon values can also be seen as a representative sample of year-round to multi-year

SOC because SOC can take up to decades to degrade. However, as mentioned above, we need more data points at different parts of the park before scaling our findings to a large scale as the soils are at different levels of erosion and degradation.

Lastly, we used a soil coring method to take a representative sample of each ERC-RG plot but scaled soil carbon stock to per hectare. There may be variability in soil carbon stock values within a sampling site or across sampling sites. Scaling to a per hectare scale is a rough estimate of carbon stock and should not be viewed as an absolute value.

5.2. Future Directions:

Our study quantified the changes of soil organic matter inputs and subsequent changes to soil organic matter storage beneath 25–30-year-old ERC trees in a remnant grassland ecosystem. Our work leveraged stable carbon isotopes to track changes in SOC and SIC distribution and litter input due to the shift in vegetation type - the C_4-dominant grassland and encroaching ERC at KAEFS. It was beyond the scope of the present study to quantify the relative stability or carbon loss of the introduced and native carbon in the system. In future studies, separation of SOC into mineral-associated organic matter (MAOM) and particulate organic matter (POM) could be conducted to determine into which soil pools ERC litter carbon are being incorporated in the soil matrix and on what mineral forms. Using a MAOM/POM approach, Liao et al. (2006b) and Filley et al. (2008) showed that over time, WPE by honey mesquite increased the portion of carbon stored in the POM fractions, indicating a potential decrease in carbon stability with stand age. Similarly, soil aggregation is known to change with WPE (Liao et al., 2006b; Creamer et al., 2011; Sauer et al., 2023) and shift the allocation of SOC to different aggregate protection pools. For example, Sauer et al. (2023) found an increase in wet mean weight diameter in the 0-7.5 cm soil layer after WPE by ERC in comparison to agricultural fields in

Oklahoma, indicating an increase in soil stability. However, carbon was not found to be stable with an increase in C allocation in macroaggregates after WPE by honey mesquite (Creamer et al., 2011). Therefore, using a MAOM/POM method can help determine how ERC allocates carbon in the soil matrix and whether it is stable.

Secondly, sampling CO_2 gas effluxes above- and below-ground will be beneficial to determine if soil respiration increases after ERC encroachment, which could be a potential major loss of soil C (McCulley et al., 2004; Creamer et al., 2011). Both in-lab incubations and field measurements have been used to quantify soil respiration after WPE to provide insight into the relative stability of introduced carbon (McCulley et al., 2004; Creamer et al., 2011). For example, McCulley et al. (2004) utilized an in-field infrared gas analyzer and soil chemical analysis to determine that soil respiration increases while SOC also increases, indicating the creation of both stable and unstable forms of carbon after WPE by honey mesquite. Conducting respiration experiments in tandem with stable isotopes would help better identify the fluxes of carbon at KAEFS.

Next, integrating a chronosequence approach may be beneficial in this area to track any age-related effects of ERC encroachment on soil carbon cycling. The chronosequence approach involves studying soil properties under tree stands of increasing age to determine the effects of WPE on the original ecosystem, where an implicit assumption is that the remnant ecosystems (grasslands in this case) measured in the present is the true reflection of the past ecosystem when the woody encroachment began (Liao et al., 2006a). In many WPE studies, authors have used the chronosequence approach to track changes in soil properties, including mineralogy and carbon and nitrogen stock and chemistry (i.e., Liao et al. 2006b; Boutton et al., 2009; Mellor et al. 2013). In a chronosequence study in south Texas, Boutton et al. (2009) demonstrated that SOC

storage increased by 200% with groves and clusters of trees that ranged from 10 to 130 years old. Sampling over time may show increases in soil organic carbon stock that we didn't see at 25-30-year-old trees, and comparing stands to individual trees may show important compounding effects that we did not note in this study.

Our study quantified the changes in root and litter biomass in young eastern red cedar trees, filling in a knowledge gap related to litter dynamics. However, our work did not assess how litter chemistry, such as tannins, lignin, allelopathic chemicals, and aromatic compounds, may have changed with ERC encroachment (Filley et al., 2008; Creamer et al., 2011; Klotzbucher et al., 2011). For example, Filley et al. (2008) used alkaline cupric-oxide oxidation to find that honey mesquite litter had more syringyl (S)- and vanillyl (V)-based lignin than grassland litter had while grassland litter had more cinnamyl (C)-based lignin. The authors concluded that the new tree litter was more resistant to decay by that particular microbial consortia (Filley et al., 2008). We also did not assess changes in other litter nutrients such as K, Ca, and Mg that are important for soil cation exchange (Kleber et al., 2007; Mellor et al., 2013; Hodges et al., 2023). Mellor et al. (2013) demonstrated that ERC stands increased soil Ca content by 102% in comparison to prairie soils in a Nebraska sand prairie, indicating that 90% of the variation in SOC was explained by Ca, N, and Mg concentrations. Using these methods may be helpful in discussing how ERC alters litter quality and, in turn, SOC stability.

Lastly, conducting microbial community analysis and microbial respiration studies may be informative to how ERC has the potential to alter soil carbon storage (Miller and Jastrow, 1990; Williams et al., 2013; Creamer et al., 2016; Li et al., 2023; Sams, 2025). Arbuscular mycorrhizal fungal biomass was found to be higher with ERC roots than with species in a C_4-dominant grassland and an Oakland forest in Oklahoma using phospholipid fatty acid analyses

(Williams et al., 2013; Sams, 2025). Alterations to microbial populations have implications for soil nutrient availability, weathering, and soil carbon storage, so it is important to quantify. Combinations of microbial and litter interactions could be a driving force for soil carbon storage, so future studies should prioritize quantifying those changes.

5.3. Works Cited

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Appendices:

Appendix A: Soil texture

Soil samples were sent to Ward Labs Inc. (Kearney, NE) for soil texture analysis.

Site Name	Average Depth (cm)_	Soil Texture	Sand (%)	Silt (%)	Clay (%)
Tree 4	2.5	Sandy Loam	56	33	11
Tree 5	2.5	Sandy Loam	57	33	10
Tree 6	2.5	Sandy Loam	57	29	14
Tree 7	2.5	Sandy Loam	65	21	14
Tree 9	2.5	Sandy Loam	61	21	18
Tree 10	2.5	Sandy Clay Loam	57	21	22
Tree 11	2.5	Clay Loam	33	37	30
Tree 12	2.5	Loam	37	37	26
Tree 13	2.5	Clay Loam	29	41	30
Tree 14	2.5	Clay Loam	33	33	34
Tree 15	2.5	Loam	37	41	22
Tree 16	2.5	Loam	48	35	17
Tree 17	2.5	Sandy Loam	53	33	14
Tree 18	2.5	Sandy Loam	69	17	14
Tree 21	2.5	Sandy Loam	65	21	14
Grass 4	2.5	Sandy Loam	57	29	14
Grass 5	2.5	Sandy Loam	61	25	14
Grass 6	2.5	Loam	49	33	18
Grass 7	2.5	Sandy Loam	65	21	14
Grass 9	2.5	Sandy Loam	65	17	18
Grass 10	2.5	Sandy Loam	65	21	14
Grass 11	2.5	Clay Loam	29	37	34
Grass 12	2.5	Loam	33	45	22
Grass 13	2.5	Clay Loam	21	45	34
Grass 14	2.5	Clay Loam	37	33	30
Grass 15	2.5	Loam	33	45	22
Grass 16	2.5	Loam	45	37	18
Grass 17	2.5	Loam	45	41	14
Grass 18	2.5	Sandy Loam	68	21	11
Grass 21	2.5	Sandy Loam	65	17	18

Tree 4	10	Sandy Loam	54	33	13
Tree 5	10	Sandy Loam	60	27	13
Tree 6	10	Sandy Loam	60	25	15
Tree 7	10	Sandy Loam	64	17	19
Tree 9	10	Sandy Clay Loam	58	19	23
Tree 10	10	Sandy Loam	66	17	17
Tree 11	10	Clay Loam	28	41	31
Tree 12	10	Loam	40	37	23
Tree 13	10	Clay Loam	26	43	31
Tree 14	10	Clay	22	31	47
Tree 15	10	Loam	28	47	25
Tree 16	10	Loam	46	35	19
Tree 17	10	Loam	52	35	13
Tree 18	10	Sandy Loam	68	19	13
Tree 21	10	Sandy Loam	58	25	17
Grass 4	10	Sandy Loam	62	25	13
Grass 5	10	Sandy Loam	62	25	13
Grass 6	10	Sandy Loam	56	27	17
Grass 7	10	Sandy Loam	60	23	17
Grass 9	10	Sandy Clay Loam	61	18	21
Grass 10	10	Sandy Loam	66	21	13
Grass 11	10	Clay Loam	20	41	39
Grass 12	10	Loam	32	43	25
Grass 13	10	Silty Clay Loam	16	49	35
Grass 14	10	Clay	26	33	41
Grass 15	10	Loam	28	47	25
Grass 16	10	Loam	44	37	19
Grass 17	10	Loam	38	45	17
Grass 18	10	Sandy Loam	62	23	15
Grass 21	10	Sandy Loam	62	19	19
Tree 4	22.5	Sandy Loam	58	23	19
Tree 5	22.5	Sandy Loam	60	27	13
Tree 6	22.5	Sandy Loam	66	23	11
Tree 7	22.5	Sandy Clay Loam	64	13	23

Tree 9	22.5	Sandy Clay Loam	54	19	27
Tree 10	22.5	Sandy Clay Loam	54	21	25
Tree 11	22.5	Clay Loam	22	47	31
Tree 12	22.5	Loam	48	31	21
Tree 13	22.5	Silt Loam	28	51	21
Tree 14	22.5	Clay Loam	28	33	39
Tree 15	22.5	Clay Loam	20	51	29
Tree 16	22.5	Loam	36	43	21
Tree 17	22.5	Loam	32	47	21
Tree 18	22.5	Sandy Loam	72	15	13
Tree 21	22.5	Clay Loam	44	25	31
Grass 4	22.5	Sandy Loam	62	23	15
Grass 5	22.5	Sandy Loam	66	23	11
Grass 6	22.5	Loam	50	29	21
Grass 7	22.5	Sandy Loam	54	29	17
Grass 9	22.5	Sandy Clay Loam	52	21	27
Grass 10	22.5	Sandy Loam	68	17	15
Grass 11	22.5	Silty Clay Loam	18	47	35
Grass 12	22.5	Clay Loam	29	39	32
Grass 13	22.5	Silt Loam	22	53	25
Grass 14	22.5	Silty Clay	14	43	43
Grass 15	22.5	Clay Loam	20	51	29
Grass 16	22.5	Clay Loam	22	45	33
Grass 17	22.5	Silt Loam	20	55	25
Grass 18	22.5	Sandy Loam	68	15	17
Grass 21	22.5	Sandy Clay Loam	46	25	29

Appendix B: Slope, elevation, and aspect of each ERC-RG plot

Data was retrieved using the Digital Elevation Model (DEM) from the United States Geological Survey.

U.S. Geological Survey, 2025, 3D Elevation Program 1-Meter Resolution Digital Elevation Model (published 20200606), accessed December, 2025 at URL https://www.usgs.gov/the-national-map-data-delivery

Plot ID	Elevation (m)	Slope (º)	Aspect (º)	Aspect (cardinal direction)
4	360.9	3.55	289.9	W
5	359.8	3.24	297.3	NW
7	365.0	1.61	31.3	NE
9	361.4	4.40	19.8	N
10	362.9	5.40	72.5	E
11	351.5	3.22	316.6	NW
12	350.6	3.49	352.6	N
13	349.6	3.18	18.5	N
14	348.0	2.53	55.8	NE
15	351.7	6.09	88.0	E
16	359.8	1.21	354.4	N
17	360.2	1.40	344.9	N
18	362.7	3.12	297.1	NW
21	365.3	1.54	54.2	NE
22	361.6	3.59	292.4	W