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Eastern Redcedar (*Juniperus Virginiana* L.) Encroachment on South-Central South Dakota Rangelands: Impact on Plant Communities

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EASTERN REDCEDAR (*JUNIPERUS VIRGINIANA* L.) ENCROACHMENT ON SOUTH-CENTRAL SOUTH DAKOTA RANGELANDS: IMPACT ON PLANT COMMUNITIES

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

EASTERN REDCEDAR (*JUNIPERUS VIRGINIANA* L.) ENCROACHMENT ON SOUTH-CENTRAL SOUTH DAKOTA RANGELANDS: IMPACT ON PLANT COMMUNITIES

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Grasslands historically covered 46 million km^2 of Earth's surface representing nearly 42% of the living vegetation. Encroachment of woody species onto grasslands and savannas is a widely researched global phenomenon, with eastern redcedar (ERC) (*Juniperus virginiana* L.) trees being the most prominent woody encroacher in the Northern Great Plains (NGP) due to the lack of prescribed fire and planted ERC escaping from shelterbelts. This encroachment poses a threat to native plant communities in terms of their reproduction, regeneration, and diversity. ERC are fierce competitors and can establish in most soil types, are drought tolerant, and prolific seed reproducers. These qualities coupled with their dense canopies that retain foliage year-round generate the perfect storm against native plant communities. The overall objective of this study was to determine how ERC encroachment in the NGP mixed-grass prairie is impacting plant communities. More specifically, we aimed to determine how ERC impact herbaceous species 1) biomass production, 2) composition and diversity, 3) the soil seed bank, and 4) the belowground bud bank. In addition, we classified, quantified, and weighed ERC seeds accumulated in the soil seed bank. Therefore, we attempted a holistic approach by assessing both the aboveground and belowground vegetation production and composition. This research was conducted in south-central South Dakota on two private ranches in 2020 and 2021. Biomass and foliar cover sampling were conducted during August whereas the soil seed bank and belowground bud bank were sampled in October

following seed rain. We found ERC canopies to impact all of our objectives studied (1-4) compared to grassland control locations. However, impacts were limited to primarily underneath ERC canopies. We found exponential reduction in herbaceous biomass underneath ERC canopies based on canopy diameter (m) and a linear reduction in ERC stands based on stand canopy cover (%). Aboveground vegetation composition was negatively impacted underneath ERC canopies compared to the grassland control in terms of species richness, foliar cover, and frequency of native C4 grasses. The soil seed bank underneath ERC canopies was not altered in terms of seedling emergence, but exhibited higher proportions of annual/biennial and introduced forb species. We found a large quantity (52,000 seeds/0.1m^3) of ERC seeds accumulated in the soil seed bank underneath ERC canopies and decreased in density with increasing distance from ERC stems. The belowground bud bank was significantly reduced underneath ERC canopies compared to other treatments with 70-99% fewer live buds produced and smaller proportions of native graminoid buds. This research contributes and builds upon previous literature, but also contains novel research conducted on the soil seed bank and belowground bud bank yet to be studied simultaneously to our knowledge in the NGP. Assessing the aboveground and belowground vegetation systems underneath and surrounding ERC canopies provides a holistic approach in determining current impacts of ERC encroachment and ideas for future management techniques or research needs. We recommend continued research on these topics post-removal of ERC on grassland systems.

CHAPTER 1: INTRODUCTION

The Northern Great Plains (NGP)

The Northern Great Plains (NGP) began their formation 10,000 years ago during the glacial retreat of the last ice age. The land was quickly inhabited by a forest dominated landscape, but after a few thousand years the climate became warm and dry with frequent wetting and drying cycles (Anderson 2006, Wells 1970). This new climate increased the prevalence of wildfires, and while coupled with mammalian grazing, resulted in a grassland dominated landscape that flourished under semi-arid conditions. The NGP spans from southern Canada down through much of Nebraska and west from the foothills of the eastern Rocky Mountains to western border of Minnesota, encompassing a majority of the Dakotas, Montana, Nebraska, and the northeast corner of Wyoming (Johnson and Briske 2009).

The NGP was historically maintained through a combination of frequent fires, periodic droughts, annual diebacks, and free roaming mammalian grazers, primarily *Bison bison* (Anderson 2006, Wells 1970). In addition, the gently rolling topography and continuity of the NGP increased the effectiveness of fire. With a little wind, fire could burn for miles until it was stopped by a hard fire break, such as a body of water, or natural precipitation extinguished it. This topography and continuity allowed for a single ignition to successfully burn and properly manage the grassland ecosystem on large areas of land (Wells 1970). However, following the Homestead Act of 1862, human settlement in the NGP quickly increased with settlers expanding westward to claim their government issued land. This rapid settlement disrupted the functioning of the grassland ecosystem.

During this time, land was plotted off in 65-hectare parcels, fences were built, bison were extirpated, and grazing was replaced by domestic cattle (Kopp 2004). Often times grazing enclosures were overstocked which led to overgrazing and a loss of grassland diversity. In addition, fire was not used as a management tool and wildfires were extinguished due to the fear of losing infrastructure, concerns on safety, loss of forage production, and the lack of education on ecological benefit of fire (Anderson 2006, Toledo et al. 2014). Many landowners not only lacked the equipment, labor, and insurance to conduct a prescribed fire, but it was also not accepted by others in their community (Toledo et al. 2014). Even today with the understanding of prescribed fire benefits, convincing landowners to change their societal, cultural, and social norms is a difficult task to accomplish in the NGP (Toledo et al. 2014). Through many years of fire suppression, drought, overgrazing, and planting trees around homesteads, the biodiversity of NGP has declined and encroachment by shrub and tree species has increased which were historically managed through frequent fire (Toledo et al. 2014).

The grasslands of the Great Plains exist in a wide array of climatic conditions, ranging from north to south average annual temperatures of 3-20°C and a west to east precipitation gradient of 260-1200 mm (Anderson 2006). This increasing precipitation gradient from the foothills of the Rocky Mountains in the west to the eastern border of Illinois is responsible for the change in plant community structure that creates the three grassland types in the Great Plains: short-grass prairie, mixed-grass prairie, and the tallgrass prairie. The mixed-grass prairie occurs in the range separating the short-grass and tallgrass prairies, spanning through eastern Montana, most of the Dakotas, and thinning out as it stretches through central Nebraska (Anderson 2006).

The NGP mixed-grass prairie experiences a continental semiarid climate consisting of warm summers and cold winters. Mean annual temperatures range from 1 to 18˚C with an average low of -13˚C in January and an average high of 21˚C in July (Biondini et al. 1998, Coupland 1992). Annual precipitation is highly variable and consecutive years of drought are not uncommon. Mean annual precipitation ranges from 400-600 mm, with a majority of the precipitation occurring during the growing season between April and September (HPRCC 2022). Net primary production and species composition rely heavily on the timing and amount of precipitation, where uncharacteristic wet winters and dry summers in the NGP can have significant impacts on grassland biodiversity (Biondini et al. 1998).

Vegetation in the NGP Mixed-Grass Prairie

Aboveground Vegetation and Forage Production

The NGP mixed-grass prairie is located in the space separating the short-grass and tallgrass prairies which causes it to possess characteristics similar to both the short-grass and tallgrass prairies with short grasses mixed throughout the uplands and tallgrasses in the depressions. The midgrasses dominant on the mixed-grass prairie are roughly 0.8-1.2 m tall and consist of *Nassella viridula* Barkworth (green needlegrass), *Pascopyrum smithii* (western wheatgrass), *Hesperostipa comata* (needle and thread), and *Schizachyrium scoparium* (little bluestem). Various forb and few shrub species add to the heterogeneity of the mixed-grass prairie, such as *Achillea millefolium* (common yarrow), *Echinacea angustifolia* (blacksamson echinacea), *Solidago missouriensis* (Missouri goldenrod), *Ratibida columnifera* (upright prairie coneflower), *Amorpha canescens* (leadplant), and *Artemisia frigida* (prairie sagewort). Both C3 (cool season) and C4 (warm season) plants are present on the mixed-grass prairie with cool season plants being more common and maturing in the early growing season and warm season plants during the later growing season (Epstein et al. 1998).

Grazing, both wild and domestic, has played an important historical role in the NGP mixed-grass prairie (Johnson and Briske 2009). Much of the mixed-grass prairie is not suitable for row-crop agriculture and is primarily used for cattle operations (Johnson and Briske 2009), which are often more profitable long term on low-fair condition rangelands compared to excellent condition rangelands (Dunn et al. 2010). As a result, rangeland forage production is critical to the NGP economy and reductions could be detrimental to some operations (Johnson and Briske 2009, Smart et al. 2007). Productivity in the mixed-grass prairie is proportionally impacted by annual variability in precipitation (Knapp and Smith 2001) with spring precipitation often being accurate in estimating annual forage production in the mixed-grass prairie due to dominance by C3 perennial grasses (Biondini and Manske 1996). However, Smart et al. (2007) found that annual production is not always proportional to annual precipitation and may also fluctuate within an ecological site based on soil water content, aspect, and slope or can be explained by other climatic variables including previous spring precipitation and days until last spring freeze. Forage production in the mixed-grass prairie ranges from 960-2100 kg/ha (Redmann 1975) with annual reported means of 1600 kg/ha in South Dakota (Smart et al. 2007) and 1430 kg/ha in Wyoming (Andales et al. 2006). Stocking rates may vary annually between 0.88-1.02 animal unit months (AUM)/ha based on annual production, rangeland condition, and other operation variables (Dunn et al. 2010). Aboveground vegetation production and composition is dependent on the belowground processes including regeneration from asexual reproduction through crown and rhizome buds (bud bank) and sexual reproduction through seed propagation (seed bank) (Benson and Hartnett 2006).

Seed Bank

Soil seed banks provide temporal reserves of historic plant populations and can influence genetic heterogeneity within a population, species richness, and species diversity (Perkins et al. 2019, Plue and Cousins 2013). Seed banks are composed of seeds produced from onsite sexual reproduction and off-site seed immigration by dispersal through wind, water, or other vectors (e.g. animals, humans, etc.) (Soons et al. 2004), although most seeds are dispersed short distances (< 30 m) from their parent source (Wilson 1993). Some seeds even possess adaptations to allow for further dispersal distances including fluffy pappas (Skarpaas et al. 2004), wing-like structures, or seed hooks (Nathan 2006). Seed production is variable among species and is usually dependent on life span, where some annual plants, such as *Bromus tectorum* (cheatgrass), can produce hundreds of seeds per individual (Pyke and Mack 1983). Annual plants rely heavily on seed production and success, being it's their sole method of reproduction (Burnside et al. 1996). Perennial plants may have minimal to high seed production, with some plants producing only a few seeds in their lifetime (Arizaga et al. 2000). Seed longevity is influenced by disturbance (fire, grazing, and seed predation) and varies among species, with forbs often outlasting grasses due to their hard seed coat (Baskin and Baskin 2001, Snyman 2010). Forbs can germinate after sown in the seed bank for up to 17 years (Burnside et al. 1996) whereas grasses can persist up to 5 years, but viability of most grasses doesn't last more than a year (Baskin and Baskin 2001, Burnside et al. 1996, Snyman 2010). Seed banks can be important in perennial dominated grasslands, especially following disturbance events or in extremely disturbed landscapes, but the bud bank is responsible for a majority of plant regeneration in an undisturbed system and seedling establishment from the seed bank is rare (Benson et al. 2004, Benson and Hartnett 2006, Rogers and Hartnett 2001).

Bud Bank

Vegetative reproduction via the bud bank is produced asexually by perennial plants and is their primary method of regeneration in the NGP mixed-grass prairie (Ott and Hartnett 2015), where researchers have found bud banks contributing up to 99% of new vegetation (Benson and Hartnett 2006). Parent individuals (genets) produce genetically identical offspring (ramets) asexually through vegetative propagation from dormant buds including rhizomes/stolons, tillers, bulbs, tubers, and corms (Harper 1977). These independent ramets are commonly connected through rhizomes (below soil surface) and stolons (above soil surface) to the genet where they can access and store nutrients, water, and carbohydrates (Alpert and Mooney 1986). Rhizomes and stolons provide the genet with the ability to spread horizontally to find new resource pockets including increased growing space, light availability, and soil moisture (Harper 1977). Bud bank production varies within and among species (Lehtilä 2000, Ott and Hartnett 2012) in perennial dominated ecosystems and can be impacted by competition, climate, resource availability, or disturbance regime (Ott and Hartnett 2015) including grazing, fire, and drought (Klimešová and Klimeš 2007). Healthy perennial grassland populations rely on vegetative reproduction via the bud bank for population persistence and resiliency to climate change, disturbance, and extreme weather events (Ott and Hartnett 2015).

Woody Encroachment

Woody encroachment is a global phenomenon impacting grassland and savanna biomes with increasing severity caused by fire suppression, climate change, and anthropogenic influences (Van Auken 2009). Over the years, woody encroachment has increased throughout the Great Plains grasslands primarily due to fire suppression (Twidwell et al. 2013). Prairie ecosystems tend to be the most at risk due to being mainly dominated by warm season plants, which require adequate light, high temperatures, and decreased moisture (Twidwell et al. 2021). This woody encroachment comes in many forms, such as cottonwood (*Populus spp.*), dogwood (*Cornus spp.*), buckthorn (*Rhamnus spp.*), sumac (*Rhus spp.*), pines (*Pinus spp.*), and junipers (*Juniperus spp.*) (Stritzke and Bidwell 1989, Twidwell et al. 2013, Van Auken 2009), with junipers (*Juniperus spp.*) and pines (*Pinus spp.*) being the most common woody encroachers in the United States (Miller et al. 2000). However, in the Great Plains, ashe juniper (*Juniperus ashei*) and ERC are the dominate encroachers, specifically ERC in the NGP of South Dakota (Meneguzzo and Liknes 2015, Schmidt and Leatherberry 1995, Twidwell et al. 2013). As woody densities increase on grasslands and shrub lands, we see a response in herbaceous communities in terms of reduced production, altered composition, and decreased diversity (Van Auken 2009).

Impact on Aboveground Vegetation

The impacts of woody invaders on grassland communities are species dependent and vary spatially but are overall detrimental to prairie ecosystems (Van Auken 2009). The most prominent impact of woody encroachment on grassland ecosystems is the reduction in forage biomass (Dye et al. 1995) resulting in decreased livestock production

(Anadón et al. 2014). Many researchers have found reductions in under canopy biomass production due to shading impacts in *Juniperus ashei* (Ashe juniper), *Juniperus monosperma* (one-seed juniper), *Juniperus occidentalis* (western juniper), *Juniperus pinchotii* (redberry juniper), *Juniperus virginiana* (eastern redcedar), and *Cornus drummondii* (rough-leaved dogwood) in the Great Plains (Arnold 1964, Bates et al. 2000, Dye et al. 1995, Fuhlendorf et al. 1997, Lett and Knapp 2005, Limb et al. 2010, McPherson and Wright 1990). These reductions in biomass production are largely due to the decrease in herbaceous plant cover (Allen and Nowak 2008) resulting from decreased light and soil moisture (Owens et al. 2006). Woody plant canopy cover and herbaceous biomass production are inversely related and commonly explained by linear (Limb et al. 2010), logarithmic (McPherson and Wright 1990), or second-degree polynomial (Alford et al. 2012, Pieper 1990) relationships. Researchers also found woody encroachment to decrease flora species diversity (Briggs et al. 2002), evenness (Limb et al. 2010), richness (Dye et al. 1995, Lett and Knapp 2005, Knapp et al. 2008), foliar cover (Arnold 1964, Dye et al. 1995), and shift communities from warm season to cool season species (Gehring and Bragg 1992). Proper management of these woody invaders has proven to reverse their negative effects rather quickly in most cases (Alford et al. 2012, Bates et al. 2017, Dittel et al. 2018, Fuhlendorf et al. 1997, Limb et al. 2010, McPherson and Wright 1990), but requires patience and may take multiple years at other sites (Bates et al. 2000, Gehring and Bragg 1992).

Impact on Belowground Propagules

Few studies exist that evaluated the impact of woody encroachment on the soil seed bank (Allen and Nowak 2008), with only one study to our knowledge that assesses how it affects the belowground bud bank (Ferarro et al. 2020). In central Nevada on encroached sagebrush communities, Pinyon-juniper tree cover did not impact seed density and species diversity in the soil seed bank as tree cover increased (Allen and Nowak 2008). The seed bank community based on seed density by species and by life form was not different between tree cover classes (Allen and Nowak 2008) and its common for the soil seed bank to be homogeneous among microhabitats (Allen and Nowak 2008, Torres, et al. 2012). Low correspondence existed between aboveground vegetation and seed bank composition (Allen and Nowak 2008), indicating limited potential for restoration from seed bank alone following woody encroachment control (Gorzen et al. 2019, Lang and Halpern 2007). Over 62.5% of the species in the seed bank did not occur in the standing vegetation, most of these being annual forbs (Allen and Nowak 2008, Koniak and Everett 1982). In contrast, *Leptospermum scoparium* (Manuka) trees in southern Australia were found to reduce seed bank species richness and abundance underneath their canopies (Price and Morgan 2008). Similar results were also found in the western Cascade Range of Oregon from conifer encroachment on mountain meadows (Lang and Halpern 2007) and *Quercus suber* (cork oak) in central Spain (Torres et al. 2012). Although some research exists on woody encroachment and the soil seed bank, how woody encroachment may impact seed and bud bank production and composition in the NGP is a research need.

Belowground bud bank research is rare, especially studies that focus on woody encroachment on grassland or savanna ecoystems. In the Brazlian savanna, massive reductions in bud bank production were observed in 50-year-old *Pinus elliottii* L. (slash pine) plantations compared to open non-encroached savannas (Ferraro et al. 2020). This reduction in bud bank production leads to a loss in savanna resiliency and may require active interventions for restoration after the removal of slash pines (Ferarro et al. 2020).

Eastern Redcedar

Eastern redcedar (*Juniperus virginiana* L.) (hereafter "ERC") is a widely distributed conifer tree that is variably and vastly spread throughout the United States (Van Haverbeke and Read 1976). The morphological structure of ERC is similar to a cone-pyramid and can reach heights of 10-20 meters at maturity. ERC grow 20-40 cm a year, both vertically and horizontally, and are considered "fast-growing" for a conifer species (Van Haverbeke and Read 1976). ERC are commonly dark green to bluish green in color and have a deep red colored bark. These trees are strictly dioecious, meaning their male and female reproductive flowers are found on separate trees (an individual tree is either male or female). Male staminate cones are small, scaly, found at the tip of branches, and are about an inch long. Female ovulate cones are found solely at the tip of branches, dark blue to purple in color, and berry-like. This fruit is about 4 to 8 mm in diameter, which contains anywhere between 2-4 seeds (Stritzke & Bidwell 1989, Van Haverbeke and Read 1976). The seeds range between 2-4 mm in diameter, are yellow/brown in color, have a hard seed coat, and may have shallow pits (Van Haverbeke and Read 1976). ERC rely on sexual reproduction and seed dispersal for recruitment (Holthuijzen and Sharik 1985). ERC may have low viability and germination in their seeds, but one female tree can produce up to 1.5 million berry-like cones each year (Holthuijzen and Sharik 1985, Twidwell et al. 2021). If an ERC is severed at the trunk below the bottom-most branch, it is not able to resprout (Ortmann et al. 1998).

In the Great Plains, ERC is the most prominent woody encroacher (Meneguzzo and Liknes 2015, Schmidt and Leatherberry 1995). ERC are spreading at alarming rates and have been termed the "green glacier" by researchers, occupying up to seven million hectares of rangeland and increasing exponentially in some areas (Bidwell et al. 1996, Engle et al. 2008, McKinley et al. 2008). ERC is an early successional native conifer species in North America present in every state east of the 100th meridian, with higher densities in Oklahoma, Kansas, Nebraska, Missouri, and South Dakota (Meneguzzo and Liknes 2015, Twidwell et al. 2021). ERC have increased by nearly 125,000 hectares in an eight-state region in the Northern Great Plains (NGP) between 2007-2012, by 2.3% per year in portions of the Kansas Flint Hills, and at a rate of 8% or 110,000 hectares per year in Oklahoma (Briggs et al. 2002, Meneguzzo and Liknes 2015, Wang et al. 2018, Zang and Hizirolgu 2010). Fire historically controlled and confined this native conifer species primarily to riparian areas or steep, rocky slopes (Lawson 1990). ERC are drought tolerant, have an extensive root system, require minimal nutrients, thrive in all soil types, and produce up to 1.5 million berry-like cones from a mature female tree (Engle et al. 1987, Holthuijzen and Sharik 1985). These attributes of ERC coupled with fire suppression, overgrazing, periodic drought, and planting ERC in shelterbelts have allowed ERC to successfully encroach and spread rapidly on grasslands in the Great Plains (Briggs et al. 2002, Lawson 1990). Avian generalists, small mammals, and white-tailed deer are known to eat the berry-like cones off ERC contributing to its seed dispersal and propagation on our grassland systems (Bidwell et al. 1996, Holthuijzen and Sharik 1985, Horncastle et al. 2004) resulting in a potential closed canopy in as little as 40 years (Briggs et al. 2002).

Impact of ERC on Aboveground Vegetation

Cattle operations are vital to the NGP culture and economy, which rely heavily on forage biomass production on their rangelands. ERC canopies reduce biomass production by limiting light penetration and inhibiting precipitation from reaching the soil surface beneath the canopy (Engle et al. 1987, Starks et al. 2014). Biomass reduction is limited primarily to underneath individual ERC canopies, with little reduction occurring at the canopy edge in comparison to open grassland sites (Briggs et al. 2002, Engle et al. 1987, Engle and Kulbeth 1992, Limb et al. 2010). Up to 70-99% reduction in biomass production is common underneath ERC canopies in comparison to open non-encroached grassland (Briggs et al. 2002, Engle et al. 1987, Smith and Stubbendieck 1990). In 2001, an estimated $100 million was lost in Oklahoma due to juniper encroachment and is expected to reach $205 million by 2013 (Hendrix 2002). To understand the heterogeneity of ERC stand encroachment on biomass production, Limb et al. (2010) studied how ERC stand densities impact overall biomass production. A linear relationship between ERC canopy cover (%) and herbaceous biomass production (kg/ha) was found in the tallgrass prairie of Oklahoma, also supported by Bidwell et al. (1996), with about 450 kg/ha in biomass lost for every 10% increase in ERC stand canopy cover (Limb et al. 2010). This ERC encroachment in the Great Plains leads to decreased livestock carrying capacities, stocking rates, pasture visibility, and increases labor hours, production costs, and extreme wildfire risk (Archer and Predick 2014, Bidwell et al. 1996).

Limited light, litter accumulation, and reduced soil moisture alters the plant community composition underneath ERC canopies (Engle et al. 1987, McKinley et al. 2008, Starks et al. 2014). In the Platte River Valley of Nebraska, open non-encroached plots were dominated by *Schizachyrium scoparium* (Michx.) Nash (little bluestem) whereas shaded plots underneath ERC were dominated by *Poa pratensis* L. (Kentucky bluegrass), implying a shift from C4 to C3 grasses resulting from ERC encroachment (Briggs et al. 2002, Gehring and Bragg 1992). However, this trend was not present in all C4 and C3 grass species from grassland plots to shaded plots (Gehring and Bragg 1992). This suggests the impact of ERC canopies might be species dependent or may rely on other environmental variables rather than solely on photosynthetic pathways. In contrast, Limb et al. (2010) found a decline in both C4 and C3 grasses and forbs along an ERC encroachment gradient, suggesting all herbaceous species decrease in cover resulting from ERC canopies. Cover of species is dependent on individual ERC canopy diameter, with a rare increase in some species (*Carex spp.*) and a decrease in most (Buehring et al. 1971, Coppedge et al. 2001, Gehring and Bragg 1992). Species diversity, evenness, and richness decreased underneath ERC canopies (Briggs et al. 2002, Horncastle et al. 2004, Meneguzzo and Liknes 2015) and within ERC stands (Limb et al. 2010) in comparison to non-encroached grassland. The change in plant communities resulting from ERC encroachment threatens the resiliency of our grasslands and has shown to alter native fauna diversity, displacing endemic grassland species (Coppedge et al. 2001, Engle et al. 2008).

ERC Seed Dispersal

Research thus far has focused on the dispersal agents, viability, longevity, germination success, and predation of ERC seeds (Holthuijzen and Sharik 1984; 1985, Holthuijzen et al. 1986, Horncastle et al. 2004, Livingston 1972, Parker 1952, Phillips 1910, Van Dresal 1938), with only one study to our knowledge on the accumulation and density of ERC seeds in the soil seed bank (Tunnell et al. 2004). In addition, whether ERC encroachment impacts the herbaceous soil seed bank has yet been studied. Birds and small mammals are responsible for ERC seed predation and dispersal, where small mammals, such as Opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*), commonly feed on cones found at the soil surface and birds remove cones from ERC branches (Horncastle et al. 2004). Seventy-one species were reported to feed on ERC berries and foliage (Van Dersal 1938) with birds responsible for 60-90% of seed dispersal (Phillips 1910) due to their increased mobility (Holthuijzen and Sharik 1985). American robins (*Turdus migratorius*) and cedar waxwings (*Bombycilla cedrorum*) are among the most abundant dispersers of ERC seeds (Livingston 1972) where an individual tree may receive visits from 37.1 birds/hr and 59.1 birds/hr in October and December, respectively (Holthuijzen and Sharik 1985). In Virginia, mature ERC were found to produce 87,000-1,592,000 seed bearing cones per tree with an average ripe (mature seed) and viable percentage of 58.6% and 35%, respectively (Holthuijzen and Sharik 1985). Of this seed crop, up to 67.6% is dispersed (>12 m) away from the tree (Holthuijzen and Sharik 1985) commonly dropped along fencerows and power lines where birds perch (Holthuijzen et al. 1986). ERC seeds were found in feces of yellow-rumped warblers (*Dendroica coronata*), cedar waxwings, and starlings (*Sturnus vulgaris*) which yielded mixed results in germination success where Holthuijzen and Sharik (1985) found a 1.5-3.5 fold increase while others found avian passage to have an inhibitory effect on ERC germination (Livingston 1972). Mixed results exist of ERC seed predation in the soil where over 50% predation has been reported (Livingston 1972) while others found no evidence of seed predation (Holthuijzen and Sharik 1984, Parker 1952). Longevity and viability of ERC seeds in the soil exponentially decreases with time where only 5.5% of sown seeds are viable after 14 months (Holthuijzen and Sharik 1984) and 5-10% are viable when collected directly from the seed bank (Tunnell et al. 2004), which is surprising due to their hard seed coat (Van Haverbeke and Read 1976). Although accumulation of viable ERC seeds in the soil is low (Holthuijzen and Sharik 1984, Tunnel et al. 2004), the factors of dispersal and increased germination allow ERC to successfully establish and encroach new territory (Holthuijzen and Sharik 1985, Holthuijzen et al. 1986, Horncastle et al. 2004).

Impact of ERC on Wildlife

As ERC encroach on grassland ecosystems, they alter the structure and composition of the landscape, in turn impacting wildlife populations (Smith 2011). ERC encroachment on grasslands is usually detrimental to native wildlife populations (Chapman et al. 2004, Coppedge et al. 2001), but can be beneficial to some species depending on the density of an ERC stand and the size of individual trees (Strizke and Bidwell 1989). Small, isolated stands of ERC can be beneficial through providing thermal cover, wind resistance, nesting cover, and a foraging source in their cone berries (Bidwell et al. 1996). In contrast, most native (especially endemic) wildlife species are overall negatively impacted by significant ERC encroachment, such as northern bobwhite quail (*Colinus virginianus*), lesser and greater prairie chickens (*Tympanuchus* spp.), and mule deer (*Odocolieus hemionus*) (Chapman et al. 2004, Coppedge et al. 2001). Ideally, planting a small stand of sterile individuals or only male trees on a given parcel of land would be the most beneficial towards wildlife (Strizke and Bidwell 1989).

Many different generalist avian species use ERC for foraging and nesting opportunities, such as mockingbirds (*Mimus polyglottos*), chipping sparrows (*Spizella passerina*), mourning doves (*Zenaida macroura*), cedar waxwings, and robins (Smith 2011). In terms of forage opportunities, ERC are commonly exploited for their berry-like cones with minimal browsing on their twigs and foliage. Mourning doves are the most common species to use ERC for nesting cover, usually building their nests just a few feet off the ground and near agriculture fields due to nearby foraging options on prairie seed or agriculture grain (Bidwell et al. 1996).

ERC are displacing native wildlife species that have historically relied on open continuous grasslands and shrublands (Coppedge et al. 2001, Smith 2011). Dense stands can impact waterfowl and upland gamebird nesting, turkey roosting, and historic lekking grounds (Bidwell et al. 1996, Coppedge et al. 2001, Smith 2001, Smith 2011). Endemic grassland species are many times specialists and impacted the most by woody encroachment (Bidwell et al. 1996). As little as 25% juniper cover can force grassland bird abundance and richness to nonexistence (Coppedge et al. 2001) and displace entire turkey flocks (Smith 2001). This altered habitat and wildlife displacement also has an economic impact. Throughout the Midwest, many ranches rely on pristine grasslands to leased hunting experiences (Bidwell et al. 1996, Hendrix 2002). In Oklahoma, an estimated $52 million was lost in hunting leases in 2001 due to ERC encroachment and could reach $124 million by 2013 at current encroachment rates (Hendrix 2002).

Impact of ERC on Abiotic Factors

ERC encroachment alters abiotic environmental factors including soil moisture, groundwater levels, and surface runoff/infiltration. (Adane and Gates 2015, Caterina et al. 2014, Thurow and Carlson 1994, Zou et al. 2015). ERC have a large root system, extending both laterally and vertically (Thurow and Hester 1997). In addition to their deep taproots, they have an extending blanket of fibrous roots that exist just beneath the soil surface, allowing ERC to extract water from soils with low moisture levels and at depths herbaceous vegetation is unable to access (Acharya et al. 2017a). ERC tend to deplete more from deep soil water reserves (Acharya et al. 2017a) and commonly pull from deep soil water during dry periods and shallow soil water during moist periods (Eggemeyer et al. 2009). ERC use on average 24 L of water daily, with some large trees exceeding 60 L (Caterina et al. 2014). Soil moisture levels underneath ERC canopies are lower than open grasslands (Engle et al. 1987, Smith and Stubbendieck 1990) with an average moisture level of 2.5% underneath the canopy compared to 9.7% in an open Nebraska grassland (Adane and Gates 2015). This reduced soil moisture under ERC canopies is likely due to over 35% of precipitation fails to reach the soil surface due to ERC canopy interception (Dueserhaus 2008, Starks et al. 2014, Zou et al. 2015) and only up to 8% of the precipitation that reaches the soil surface is retained in the litter prior to reaching the mineral soil (Acharya et al. 2017b). The amount of rainfall that reaches the soil surface depends on the density of foliage on the tree and the intensity of the rainfall event (Thurow and Hester 1997). For the precipitation that reaches the soil surface, infiltration rates triple, and surface runoff is reduced by 80% in ERC forests due to an increase in soil organic matter and a decrease in bulk density (Zou et al. 2014). However, due to their high water usage, ERC reduce groundwater recharge up to 85% (Adane and Gates 2015) increasing the risk of a future water crisis in the Great Plains at current encroachment rates (Zou et al. 2018).

ERC Control

Wildfire historically managed woody encroachment on our rangelands and inhibited the spread of forest from northern latitudes or riparian areas (Higgins 1986). The lack of resources, public outreach, and the fear of prescribed fire in the NGP is responsible for limited fire use resulting in extensive ERC encroachment (Twidwell et al. 2013). Using prescribed fire to control ERC was found successful at early stages of encroachment in multiple studies (Buehring et al. 1971, Engle et al. 1987, Martin and Crosby 1955, Nippert et al. 2021, Ortmann et al. 1998, Owensby et al. 1973, Rollins 1985), even in the understory of mature hardwoods using deciduous leaf litter as a fuel (Engle and Stritzke 1995). Up to 90% mortality is possible for ERC <1 m tall using prescribed fire as a treatment, but its efficacy falls with increasing tree height resulting in a range of 10-50% morality for trees taller than 3 m (Nippert et al. 2021, Ortmann et al. 1998). Contrasting results were found in Buehring et al. (1971) for larger trees where mortality rates in trees less than a meter were above 90% and 50-80% in trees near 3 meters. However, tree kill did decrease with height, supporting the findings of Ortmann et al. (1998). The reasoning behind reduced kill on larger trees may be due to minimal fuel loads underneath the canopy, thick bark, and the lethal flame threshold not reaching higher foliage. These findings suggest broadcast prescribed fire can only be used effectively for young ERC stands (Buehring et al. 1971, Ortmann et al. 1998, Owensby et al. 1973). Mechanical removal of ERC is nearly 100% effective through shearing, mechanical diamond blades, chaining, or manual chainsaws, but can be extremely costly (Bidwell et al. 1996, Ortmann et al. 1998). The effectiveness of chemical treatments resembles that of prescribed fire with efficacy severely decreasing with trees taller than 3 m (Ortmann et al. 1998). As a result, a combination of prescribed fire, mechanical removal, and chemical removal is often required to successfully combat dense stands of ERC and is the most cost-effective strategy (Bidwell et al. 1996, Engle and Stritzke 1992, Ortmann et al. 1998). At an ERC encroached site, researchers suggest building a fine fuel load through deferred grazing, cutting and stuffing ERC near grassland/woodland transition points, conducting a prescribed burn, and then following up with a mechanical or chemical treatment to remove survivors (Bragg and Hulbert 1976, Ortmann et al. 1998, Rollins 1985).

Fine fuel load influences ERC mortality through the size and intensity of the prescribed fire. Strizke & Bidwell (1989) discussed the influence of fine fuel load on ERC mortality. Seedlings and trees less than 50 cm in height will usually be killed regardless of fire severity. In this case, minimal fuel loads of as low as 2,250 kg/ha can be used to kill small trees but those between 1-3 meters may only have a mortality of 60%. In addition, trees less than 3 meters tall can consistently be managed with fuel loads above 4,500 kg/ha (Strizke & Bidwell 1989). Rangelands that consist of ERC that are greater than 3 meters in height will rarely have an adequate fine fuel load to obtain a mortality rate of 50% (Strizke & Bidwell 1989). Canopy cover and density also influence the success of a prescribed burn. Increasing ERC canopy cover reduces the effectiveness of prescribed burning (Engle et al. 1987). As canopy cover increases and trees become larger, the fine fuel load required for an effective prescribed burn increases (Engle et al. 1987). As a result, some rangelands will need to lower their stocking rates or defer grazing for one season to keep an adequate fuel load available for a future prescribed burn. At some point, ERC stands will reduce herbaceous forage production to where there is not enough fine fuel to effectively burn the stand even without grazing pressure (Engle et al. 1987, Smith 2011).

Weather conditions and fine fuel loading are not the only variables to consider when controlling ERC with prescribed burning (Bidwell et al. 1996, Strizke and Bidwell 1989). The time of year and ERC leaf water content can impact the success of the burn and the succession following the burn disturbance (Engle et al. 1987). Early burns may reduce future forage production, so burning between April 15-May 1 is recommended (Engle et al. 1987). Leaf water content and surrounding soil water content may also influence the flammability of ERC and the intensity of the fire. Results from Engle et al. (1987) suggest that leaf water content and soil water content reflect the growing seasons. In other words, values tended to be high in the late spring and early summer months, with lower, consistent values the rest of the year (Engle et al. 1987). For the study years, the leaf and soil water content spiked in May – June. This suggests that prescribed burning would be most effective prior to that spike, which supports previous recommendations (Engle et al. 1987).

In general, removal of *Juniperus spp.* increases herbaceous biomass production, species diversity, species evenness, and total flora and fauna richness (Alford et al. 2012, Bates et al. 2000, Evans and Young 1985). Whether rangelands will quickly return to pre-encroachment conditions following the removal of ERC has been widely debated in previous research. Some research suggests rangelands will experience a "legacy effect" following the removal of ERC (Gehring and Bragg 1992) whereas others indicate a swift return to a pre-encroachment grassland site is possible (Alford et al. 2012, Limb et al. 2010, Owensby et al. 1973, Pierce and Reich 2010). The response of rangeland plant communities following ERC removal has not yet been studied in the NGP. Understanding how plant communities respond to ERC removal will be essential when discussing potential ERC management with landowners.

Future of the Great Plains

Historically, the Great Plains was developed and maintained through a regime of fire and grazing (Anderson 2006, Higgins 1986). Today, the suppression of wildfire and improper land management has led to woody encroachment, loss in diversity, and ecosystem peril on grasslands throughout the Midwest. With what little grassland remains in the Great Plains, we as managers need to unite to properly manage and sustain these prairie ecosystems. These issues we face on our grasslands will continue if proper land management is not implemented. Woody encroachment alone is deeply impacting our prairie ecosystems through a loss in forage production, habitat fragmentation, wildlife displacement, change in vegetative composition, and hydrological health (Van Auken 2009). To eliminate future woody encroachment, management techniques, such as prescribed fire, should be applied every 5-10 years, since ERC trees begin seed-bearing at roughly 10 years old (Strizke & Bidwell 1989). Prescribed fire is the most cost effective and ecosystem friendly form of land management in the Great Plains (Toledo et al. 2014). The future of the Great Plains should revolve around implementing prescribed fire as a management tool on our prairie ecosystems. In addition, we need to stress the importance of establishing non-profit prescribed burn associations (PBAs). Often times private landowners may be aware of the importance of using fire as a management tool, but do not have the means of implementing fire themselves, due to a lack of equipment, labor, insurance, or fire knowledge (Toledo et al. 2014, Twidwell et al. 2013). Since most state and federal agencies cannot legally aid private landowners with conducting a prescribed fire, private landowners with common management goals need to pool their knowledge and labor together (in PBAs) to implement proper prescribed burning techniques on their lands (Toledo et al. 2014, Twidwell et al. 2013). We acknowledge that the Great Plains is responsible for the export of many goods and services (ie. livestock, biofuel, forage), but understanding current issues we face on our grasslands is essential for future management and sustainability (Johnson and Briske 2009). Restoration and preservation of healthy grasslands should be a moral responsibility for all ecologists, rangeland managers, and habitat enthusiasts.

Research Overview

The overall purpose of this study is to evaluate the effects of ERC encroachment on plant communities in the NGP mixed-grass prairie. More specifically, we aim to determine how ERC at the individual and stand level impact plant communities in terms of herbaceous biomass production, plant community composition, soil seed bank, and the bud bank. Zou et al. (2018) documented the research conducted thus far on ERC in the Great Plains. Most of the research on ERC was conducted in the Southern Great Plains, primarily in Oklahoma, with little research in the NGP, especially South Dakota (Zou et al. 2018). Our research on the impact of ERC encroachment on herbaceous biomass production and plant community composition builds off previous work (Briggs et al. 2002, Engle et al. 1987, Gehring and Bragg 1992, Limb et al. 2010), but it is the first of its kind in the NGP mixed-grass prairie and with substantial replication. In addition, our research on how ERC encroachment impacts the soil seed bank and bud bank is novel and will contribute to filling the current knowledge gap on these subjects in the literature.

The objectives of this project were to evaluate the impact of ERC encroachment on 1) herbaceous biomass production, 2) aboveground plant community composition and structure, 3) soil seed bank production and composition, 4) the density and classification of ERC seeds in the soil seed bank, and 5) the bud bank production and composition. The alternative hypotheses based on previous or related literature for this study were:

1. Herbaceous biomass production
    a. Herbaceous biomass production will not significantly differ underneath ERC trees with canopy diameters less than two meters in comparison to grassland control plots. ERC tress with canopy diameters greater than two meters will significantly reduce herbaceous biomass production with minimal biomass production resulting from trees with canopies greater than six meters.
    b. As ERC stand canopy cover (%) increases, herbaceous biomass production (kg/ha) will be reduced linearly at nearly a 1:1 ratio with ERC stand canopy cover (%).
2. Aboveground plant community composition and structure
    a. Species diversity, evenness, richness, and floristic quality index will decrease with increasing ERC canopy diameters. More specifically, the frequency and cover of introduced graminoids, some annual/introduced forb species, and ERC seedlings will increase and native graminoids and forbs will decrease as ERC canopy diameter increases.
3. Soil seed bank production and composition

a. Total seed bank production will not differ between samples collected underneath or near ERC trees in comparison to open grassland control samples.

b. The soil seed bank composition will be significantly different directly underneath ERC canopies due to an increase in annual forbs and introduced species in comparison to the seed bank at the canopy edge, two meters from the canopy edge, and grassland control. Soil seed bank production and composition will not differ between the canopy edge, two meters from the canopy edge, and grassland control.

4. Density and classification of ERC seeds in the soil seed bank

    a. ERC seed density will be highest underneath the canopy and a majority of seeds will be classified as damaged or unviable.

    b. ERC seed density will be minimal in samples collected two-meters from ERC canopies and in grassland control locations.

    c. Based on previous literature (Holthuijzen and Sharik 1984, Parker 1952), minimal seed predation will occur in ERC seeds accumulated in the soil seed bank.

5. Vegetative bud bank production and composition

    a. Live bud production will be minimal underneath ERC canopies and will be similar between the canopy edge, two meters from the canopy edge, and grassland control samples.

    b. ERC canopies will negatively affect bud composition by increasing the percent of introduced grasminoid buds.

This study as a whole attempts to evaluate the full picture of ERC encroachment on grassland plant communities by assessing both aboveground and belowground plant communities. We conducted this research on two private ranches in south-central South Dakota but were unable to have temporal replication due to the severe drought during the spring and summer of 2021 inhibiting prescribed fire. However, we were able to collect data during two years with very different precipitation regimes occurring during the growing season. The total precipitation during the growing season was 15% higher than and 37% below the 30-year average for 2020 and 2021, respectively. This allowed us to evaluate the relationship between ERC encroachment and plant communities during a wet (2020) and an abnormally dry (2021) growing season. The results of this study will aid in future management of ERC in the NGP mixed-grass prairie and shed light on how plant communities might respond following the removal and control of ERC on encroached grasslands.

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CHAPTER 2: IMPACT OF EASTERN REDCEDAR CANOPY DIAMETER AND STAND CANOPY COVER ON ABOVEGROUND PRODUCTION AND COMPOSITION IN THE NORTHERN GREAT PLAINS MIXED-GRASS PRAIRIE

ABSTRACT

Eastern redcedar (ERC) (*Juniperus virginiana* L.) trees are invading prairies throughout the Great plains due to fire suppression and escaping from planted ERC shelterbelts. This encroachment poses a threat to native plant communities in terms of their reproduction, regeneration, and diversity. The objectives of this study were to determine how ERC canopies impact herbaceous biomass production and composition. Square quadrats (0.25 m^2) were placed in four cardinal directions underneath canopies of ERC trees ranging from 0.1-10 m in diameter and at grassland control locations. We collected herbaceous biomass samples and foliar cover estimates underneath 326 ERC trees and at 240 grassland control locations among two sites totaling 1381 samples overall. Samples were averaged by ERC tree as the sample unit for herbaceous biomass samples and with a quadrat as the sample unit for foliar cover analyses. We found herbaceous biomass production underneath ERC canopies to decrease exponentially with increasing ERC canopy diameter with 63-97% reduction under trees with canopies larger than 2 meters. Also, biomass production decreased linearly with increasing ERC stand canopy cover (%) at nearly a 1:1 ratio. Mean foliar cover for all species, floristic quality index, species richness, and native species richness decreased as ERC canopy diameter increased. Our results indicate that ERC encroachment is not only reducing herbaceous biomass production, but it is also altering the composition of plant communities. This highlights the importance of ERC control on our grasslands and provides landowners

with data that can be applied to their individual operation. To maintain or restore our native grasslands, we suggest the removal of ERC through prescribed fire and/or mechanical removal every 5-10 years. Following these management strategies should maintain a healthy grassland system.

INTRODUCTION

Grasslands historically covered 46 million km^2 of Earth's surface representing nearly 42% of the living vegetation (Anderson 2006). Encroachment of woody species onto grasslands and savannas is a widely researched global phenomenon, with junipers (*Juniperus spp.* L.) and pines (*Pinus spp.* L.) being the most common woody encroachers in the United States (Miller et al. 2000). Historic grasslands in North America were maintained through a combination of wildfire and grazing, inhibiting the spread of forest from northern latitudes or riparian areas and resulting in a grass dominated landscape for the last 5000-8000 years (Higgins 1986, Twidwell et al. 2013). Since European settlement, dramatic changes have converted our grasslands from their historical state through fire suppression, cultivation, and woody encroachment (Engle et al. 2008). This woody encroachment in North America has led to grassland systems being one of the most endangered ecosystems (Engle et al. 2008). The increase of woody plants on grasslands alters nutrient cycling, forage production, flora and fauna species composition, landscape heterogeneity, and risk of wildfire (Belsky 1994, DeSantis et al. 2011, Knapp et al. 2008, Limb et al. 2010, Miller et al. 2000, Van Auken 2009, Van Els et al. 2010, Wang et al. 2018, Williams et al. 2017).

In the Great Plains, eastern redcedar (*Juniperus virginiana* L.) (hereafter ERC) is the most prominent woody encroacher (Meneguzzo and Liknes 2015, Schmidt and

Leatherberry 1995). ERC are spreading at alarming rates and have been termed the "green glacier" by researchers, occupying up to seven million hectares of rangeland and increasing exponentially in some areas (Bidwell et al. 1996, Engle et al. 2008, McKinley et al. 2008). ERC is an early successional native conifer species in North America present in every state east of the 100^{th} meridian, with higher densities in Oklahoma, Kansas, Nebraska, Missouri, and South Dakota (Meneguzzo and Liknes 2015, Twidwell et al. 2021). ERC have increased by nearly 125,000 hectares in an eight-state region in the Northern Great Plains (NGP) between 2007-2012, by 2.3% per year in portions of the Kansas Flint Hills, and at a rate of 8% per year in Oklahoma (Briggs et al. 2002, Meneguzzo and Liknes 2015, Wang et al. 2018). Fire historically controlled and confined this native conifer species primarily to riparian areas or steep, rocky slopes (Lawson 1990). Fire suppression, overgrazing, and planting ERC in shelterbelts has allowed ERC to successfully encroach and spread rapidly on grasslands in the Great Plains due to its ability to compete for scarce resources and its high reproductive rate, with female trees producing up to 1.5 million berry-like cones on productive years (Briggs et al. 2002, Engle et al. 1987, Holthuijzen and Sharik 1985, Lawson 1990). Avian generalists, small mammals, and white-tailed deer are known to eat these fruit-like cones off ERC contributing to its seed dispersal and propagation on our grassland systems (Bidwell et al. 1996, Holthuijzen and Sharik 1985, Horncastle et al. 2004) resulting in a potential closed canopy in as little as 40 years (Briggs et al. 2002).

Cattle operations are vital to the NGP culture and economy, which rely heavily on forage biomass production on their rangelands. Juniper canopies reduce herbaceous biomass production by limiting light penetration and inhibiting precipitation from reaching the soil surface beneath the canopy (Engle et al. 1987, Fuhlendorf et al. 1997, Starks et al. 2014). Biomass reduction is limited primarily to underneath individual ERC canopies, with little reduction occurring at the canopy edge in comparison to open grassland sites (Briggs et al. 2002, Engle et al. 1987, Engle and Kulbeth 1992, Limb et al. 2010). Up to 70-99% reduction in biomass production is common underneath ERC canopies in comparison to open non-encroached grassland (Briggs et al. 2002, Engle et al. 1987, Smith and Stubbendieck 1990). Similar reductions in below-canopy biomass production were found from shading impacts in *Juniperus ashei* (Ashe juniper), *Juniperus monosperma* (one-seed juniper), *Juniperus occidentalis* (western juniper), *Juniperus pinchotii* (redberry juniper), and *Cornus drummondii* (rough-leaved dogwood) in other locations in the Great Plains (Arnold 1964, Bates et al. 2000, Dye et al. 1995, Fuhlendorf et al. 1997, Lett and Knapp 2005, McPherson and Wright 1990). In 2001, an estimated $100 million was lost in Oklahoma due to juniper encroachment and is expected to reach $205 million by 2013 (Hendrix 2002). To understand the heterogeneity of ERC stand encroachment on biomass production (ie. non-uniform sizes of ERC and level of encroachment), Limb et al. (2010) studied how ERC stand densities impact overall biomass production. A linear relationship between ERC canopy cover (%) and herbaceous biomass production (kg/ha) was found in the tallgrass prairie of Oklahoma, also supported by Bidwell et al. (2016), with about 450 kg/ha in biomass lost for every 10% increase in ERC stand canopy cover (Limb et al. 2010). This ERC encroachment in the Great Plains leads to decreased livestock carrying capacities, stocking rates, pasture visibility, and increases labor hours, production costs, and extreme wildfire risk (Archer and Predick 2014, Bidwell et al. 1996).

Limited light, litter accumulation, and reduced soil moisture alters the plant community composition underneath juniper canopies (Engle et al. 1987, McKinley et al. 2008, Starks et al. 2014). In the Platte River Valley of Nebraska, open non-encroached plots were dominated by *Schizachyrium scoparium* (Michx.) Nash (little bluestem) whereas shaded plots underneath ERC were dominated by *Poa pratensis* L. (Kentucky bluegrass), implying a shift from C4 to C3 grasses resulting from ERC encroachment (Briggs et al. 2002, Gehring and Bragg 1992). However, this trend was not present in all C4 and C3 grass species from grassland plots to shaded plots (Gehring and Bragg 1992). This suggests the impact of ERC canopies might be species dependent or may rely on other environmental variables rather than solely on photosynthetic pathways. In contrast, Limb et al. (2010) found a decline in both C4 and C3 grasses and forbs along an ERC encroachment gradient, suggesting all herbaceous species decrease in cover resulting from ERC canopies. Cover of species is dependent on individual ERC canopy diameter, with an increase in some species (*Carex spp.*) and a decrease in others (Coppedge et al. 2001, Gehring and Bragg 1992). Species diversity, evenness, and richness decrease underneath ERC canopies (Briggs et al. 2002, Horncastle et al. 2004, Menguzzo and Liknes 2015) and within ERC stands (Limb et al. 2010) in comparison to non-encroached grassland. The change in plant communities resulting from ERC encroachment threatens the resiliency of our grasslands and has shown to alter native fauna diversity, displacing endemic grassland species (Coppedge et al. 2001, Engle et al. 2008).

Zou et al. (2018) documented the research conducted thus far on ERC in the Great Plains. Most of the research on ERC was conducted in the Southern Great Plains (SGP), primarily in Oklahoma, with little research in the NGP, especially South Dakota (Zou et al. 2018). This research builds upon previous studies (Briggs et al. 2002, Engle et al. 1987, Gehring and Bragg 1992, Limb et al. 2010), but is the first to our knowledge of this magnitude and in the NGP mixed-grass prairie of South Dakota. The objectives of our study were to 1) determine the impact of individual ERC canopy size in terms of diameter diameter (m) on herbaceous biomass production underneath ERC canopies, 2) calculate the herbaceous biomass production in relation to ERC stand canopy cover (%), and 3) assess the aboveground plant community structure under ERC with canopy diameters greater than two meters in terms of species composition, richness, diversity, and evenness. Based on knowledge from previous studies, the alternative hypotheses for this study were:

1. Herbaceous biomass production will not significantly differ underneath ERC trees with canopy diameters less than two meters in comparison to grassland control plots. ERC tress with canopy diameters greater than two meters will significantly reduce herbaceous biomass production with minimal biomass production resulting from trees with canopies greater than six meters.
2. As ERC stand canopy cover (%) increases, herbaceous biomass production (kg/ha) will be reduced linearly at nearly a 1:1 ratio with ERC stand canopy cover (%).
3. Species diversity, evenness, richness, and floristic quality index will decrease with increasing ERC canopy diameters. More specifically, the frequency and cover of introduced graminoids, some annual/introduced forb species, and ERC seedlings will increase and native graminoids and forbs will decrease as ERC canopy diameter increases.

MATERIALS AND METHODS

STUDY AREA

This study was conducted on two separate private ranches in south-central South Dakota in the Northern Great Plains mixed-grass prairie. Ranch 1, referred to as Site 1 (285 ha), is located in the Bijou Hills of Brule County along the east side of the Missouri River near Academy, South Dakota. This ecoregion contains a mixture of steep hills (15-40% slopes) surrounded by rolling mixed-grass prairie, cropland, and rangeland pastures. Soils primarily consist of Okaton bouldery silty clay (clayey residuum weathered from shale) where sampling was conducted (Soil Survey Staff 2022). Elevation ranges from 400 to 500 meters above sea level. Ranch 2, referred to as Site 2 (70 ha), is located in Charles Mix County along the east side of the Missouri River near Platte, South Dakota. This ecoregion contains a mixture of steep valleys and drainages (15-40% slopes) surrounded by rolling mixed-grass prairie, flat-topped ridges, cropland, and rangeland pastures. Soils primarily consist of Betts-Ethan loams (fine-loamy till) with abundant moraine at or near the soil surface (Soil Survey Staff 2022). Elevation ranges from 340 to 680 meters above sea level.

Sites 1 and 2 are close in geographic proximity (<40 km), therefore the same data was used to describe their climate. The landscape experiences a semiarid climate, consisting of hot, dry summers and cold, wet winters. The average annual temperature in 2020 was 8.5□C with a low of -24.4□C (February) and a high of 35.5□C (June). The total annual precipitation in 2020 was 445 mm with 86% of the precipitation occurring during the growing season (May – August), which was 15% higher than the 30-year average (1990-2019) during the growing season (HPRCC 2022, Mesonet 2022). The average annual temperature in 2021 was 9.1°C with a low of -31.7°C (February) and a high of 40.6°C (June). The total annual precipitation in 2021 was 399.8 mm with 53.7% of the precipitation occurring during the growing season (May – August), which was 36.4% lower than the 30-year average during the growing season indicating a drought (HPRCC 2022, Mesonet 2022). Deviations of monthly temperature and precipitation from the 30-year (1990-2019) average are shown in Appendix Tables A.1, A.2 and Figures A.1, A.2.

Site 1 consists of a disturbed mixed-grass prairie with ERC encroachment and no previous cattle grazing activity or prescribed fire within the past five years. The vegetation at this site is dominated by introduced graminoids including *Poa pratensis* L. (Kentucky bluegrass) and *Bromus inermis* Leyss. (smooth bromegrass) with native graminoids mixed throughout including *Nassella viridula* (Trin.) Barkworth (green needlegrass), *Andropogon gerardii* Vitman (big bluestem), *Sporobolus compositus* (Poir.) Merr. (composite dropseed), *Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould (Scribner's rosette grass), and *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass). Various forb species are present adding to the diversity of the site including *Solidago missouriensis* Nutt. (Missouri goldenrod), *Monarda fistulosa* L. (wild bergamot), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), and *Artemisia ludoviciana* Nutt. (white sagebrush).

Site 2 consists of a disturbed mixed-grass prairie with ERC encroachment and previous cattle grazing from April – June at a stocking rate of 75 cow-calf pairs on 70 hectares. However, this land was deferred (not grazed) in 2021 for sampling and to build fuel for a prescribed fire in spring 2022. The vegetation at this site is dominated by a mixture of native graminoids such as *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needle and thread), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama), *Bouteloua dactyloides* (Nutt.) J. T. Columbus (buffalograss), and *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama). Numerous forb species add to the diversity of the landscape, primarily dominated by natives, such as *Echinacea angustifolia* DC. (blacksamson echinacea), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), *Symphyotrichum ericoides* (L.) G.L. Nesom (white heath aster), *Verbena stricta* Vent. (hoary verbena), and some introduced species including *Cirsium arvense* (L.) Scop. (Canada thistle) and *Verbascum thapsus* L. (common mullein).

TREATMENTS

Nine treatments were compared based on ERC canopy diameter. The treatments were grassland control (grassland location with no ERC encroachment present) and ERC canopy diameter classes of 0-1 m (A), 1-2 m (B), 2-3 m (C), 3-4 m (D), 4-5 m (E), 5-6 m (F), 6-7 m (G), and >7 m (H).

FIELD METHODS & DATA COLLECTION

Plot Establishment, ERC Measurements, & Mapping

In late July 2020 (Site 1) and 2021 (Site 2), twelve 25 m x 25 m permanent plots were established at each site consisting of increasing percentages of ERC stand canopy cover. To achieve a range in tree cover, three categories were used to classify ERC canopy cover based on field estimates of low, moderate, and high canopy cover densities (Allen & Nowak 2008). At each 25 m x 25 m plot, a metal T-post was placed at each of the four corners, marked with colored tape, and its GPS position recorded. Within each plot, 25 temporary subplots (5 m x 5 m) were created by placing a step-in fence post every 5 meters to make a grid structure throughout the plot (Figure 2.1). These temporary posts were used as reference points when determining the approximate location and canopy size of each individual ERC tree in the plots. Starting in grid 1 (Figure 2.1) and working towards grid 25, we searched for ERC trees and collected the following data on those found: approximate tree location, height (m), canopy diameter (m), diameter breast height (DBH) (mm), basal diameter (mm), and visual interpretation of sex (if mature). Canopy diameter was measured in two perpendicular transects (axis A & axis B) where axis A is the longest canopy diameter and axis B is perpendicular to axis A. The average canopy diameter of axis A and axis B was used for analysis and canopies were assumed circular. With approximate tree location and canopy diameter measurements, we created hand-drawn aerial maps of each plot to represent ERC stand canopy cover and used as reference during future sampling (Figure 2.2).

Tree Selection & Herbaceous Biomass Collection

Once ERC measurements were recorded for individual trees in all twelve plots, eight tree classes were created based on canopy diameter for herbaceous biomass sampling. The eight tree classes were 0-1 m (A), 1-2 m (B), 2-3 m (C), 3-4 m (D), 4-5 m (E), 5-6 m (F), 6-7 m (G), and >7 m (H). Within each of the twelve plots, up to three trees from each tree class (if present in each plot) were randomly selected for sampling. This totaled a potential of 24 trees sampled in each plot (8 classes x 3 trees). In addition, ten open grassland samples (control) were selected at random for sampling in each plot. Open grassland locations were at least two meters away from surrounding ERC trees and spread out randomly throughout each plot. For the eight tree classes, biomass samples were collected under the ERC canopy at half canopy radius in four cardinal directions. Therefore, four biomass samples were collected under each ERC tree except for tree class A where only two samples were collected under each ERC tree due to limited area under the canopy. Distance from ERC canopy was not evaluated in this study due to previous research by Engle et al. (1987) finding biomass reduction is limited to primarily underneath ERC canopies.

Herbaceous biomass samples were collected in the middle of August 2020 (Site 1) and 2021 (Site 2) during peak standing crop. A quadrat (50 cm x 50 cm) was used for sampling grassland control samples and tree classes C, D, E, F, G, and H (canopy diameters >2 m). A smaller quadrat (25 cm x 25 cm) was used for sampling tree classes A and B to ensure the samples were collected primarily under the tree canopy and to exclude biomass not impacted by shading. Sampled trees were tagged with aluminum tree tags and wire for future reference. Prior to clipping biomass samples, ocular percent foliar cover by species was estimated and recorded within each quadrat. Botanical nomenclature for each species followed the USDA Plants Database (USDA 2006). Herbaceous biomass samples were clipped using hand-held grass shears at ground level. Litter, dead plant material, and woody components were removed from each sample. Samples were labeled, placed in paper bags, and transported back to South Dakota State University in Brookings, SD for further lab procedures. At the lab, biomass samples were oven dried at 60°C for 72 hours and their dry mass were weighed with an electric balance and recorded to the nearest hundredth of a gram.

DATA ANALYSIS

Site 1 and Site 2 were only sampled one year each (ie. spatial replication but no temporal replication), so neither the interaction between site and treatment nor year and treatment analyses were conducted. Analyses were conducted among and between treatments within each site for a given year. If normality of dependent variables was not met through transformations, such as $\log_{10}$, square root, and squared, required for analysis of variance (ANOVA), we used the non-parametric Kruskal-Wallis approach to compare medians among treatments to test for significance ($P<0.05$) within the year. When significance was found using Kruskal-Wallis analysis, Dunn's post hoc test with a Bonferroni p-value adjustment was used to determine significance between treatment medians through assigning different lettering.

ERC Stand Canopy Cover

Within each plot, canopy cover was calculated using canopy diameter measurements and approximate location for each individual ERC tree. Canopies were assumed circular from an aerial view. Individual canopy cover (m^2) of an ERC tree was calculated with the formula:

$$C = \pi * \left(\frac{d}{2}\right)^2 * \left(\frac{100 - p}{100}\right)$$

where π is the Greek letter pi and is a mathematical constant, d is the canopy diameter (m), and p is the percent of the tree canopy that is not included in the plot and/or overlaps with another tree canopy. Some tree canopies extended outside the plot or overlapped with other canopies (especially in high density plots). To ensure proper canopy calculations, field estimations were conducted on all trees to determine how much canopy should be excluded in analysis. For individual trees that occurred on plot borders, we estimated the proportion of the tree canopy that lied inside the plot border. For individual trees that overlapped other ERC tree canopies or grew in clusters, we estimated the amount of tree canopy as a percentage that should be excluded in analysis.

Within each plot, individual tree canopies were summed together to find the total canopy area. Our plots measure 25 m x 25 m with a total area of 625 m^2. Total plot canopy cover (%) of the ERC stand was calculated with the formula:

$$Plot\ Canopy\ Cover\ (\%) = \left(\frac{\sum_{i=1}^{n} C_i}{625}\right) * 100$$

where C_i is the canopy cover (m^2) of the *i*th ERC tree, *n* is the number of ERC trees, and 625 is the area of the plot in square meters.

Herbaceous Biomass Production by ERC Canopy Diameter

Herbaceous biomass samples collected under an individual ERC canopy were averaged per tree and analyzed with an individual ERC as the sample unit. Data analysis was conducted using program R (R Development Core Team 2015). The independent factor observed was treatment including ERC canopy diameter classes A-H and grassland control. The dependent factor observed was herbaceous biomass production in g/m^2. Our data from Site 1 and Site 2 did not meet normality required for ANOVA analysis even after $\log_{10}$, square root, and squared transformations on the dependent variable. As a result, Kruskal-Wallis' non-parametric test was used for comparisons among treatment medians within each site. Dunn's post-hoc test with a Bonferroni p-value adjustment was used for comparisons between treatment medians which is appropriate for treatments with unequal sample sizes (Zar 2010).

Herbaceous Biomass Production by ERC Stand Canopy Cover

Average biomass production underneath ERC canopy classes A-H were averaged within each treatment by plot. In addition, the ten biomass samples collected at the grassland control locations within each plot were averaged. With treatment averages per plot, we applied these values to the remaining classified trees and their canopy areas to determine biomass production underneath each ERC within each plot. Then, we were able to determine total biomass production per plot based on the unique trees present in each plot. We then ran regression using ERC stand canopy cover (%) as the independent variable and total biomass production by plot (kg/ha) as the dependent variable.

Species Composition and Functional Groups

Foliar cover composition was analyzed by the quadrat (0.25 m^2) as the sample unit. To determine species composition within each quadrat, we identified individual species and estimated their foliar cover as a percentage. Due to the sampling design, species composition was only analyzed for tree classes C-H (greater than 2 m canopy diameter) and grassland control samples. A smaller quadrat was used for tree classes A and B, so including these samples when analyzing the foliar cover estimates would not be fair in terms of plant community analysis. We used this cover by species data to compare functional groups among and between treatments including: life form (Forb *vs.* Graminoid *vs.* Shrub *vs.* Tree), origin (Native *vs.* Introduced), and life span (Annual *vs.* Perennial). Percent frequency and mean cover by species were calculated within each treatment with the following formulas:

$$Frequency\ (Spp_x) = \left(\frac{\#\ of\ plots\ in\ which\ Spp_x\ occurs}{Total\ \#\ of\ plots\ examined}\right) * 100$$

$$Mean\ Cover\ (Spp_x) = \left(\frac{Total\ cover\ (\%)\ of\ Spp_x}{\#\ of\ plots\ in\ which\ Spp_x\ occurs}\right) * 100$$

Floristic Quality Index (FQI) is a metric used to express the tolerance and resiliency of species in relation to disturbance, degradation, and conservation concern. FQI relies on coefficients of conservatism (C values) and species richness. Each species is given a numerical score (C value) that ranges between 0-10. Species with little conservation concern and that are well adapted to degraded habitats, such as annual or "weedy" species, are given a score of zero. Species of high conservation concern that require unchanged natural conditions, such as rare native species, are given a score of at most ten. Introduced species are given a non-numerical value of a star (*) and are excluded when calculating the mean C value for each plot. We calculated FQI using the following formula:

$$FQI = \bar{C}\sqrt{n}$$

where $\bar{C}$ is the mean C value per sample and n is the number of species per sample (Northern Great Plains Floristic Quality Assessment Panel 2001).

Diversity

Species richness, native species richness, Shannon-Wiener diversity, and Shannon-Wiener evenness were calculated at the sample level for analysis. Shannon's Diversity based on vegetation cover by species was calculated with the formula:

$$H' = -\sum_{i=1}^{S} P_i \ln P_i$$

where S is the number of species (ie. species richness), P_i is the proportion of individuals in the *i*th species, and ln is the natural logarithm (Magurran 2004). Shannon's diversity assumes all species are randomly sampled within a study area and incorporates species richness and evenness (Magurran 2004).

Shannon's evenness was calculated with the formula:

$$Evenness = \frac{H'}{\ln S}$$

where *H'* is Shannon's diversity, ln is the natural logarithm, and *S* is the species richness. Shannon's evenness quantifies how the relative abundance of species is distributed throughout a sample and ranges between 0-1. Low evenness will result from samples dominated by one or two species, whereas high evenness will result from samples with an even distribution of species (Magurran 2004, Moore 2013).

Community Analysis

Vegetation composition by functional group, FQI, Shannon-Wiener diversity, Shannon-Wiener evenness, species richness, and native species richness all failed to meet normality assumptions required for analysis of variance (ANOVA) at Site 1 and Site 2. As a result, Kruskal-Wallis' non-parametric test was used to test for differences in the dependent variable medians among treatments within each year. If the Kruskal-Wallis test found significance ($P<0.05$) among treatments, Dunn's post-hoc test with a Bonferroni p-value adjustment was used for comparisons between treatment medians which is appropriate for treatments with unequal sample sizes (Zar 2010).

RESULTS

Weather

Site 1 (2020) and Site 2 (2021) experienced very different weather regimes during their respective growing seasons. Average temperature was higher than the 30-year (1990-2019) average for both Sites, especially during the growing season with an average increase of 0.40˚C and 0.57˚C at Site 1 and Site 2, respectively (Table A.1). Total precipitation was lower than the 30-year average at both Sites consisting of 445 mm (25.2% reduction) and 400 mm (36.6% reduction) at Site 1 and Site 2, respectively (Table A.2). However, Site 1 had 15% more precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 1, monthly precipitation was lower in May and higher during the months of June, July, and August compared to the 30-year average (Table A.2, Figure A.2). Site 2 had 36.5% less precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 2, monthly precipitation was lower in May, June, and August and higher in July than the 30-year average (Table A.2, Figure A.2).

ERC Stand Canopy Cover

A total of 1,118 ERC trees were mapped and measured in the 24 plots between Site 1 (2020) and Site 2 (2021). At Site 1 in 2020, our twelve plots ranged from 8-90% ERC stand canopy cover with a total of 507 ERC trees counted, measured, and mapped (Table 2.1). At Site 2 in 2021, our twelve plots ranged from 4-67% ERC stand canopy cover with a total of 611 ERC trees counted, measured, and mapped (Table 2.1).

Herbaceous Biomass Production

A total of 326 ERC trees were selected at random for herbaceous biomass sampling underneath the canopy between our two Sites in 2020 and 2021. At Site 1 in 2020, a total of 704 biomass samples were collected from 120 grassland control locations and underneath 164 ERC tree canopies (Table 2.2). At Site 2 in 2021, a total of 677 biomass samples were collected from 120 grassland control locations and from underneath 162 ERC tree canopies (Table 2.2). Average biomass production (g/m^2) with ERC tree as the sample unit is summarized in Table 2.3 with mean, standard error, and percent reduction from the grassland control for each treatment.

Average biomass production decreased as ERC canopy diameter increased. As tree canopies reach 2-3 m in diameter, biomass production plummets (over 60% reduction) resulting in over 85% biomass reduction when canopies reach over five meters in diameter. Site 1 had nearly three times the biomass production in open grassland locations in comparison to Site 2. However, despite the difference in overall biomass production, the relationship between canopy diameter and biomass reduction was uniform between Site 1 and Site 2 (Figure 2.3). Kruskal-Wallis tests found significant differences among treatments at Site 1 in 2020 ($P<0.01$) and at Site 2 in 2021 ($P<0.01$). At Site 1, Dunn's post hoc test found that tree classes C-H significantly differed from the grassland control in terms of biomass production (Figure 2.4). Tree classes A and B were not significantly different from the grassland control and tree classes B and C were similar. Tree class C seems to be the inflection point for drastic biomass reduction (Figure 2.4). Similar to Site 1, Dunn's post hoc test for Site 2 in 2021 found that tree classes C-H were significantly different from the grassland control in terms of biomass production (Figure 2.5). Tree classes A and B were not significantly different from the grassland control (Figure 2.5). All other tree classes (C-H) were significantly different from the grassland control and tree clases A and B (Figure 2.5). Once again, the same trend of biomass reduction was found at Site 2 where an abrupt decrease in biomass production occurred in tree class C and continued into the larger tree classes. The impact of tree canopy diameter on herbaceous biomass production can be modeled through a negative exponential relationship. We found an equation of $y = 454.46e^{-0.44x}$ (R^2=0.75, n=176, *P<0.01*) for Site 1 and $y = 182.13e^{-0.53x}$ (R^2=0.80, n=174, *P<0.01*) for Site 2 to model the relationship between ERC canopy diameter in meters (x-value) and herbaceous biomass production (y-value) (Figure 2.6). We can generalize these equations to create the following model:

$$Biomass = \alpha * e^{(-0.485x)}$$

where *Biomass* is the herbaceous biomass produced in g/m^2, α is the average grassland (no ERC present) biomass production (g/m^2) at given site, *e* is the mathematical constant 2.718, *x* is the canopy diameter of an ERC tree in meters, and -0.485 is the average slope calculated from the equations at Site 1 and Site 2.

A linear relationship was found between ERC stand canopy cover (%) and herbaceous biomass production (kg/ha) (Figure 2.7). As ERC stand canopy cover increases, herbaceous biomass production decreases at 350 kg/ha and 200 kg/ha for every 10% increase in canopy cover for Site 1 and Site 2, respectively. Site 1 produced over 4500 kg/ha of biomass on grasslands without ERC encroachment, whereas Site 2 only produced 1700 kg/ha. Production at Site 2 may seem low, but similar values were reported in Smart et al. (2007) with means of 1600 kg/ha in central South Dakota.

Despite the differences in total biomass production, the relationship was similar between Site 1 and Site 2. We found a relationship expressed by y = -35.61x + 4502.8 (R^2=0.85, n=13, P<*0.01*) and y = -20.49x + 1887.6 (R^2=0.76, n=13, *P<0.01*) for Site 1 and Site 2, respectively, where "x" is the ERC stand canopy cover (%) and "y" is the herbaceous biomass produced in kg/ha (Figure 2.7). We can generalize these equations to create the following model:

$$Stand\ Biomass = -28.05x + \beta$$

where *Stand Biomass* is the herbaceous biomass (kg/ha) produced, -28.05 is the average slope from the equations created for Site 1 and Site 2, *x* is the ERC stand canopy cover (%), and β is the average grassland (no ERC present) herbaceous biomass production (kg/ha) of a given site.

As ERC stand canopy cover (%) increases, herbaceous biomass is reduced at nearly a 1:1 ratio with a 0.88% and 0.80% reduction in biomass for a 1% increase in stand canopy cover for Site 1 and Site 2, respectively (Figure 2.8). The relationship we found between ERC stand canopy cover (%) and herbaceous biomass reduction (%) can be expressed by the equations y = 0.88x - 1.35 (R^2=0.994, n=13, *P<0.01*) and y = 0.80x - 0.24 (R^2=0.996, n=13, *P<0.01*) for Site 1 and Site 2, respectively, where "x" is the ERC stand canopy cover (%) and "y" is the herbaceous biomass reduction (%) (Figure 2.8).

Species Composition and Functional Groups

A total of 100 species were present in the 1,000 foliar cover samples collected at Site 1 and Site 2. Eleven of these species at Site 1 and ten of these species at Site 2 had average frequencies among the treatments of greater than 10% (Appendix A.3, A.4). At

Site 1, grassland control plots were dominated by *Bromus inermis* (84% frequency, 25.15% cover), *Ambrosia artemisiifolia* (57%, 4.19%), *Poa pratensis* (49%, 3.35%), and *Nassella viridula* (48%, 8.63%) (Appendix A.3). In samples collected underneath ERC canopies, we found a decrease in frequency and cover of a few native species at Site 1, such as *Andropogon gerardii*, *Bouteloua curtipendula*, *Monarda fistulosa*, *Symphyotrichum spp.*, and *Dichanthelium oligosanthes*. *Andropogon gerardii* went from 28% frequency in grassland control plots to 28%, 12%, 11%, 8%, 19%, and 10% frequency underneath tree classes C-H, respectively. Its cover also drastically decreased from 16.71% in grassland control plots to 4.14%, 3.68%, 1.39%, 2.02%, 0.96%, and 2.06% underneath tree classes C-H, respectively. *Symphyotrichum spp.* decreased from 20% frequency in grassland control plots to 7%, 4%, 1%, 0%, 6%, and 4% underneath tree classes C-H, respectively. Although not as severe, its cover also reduced from 2.54% in grassland control plots to 0.76%, 1.50%, 0.10%, 0%, 0.55%, and 1% underneath tree classes C-H, respectively. Very few plants had an increasing trend in frequency or cover underneath ERC trees in comparison to grassland control plots, except for *Carex duriuscula* and *Juniperus virginiana*. *Carex duriuscula* increased from 4% frequency in grassland control plots to 10%, 17%, 14%, 27%, 19%, and 27% underneath tree classes C-H, respectively. However, we did not see the same trend in cover percentage for *Carex duriuscula*, where its cover decreased from 1.64% in grassland control plots to 0.48%, 0.85%, 0.73%, 0.76%, 0.56%, and 0.38% underneath tree classes C-H, respectively. *Juniperus virginiana* increased from 2% frequency in grassland control plots to 9%, 16%, 34%, 34%, 44%, and 46% underneath tree classes C-H, respectively (Appendix A.3).

At Site 2, grassland control plots were dominated by *Bouteloua curtipendula* (74% frequency, 3.95% cover), *Andropogon gerardii* (60%, 9.97%), *Schizachyrium scoparium* (56%, 11.36%), and *Poa pratensis* (49%, 6.12%) (Appendix A.4). In the samples collected underneath the ERC canopies, we found a decrease in frequency and cover of some important native species at Site 2, such as *Bouteloua curtipendula, Andropogon gerardii, Schizachyrium scoparium, Bouteloua gracilis,* and *Ratibida columnifera*. *Bouteloua curtipendula* decreased from 74% frequency in grassland control plots to 33%, 27%, 16%, 4%, 4%, and 7% frequency underneath tree classes C-H, respectively. Its cover also decreased from 3.95% in grassland control plots to 1.60%, 1.29%, 1.23%, 0.88%, 0.75%, and 2% in order of tree classes C-H, respectively. *Ratibida columnifera* went from 8% frequency in grassland control plots to 1%, 0%, 1%, 2%, 0%, and 0% frequency underneath tree classes C-H, respectively. Its cover also decreased from 0.93% in grassland control plots to 0.40%, 0%, 0.10%, 0.40%, 0%, and 0% underneath tree classes C-H, respectively. In contrast, some introduced C3 species increased in frequency underneath ERC canopies in comparison to the grassland control plots, such as *Poa pratensis* L. and *Bromus inermis* Leyss. *Poa pratensis* increased from 49% frequency in grassland control plots to 72%, 90%, 78%, 89%, 85%, and 79% frequency underneath tree classes C-H, respectively. *Bromus inermis* increased from 3% frequency in grassland control plots to 11%, 10%, and 12% in tree classes C-E, respectively. Most of the species that occurred solely underneath ERC canopies at Site 2 were either introduced species or weedy annuals, such as *Setaria pumila*, *Poa compressa*, *Taraxacum officinale*, *Thlaspi arvense*, and *Conyza canadensis* (Appendix A.4).

Treatment had a significant effect (*P<0.05*) on foliar cover functional group composition at Site 1 and Site 2 (Table 2.4, 2.5). Mean, standard error, and significance calculated from medians among and between treatments of foliar cover composition at Site 1 and Site 2 are displayed in Tables 2.4 and 2.5. Significance was found between treatments in most of the functional groups at Site 1, but we only see a definitive trend with increasing ERC canopy diameter in two groups: total grasminoid and native tree. Total graminoid composition decreased from grassland control plots with increasing ERC canopy diameter. Native tree composition, primarily seedling or juvenile ERC trees, increased with ERC canopy diameter in comparison to grassland control plots (Table 2.4). At Site 2, native perennial graminoids and trees were the only two functional groups to display a trend with increasing ERC canopy diameter in comparison to the grassland control plots. Native perennial graminoids decreased in cover composition as ERC canopy diameter increased in comparison to grassland control plots. In contrast, native tree composition increased from grassland control plots as ERC canopy diameters increased, with the exception of tree class H (Table 2.5).

Diversity

Treatment had a significant negative (*P<0.05*) effect on medians of FQI, total species richness, and native species richness for Site 1 and Site 2 (Table 2.6). FQI was highest in grassland control samples at both Sites. Site 1 had a mean FQI of 10.6 for grassland control samples and decreased as ERC canopy diameter increased with the lowest FQI in tree class H with a mean of 6.3 (Table 2.6). FQI medians were significantly different between grassland control samples and tree classes D-H at Site 1 (Figure 2.9). Site 2 had a mean FQI of 10.9 for grassland control samples and decreased as ERC canopy diameter increased with the lowest FQI in tree class H with a mean of 4.1 (Table 2.6). FQI medians were significantly different between grassland control samples and tree classes C-H at Site 2 (Figure 2.9). FQI decreased at a fast rate at Site 2 compared to Site 1 as ERC canopy diameter increased (Figure 2.9). Total species richness decreased from grassland control samples as ERC canopy diameter increased at Site 1 and Site 2 (Table 2.6). Grassland control samples had a mean species richness of 5 and 4.8 whereas tree class H had a mean richness of 3.3 and 2.3 for Site 1 and Site 2, respectively. Total species richness medians significantly differed between grassland control samples and tree classes C-H at Site 1 and Site 2, with a stronger negative trend occurring at Site 2 (Figure 2.10). Native species richness also decreased from grassland control samples as ERC canopy diameter increased at Site 1 and Site 2 (Table 2.6). Mean native richness was 3.2 and 4.2 for grassland control samples compared to 2.0 and 1.4 in tree class H at Site 1 and Site 2, respectively (Table 2.6). Native species richness medians significantly differed between grassland control samples and tree classes E, F, and H at Site 1 and tree classes C-H at Site 2, respectively (Figure 2.11). Similar to total species richness, the decreasing trend of native species richness was more prevalent at Site 2 compared to Site 1 (Figure 2.11).

We found mixed results of significance ($P<0.05$) for Shannon-Wiener diversity and evenness at Site 1 and Site 2 (Table 2.6). Treatment had a significant effect on Shannon's diversity at Site 2 ($P<0.05$), but not at Site 1 ($P=0.21$). At Site 2, Shannon's diversity decreased from grassland control plots as ERC canopy diameter increased with a mean of 1.07 and 0.48 in grassland control plots and tree class H, respectively. (Table 2.6). Grassland control medians significantly differed compared to tree classes D-H

(Figure 2.12). Treatment had a significant effect on Shannon's evenness at Site 1 (*$P<0.05$*), but not at Site 2 (*$P=0.02$*) where p-value adjustment did not find significance between treatment medians with Dunn's test (Table 2.6). Evenness at Site 1 did not show a definitive trend between grassland control samples and increasing ERC canopy diameter. The highest mean evenness value was observed in tree class F (0.73) and the lowest in the grassland control (0.61). Evenness medians significantly differed between grassland control samples and tree classes C, E, F, and G, with even a slight increase in evenness medians from grassland control samples to increasing ERC canopy diameters (Figure 2.13).

DISCUSSION

The grasslands of the Northern Great Plains are experiencing afforestation through ERC encroachment resulting in altered rangeland production and plant community structure. The lack of prescribed fire, overgrazing, and planting ERC in shelterbelts has contributed to its prolific expansion onto our rangelands and raises concern of future rangeland sustainability and resiliency (Limb et al. 2010). Our study evaluated the impact of ERC encroachment on South Dakota rangelands at the individual and stand level. Previous literature has documented the impact of ERC encroachment on rangeland plant communities in the Southern Great Plains, but this research is the first of its kind in the Northern Great Plains. The results of this study support the concern raised by previous research in the Great Plains (Nippert et al. 2021, Ratajczak et al. 2016) and emphasize the need of woody plant management required to save this ecosystem in peril.

Individual ERC Canopies Exponentially Reduced Herbaceous Biomass

We found herbaceous biomass production underneath ERC canopies to decrease exponentially with increasing ERC canopy diameter with 63-97% reduction in trees with canopies larger than 2 meters. This reduction underneath ERC canopies compared to grassland plots is supported by findings in Engle et al. (1987) and Strizke and Bidwell (1989) but is more comprehensive in terms of a range in ERC size. Engle et al. (1987) only observed biomass production underneath two ERC heights (2 m and 6 m) and found no difference in biomass production underneath canopies based on tree height. Although our study focused on ERC canopy diameter instead of height, our results contradict from previous studies (Engle et al. 1987) where we found significant differences in biomass production based on ERC canopy diameter. ERC with canopies greater than 2 m in diameter significantly reduce biomass production in relation to trees smaller than 2 m in canopy diameter. Our findings highlight a potential threshold important to consider when controlling ERC encroachment. The exponential regression model we developed relating ERC canopy diameter to understory biomass production can be used by land managers to determine how individual trees are reducing their forage production based on their unique rangeland production. These findings support our first hypothesis and stress the importance of early detection and management of ERC while in the juvenile stage (Engle and Strizke 1995, Ortmann et al. 1998, Owensby et al. 1973).

ERC Stand Cover Linearly Reduced Herbaceous Biomass

Herbaceous biomass production decreased linearly with increasing ERC stand canopy cover. Our results support our second hypothesis and findings from (Limb et al. 2010) where biomass was linearly reduced by 450 kg/ha for a 10% increase in ERC stand canopy cover in the tallgrass prairie. At our study sites in the mixed-grass prairie, we found our average biomass production decreased by 1% for every 1% increase in ERC stand canopy cover between our two sites (Figure 2.7). This reduction is expected to be less severe in the mixed-grass prairie due to the differences in annual production between tallgrass and mixed-grass prairie ecosystems (Ott and Hartnett 2015). Similar reductions were found in other *Juniper spp.* in the Great Plains, where grass production decreased linearly as *Juniperus pinchotii* (redberry juniper) canopy cover increased (McPherson and Wright 1990) and total herbage biomass decreased as a second-degree polynomial as pinyon-juniper canopy cover increased (Pieper 1990). Our generalized regression model of biomass production based on ERC stand canopy cover can be used by land managers to determine potential forage production on their encroached rangelands. By estimating ERC canopy cover at a site, land managers can determine proper stocking rates and potential additional forage needed to supplement their herd.

Biomass Reduction Trend Consistent Despite Variable Precipitation

Overall herbaceous biomass production at Site 1 was nearly three times greater than at Site 2 in open grassland plots. We can attribute this difference in biomass production to the drought-like conditions Site 2 experienced during the 2021 growing season and temporal effects from previous years of grazing. Total precipitation was 36.6% lower than the 30-year average in 2021, with only 53.7% of the total precipitation occurring during the growing season. Total precipitation was below the 30-year average for each month during the growing season except for July (Figure A.2), which attributed to a 36.5% reduction in total precipitation during the growing season (HPRCC 2022, Mesonet 2022). The lack of precipitation during the early growing season is likely responsible for reduced forage production in 2021 because most forage is produced in the early growing season due to the dominance of C3 perennial grasses in the northern mixed-grass prairie (Biondini and Manske 1996). Despite differences in precipitation, previous grazing, and overall biomass production between our two research sites, we found a consistent relationship between herbaceous biomass production and our independent variables of ERC canopy diameter and ERC stand canopy cover (Figures 2.3, 2.8). This suggests that the primary factors impacting under-canopy biomass production are limited to sunlight, litter accumulation, and reduced soil moisture (Engle et al. 1987, McKinley et al. 2008).

ERC Encroachment Altered Plant Community Composition

Frequency of some species decreased while others increased from grassland control plots compared to underneath ERC canopies. In contrast, average cover of all species at both sites decreased from grassland control plots to under ERC canopies, contrasting from (Gehring and Bragg 1992) and supporting findings from (Limb et al. 2010). Our results partially support findings from previous studies where we found a decrease in frequency and cover of certain C4 species in response to ERC encroachment (Gehring and Bragg 1992). At both sites, key C4 species such as *Andropogon gerardii*, *Bouteloua curtipendula*, *Schizachyrium scoparium*, *Bouteloua gracilis*, *Ratibida columnifera*, and *Symphyotrichum spp*. decreased in frequency and cover from grassland control plots to under ERC canopies. Unlike previous studies (Gehring and Bragg 1992), we found mixed results in C3 grasses *Carex duriuscula* and *Poa pratensis* (C3 species) where their frequencies underneath ERC canopies were inversely related between Sites. However, this supports conclusions in (Gehring and Bragg 1992), where a definitive trend did not exist in all C4 and C3 species. *Nassella viridula*, *Bromus inermis*, and *Hesperostipa comata* (C3 species) decreased in cover from grassland control plots to underneath ERC canopies, suggesting that additional factors influence species cover underneath ERC canopies rather than solely photosynthetic pathway.

FQI, total species richness, and native species richness declined as a result of increasing ERC canopy diameter. This supports previous literature (Briggs et al. 2002, Limb et al. 2010) where species richness declines as ERC cover increases. Our study is the first to interpret FQI underneath ERC canopies. FQI is a useful tool when determining whether ERC encroachment is creating a degraded habitat for native, rare flora species. Our results show that FQI is a function of ERC canopy diameter, indicating that the under-canopy microenvironment created by ERC encroachment is shifting from open grassland plots to a degraded, disturbed landscape of conservation concern. These results were more significant at Site 2, which was primarily dominated by natives in open grassland locations, suggesting ERC encroachment is more detrimental to plant communities on natural landscapes compared to those severely degraded with introduced species. In contrast to other studies, we did not see a uniform decrease in species diversity and evenness from grassland control plots to underneath ERC canopies (Gehring and Bragg 1992, Horncastle et al. 2005, Limb et al. 2010). Diversity was similar between all treatments at Site 1 but decreased as ERC canopy diameter increased at Site 2. These mixed results partially support findings in (Horncastle et al. 2005, Limb et al. 2010) where species diversity decreases with increasing ERC canopy cover. Unlike previous research, no trend was apparent between ERC canopy cover and species evenness (Alford et al. 2012, Limb et al. 2010). In contrast with findings from Limb et al.

(2010), species evenness increased from grassland control plots to underneath some ERC canopies at Site 2, which partially contradicts our third hypothesis.

Overall, ERC encroachment altered plant communities in comparison to grassland control plots. Our results partially support our third hypothesis, where we observed a decrease in cover and frequency of some key plant species, FQI, total species richness, and native species richness from ERC encroachment on our rangelands. However, our findings did not support a decrease in cover and frequency of all C4 species or species diversity and evenness, partially contradicting our third hypothesis. These findings highlight the transition state of our rangelands to woodland and indicate the necessity of woody plant management in the Northern Great Plains by implementing prescribed fire at broad spatial scales (Fuhlendorf et al. 2012). Mixed results have also been reported in studies of other *Juniperus spp.* where species diversity, evenness and richness decreased with increasing canopy cover (Bates et al. 2000, Dye et al. 1995, Fuhlendorf et al. 1997, Lett and Knapp 2005, Van Els et al. 2010), but herbaceous cover did not consistently decrease or increase as a function of canopy cover (Fuhlendorf et al. 1997, McPherson and Wright 1990, Miller et al. 2000).

ERC Control

Wildfire historically managed woody encroachment on our rangelands and inhibited the spread of forest from northern latitudes or riparian areas (Higgins 1986). The lack of resources, public outreach, and the fear of prescribed fire in the Northern Great Plains is responsible for limited fire use resulting in extensive ERC encroachment (Twidwell et al. 2013). Using prescribed fire to control ERC was found successful at early stages of encroachment in multiple studies (Nippert et al. 2021, Ortmann et al.

1998, Rollins 1985), even in the understory of mature hardwoods using deciduous leaf litter as a fuel (Engle and Stritzke 1995). Up to 90% mortality is possible for ERC <1 m tall using prescribed fire as a treatment, but its efficacy falls with increasing tree height resulting in a range of 10-50% morality for trees taller than 3 m (Nippert et al. 2021, Ortmann et al. 1998). Mechanical removal of ERC is nearly 100% effective through shearing, mechanical diamond blades, chaining, or chainsaws, but can be extremely costly (Bidwell et al. 1996, Ortmann et al. 1998). The effectiveness of chemical treatments resembles that of prescribed fire with efficacy severely decreasing with trees taller than 3 m (Ortmann et al. 1998). As a result, a combination of prescribed fire, mechanical removal, and chemical removal is often required to successfully combat dense stands of ERC and is the most cost-effective strategy (Bidwell et al. 1996, Engle and Stritzke 1992, Ortmann et al. 1998). At an ERC encroached site, researchers suggest building a fine fuel load through deferred grazing, cutting and stuffing ERC near grassland/woodland transition points, conducting a prescribed burn, and then following up with a mechanical/chemical treatment to remove survivors (Bragg and Hulbert 1976, Ortmann et al. 1998, Rollins 1985).

In general, removal of *Juniperus spp.* increases herbaceous biomass production, species diversity, species evenness, and total flora and fauna richness (Alford et al. 2012, Bates et al. 2000, Evans and Young 1985). Whether rangelands will quickly return to pre-encroachment conditions following the removal of ERC has been widely debated in previous research. Some research suggests rangelands will experience a "legacy effect" following the removal of ERC (Gehring and Bragg 1992) whereas others indicate a swift return to a pre-encroachment grassland site is possible (Alford et al. 2012, Limb et al.

2010, Owensby et al. 1973, Pierce and Reich 2010). The response of rangeland plant communities following ERC removal has not yet been studied in the Northern Great Plains. Understanding how plant communities respond to ERC removal will be essential when discussing potential ERC management with landowners. This research demonstrates our rangeland plant communities have shifted toward a more degraded community as a result of ERC encroachment, but further research is needed to determine how these plant communities respond to ERC removal in the Northern Great Plains.

Juniper spp. and *Pinus spp.* are the most common woody encroachers in the United States (Miller et al. 2000). The current lack of prescribed fire due to limited public resources, landowner fire experience, and prescribed burn associations could lead to widespread juniper dominated forests on our rangelands in the near future (DeSantis et al. 2011, Twidwell et al. 2013). Junipers are deemed “water wasters” by many researchers (Bidwell et al. 1996, Zou et al. 2018) and widespread juniper forests in the Great Plains could lead to a potential water crisis (Zou et al. 2018). Without proper management, woody encroachment could cause grassland and savanna ecosystem failure not only in North America, but also worldwide.

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TABLES

Table 2.1 Permanent plot summary for the number of eastern redcedar trees present in each plot and total eastern redcedar stand canopy cover (%) calculated within each plot at Site 1 in 2020 and at Site 2 in 2021.

	Site 1 (2020)		Site 2 (2021)	
Plot	# of trees	ERC Plot Canopy Cover (%)	# of trees	ERC Plot Canopy Cover (%)
1	22	8	23	4
2	21	12	33	5
3	14	9	28	14
4	15	12	22	4
5	103	42	56	22
6	15	25	35	25
7	12	25	39	18
8	12	14	71	15
9	67	49	68	45
10	45	48	46	51
11	181	68	99	54
12	124	90	91	67
Total	**507**		**611**	

Table 2.2 Treatment group summary for the number of locations/trees sampled and the total sample size (n) per treatment at Site 1 in 2020 and at Site 2 in 2021.

	Site 1 (2020)		Site 2 (2021)	
Tree Classes	Trees/Locations	Quadrats Sampled	Trees/Locations	Quadrats Sampled
Grassland Control	120	120	120	120
A (0-1m)	33	66	36	72
B (1-2m)	29	114	33	128
C (2-3m)	26	103	25	99
D (3-4m)	20	77	20	78
E (4-5m)	19	76	24	92
F (5-6m)	16	64	11	47
G (6-7m)	9	36	7	27
H (7m+)	12	48	4	14
	120|164[a]	**120|584**[b]	**120|162**[c]	**120|557**[d]

[a] Total grassland control locations | total eastern redcedar trees selected for biomass sampling at Site 1 in 2020.

[b] Total quadrats sampled where total for grassland control | total for tree classes (A-H) at Site 1 in 2020.

[c] Total grassland control locations | total eastern redcedar trees selected for biomass sampling at Site 2 in 2021.

[d] Total quadrats sampled where total for grassland control | total for tree classes (A-H) at Site 2 in 2021.

Table 2.3 Mean herbaceous biomass production (g/m^2) with standard error, sample size by treatment (n), and percent reduction from the grassland control for Site 1 in 2020 and Site 2 in 2021.

	Site 1 (2020)			Site 2 (2021)		
Tree Classes	Sample Size (n)	Biomass Production (g/m^2)	Reduction From Control (%)	Sample Size (n)	Biomass Production (g/m^2)	Reduction From Control (%)
Grassland Control	12	465.17 ± 17.49		12	167.35 ± 10.71	
A (0-1m)	33	347.13 ± 17.41	25	36	133.79 ± 8.72	20
B (1-2m)	29	301.69 ± 20.16	35	33	108.80 ± 8.22	35
C (2-3m)	26	171.28 ± 11.14	63	25	52.82 ± 3.34	69
D (3-4m)	20	115.79 ± 10.13	75	20	42.22 ± 4.34	75
E (4-5m)	19	69.40 ± 8.22	85	24	21.37 ± 3.33	87
F (5-6m)	16	54.27 ± 11.0	88	13	8.84 ± 1.78	85
G (6-7m)	9	26.43 ± 7.79	94	7	4.47 ± 0.66	97
H (7m+)	12	28.35 ± 6.02	94	4	5.54 ± 1.78	97
Total[a]	**12|164**		**Total[b]**	**12|162**		

[a] Total sample size (n) where total for grassland control | total for tree classes (A-H) at Site 1 in 2020.

[b] Total sample size (n) where total for grassland control | total for tree classes (A-H) at Site 2 in 2021.

Table 2.4 Foliar cover composition by functional group for Site 1 in 2020. Mean composition with standard error is represented for each functional group by treatment. Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using a Kruskal-Wallis test and Dunn's Post Hoc test with a Bonferroni p-value adjustment.

Tree Classes	Mean Foliar Cover Composition (%/sample) by Functional Group at Site 1 (2020)											
	Forb	Unk Forb	FIA/B	FIP	FNA/B	FNP	Graminoid	GIA	GIP	GNP	ShNP	TNP
Control	13.74 ± 1.19$_{ab}$	0$_{a}$	1.63 ± 0.38$_{a}$	0.79 ± 0.31$_{ab}$	6.37 ± 0.92$_{a}$	4.95 ± 0.70$_{a}$	81.84 ± 1.36$_{a}$	0.02 ± 0.02	52.73 ± 3.00$_{a}$	29.09 ± 2.82	4.18 ± 0.75	0.25 ± 0.21$_{a}$
C (2-3m)	15.78 ± 2.09$_{ab}$	0$_{a}$	1.02 ± 0.48$_{b}$	1.18 ± 0.77$_{a}$	3.75 ± 0.74$_{abc}$	9.83 ± 1.69$_{ab}$	73.41 ± 2.68$_{ab}$	0	40.73 ± 3.49$_{abc}$	32.68 ± 2.94	9.91 ± 2.10	0.90 ± 0.38$_{a}$
D (3-4m)	12.63 ± 1.81$_{ab}$	0.03 ± 0.03$_{a}$	0.75 ± 0.36$_{b}$	0.58 ± 0.38$_{ab}$	2.97 ± 0.85$_{bc}$	8.31 ± 1.72$_{abc}$	71.04 ± 3.01$_{ab}$	0	41.33 ± 3.89$_{abc}$	29.71 ± 3.50	15.23 ± 2.90	1.10 ± 0.46$_{ab}$
E (4-5m)	13.34 ± 2.30$_{a}$	1.02 ± 0.52$_{a}$	2.28 ± 1.02$_{ab}$	1.32 ± 0.75$_{ab}$	3.60 ± 0.92$_{bc}$	5.12 ± 1.81$_{c}$	71.14 ± 3.06$_{ab}$	0	42.47 ± 3.79$_{ab}$	28.67 ± 3.57	11.06 ± 2.58	4.46 ± 1.03$_{bc}$
F (5-6m)	19.95 ± 3.42$_{ab}$	1.51 ± 0.69$_{ab}$	4.15 ± 1.55$_{ab}$	0.53 ± 0.26$_{ab}$	6.35 ± 2.26$_{bc}$	7.42 ± 2.35$_{abc}$	60.61 ± 4.18$_{bc}$	0.14 ± 0.14	33.98 ± 3.86$_{bc}$	26.50 ± 3.49	11.98 ± 3.05	7.46 ± 2.09$_{bc}$
G (6-7m)	30.55 ± 5.07$_{b}$	1.62 ± 1.19$_{a}$	2.74 ± 1.92$_{ab}$	3.60 ± 1.69$_{ab}$	15.06 ± 3.83$_{ab}$	7.52 ± 2.26$_{abc}$	45.61 ± 5.31$_{c}$	0	24.77 ± 5.43$_{bc}$	20.84 ± 4.51	13.14 ± 4.69	10.70 ± 2.91$_{c}$
H (7m+)	21.02 ± 3.93$_{ab}$	9.23 ± 3.13$_{b}$	2.42 ± 1.48$_{ab}$	4.44 ± 2.00$_{b}$	1.90 ± 1.02$_{c}$	3.03 ± 1.21$_{bc}$	47.29 ± 5.06$_{c}$	0	23.31 ± 4.42$_{c}$	23.99 ± 4.64	16.13 ± 4.21	15.56 ± 3.71$_{c}$
P-Value	***0.02***	***<0.01***	***<0.01***	***0.02***	***<0.01***	***<0.01***	***<0.01***	***0.47***	***<0.01***	***0.18***	***0.83***	***<0.01***
Notes:	Life Form = F, forb; G, graminoid, Sh, shrub; T, tree | Origin: I, introduced; N, native | Life Span = A/B, annual/biennial; P, perennial | Other = Forb, total forb; Unk, unknown; Grasminoid, total graminoid											

Table 2.5 Foliar cover composition by functional group for Site 2 in 2021. Mean composition with standard error is represented for each functional group by treatment. Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using a Kruskal-Wallis test and Dunn's Post Hoc test with a Bonferroni p-value adjustment.

Tree Classes	Mean Foliar Cover Composition (%/sample) by Functional Group at Site 2 (2021)												
	Forb	Unk Forb	FIA/B	FIP	FNA/B	FNP	Graminoid	GIA	GIP	GNA	GNP	ShNP	TNP
Control	$4.16 \pm 0.64_{a}$	0	0	0.03 ± 0.03	0.18 ± 0.11	$3.95 \pm 0.64_{a}$	94.53 ± 0.78	0_{a}	$13.47 \pm 1.83_{a}$	0.28 ± 0.17	$80.78 \pm 1.95_{a}$	$1.15 \pm 0.45_{a}$	$0.16 \pm 0.08_{a}$
C (2-3m)	$6.06 \pm 1.51_{ab}$	0.14 ± 0.09	0.07 ± 0.07	0.12 ± 0.09	0.06 ± 0.04	$5.66 \pm 1.50_{ab}$	91.85 ± 1.57	0_{a}	$34.05 \pm 3.20_{b}$	0.70 ± 0.49	$57.10 \pm 3.12_{b}$	$1.04 \pm 0.37_{a}$	$1.05 \pm 0.29_{ab}$
D (3-4m)	$2.34 \pm 0.66_{b}$	0.06 ± 0.06	0.10 ± 0.10	0.21 ± 0.13	0.42 ± 0.31	$1.55 \pm 0.58_{b}$	93.13 ± 1.22	0_{a}	$37.95 \pm 3.17_{b}$	0.57 ± 0.55	$54.61 \pm 3.20_{bc}$	$0.09 \pm 0.07_{a}$	$4.43 \pm 1.05_{bc}$
E (4-5m)	$3.40 \pm 0.84_{b}$	0.32 ± 0.18	0.08 ± 0.08	0.21 ± 0.21	0.21 ± 0.21	$2.58 \pm 0.74_{b}$	86.18 ± 2.27	0_{a}	$42.36 \pm 3.45_{b}$	0.62 ± 0.44	$43.19 \pm 3.35_{bc}$	$2.67 \pm 1.56_{a}$	$7.75 \pm 1.46_{c}$
F (5-6m)	$5.54 \pm 2.10_{ab}$	0.03 ± 0.03	0.60 ± 0.44	0.22 ± 0.16	0	$4.69 \pm 2.09_{b}$	83.14 ± 3.57	0_{a}	$46.08 \pm 5.16_{b}$	0	$37.06 \pm 5.44_{bc}$	$3.12 \pm 1.41_{ab}$	$8.20 \pm 2.68_{bc}$
G (6-7m)	$4.56 \pm 2.42_{ab}$	0.12 ± 0.12	0.87 ± 0.87	0	0.16 ± 0.16	$3.41 \pm 2.05_{b}$	80.35 ± 5.31	0_{a}	$52.37 \pm 6.69_{b}$	0	$27.99 \pm 7.13_{c}$	$0.93 \pm 0.93_{a}$	$14.16 \pm 5.08_{c}$
H (7m+)	$3.36 \pm 1.79_{ab}$	0	0	0	1.02 ± 0.73	$2.34 \pm 1.69_{ab}$	79.84 ± 5.54	$1.02 \pm 0.73_{b}$	$54.23 \pm 7.42_{b}$	0	$24.59 \pm 7.14_{c}$	$15.64 \pm 5.73_{b}$	$1.16 \pm 0.46_{abc}$
P-Value	***<0.01***	***0.34***	***0.37***	***0.28***	***0.39***	***<0.01***	***0.04***	***<0.01***	***<0.01***	***0.87***	***<0.01***	***<0.01***	***<0.01***
Notes:	Life Form = F, forb; G, graminoid, Sh, shrub; T, tree | Origin: I, introduced; N, native | Life Span = A/B, annual/biennial; P, perennial | Other = Forb, total forb; Unk, unknown; Graminoid, total graminoid												

Table 2.6 Shannon-Wiener diversity (H’), Shannon-Wiener evenness, Floristic Quality Index (FQI), species richness, and native species richness by treatment for Site 1 in 2020 and Site 2 in 2021. Mean and standard error is represented by treatment. Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using a Kruskal-Wallis test and Dunn’s Post Hoc test with a Bonferroni p-value adjustment.

Tree Classes	Site 1 (2020)					Site 2 (2021)				
	H'	Evenness	FQI	Total Rich	Nat Rich	H'	Evenness	FQI	Total Rich	Nat Rich
Control	0.98 ± 0.04	$0.61 \pm 0.02_{a}$	$10.60 \pm 0.39_{a}$	$5.01 \pm 0.16_{a}$	$3.24 \pm 0.15_{a}$	$1.07 \pm 0.03_{a}$	0.70 ± 0.02	$10.92 \pm 0.20_{a}$	$4.81 \pm 0.14_{a}$	$4.28 \pm 0.16_{a}$
C (2-3m)	1.00 ± 0.04	$0.70 \pm 0.02_{b}$	$9.25 \pm 0.34_{ab}$	$4.20 \pm 0.15_{b}$	$2.91 \pm 0.16_{ab}$	$0.93 \pm 0.04_{ab}$	0.70 ± 0.02	$9.46 \pm 0.26_{b}$	$3.86 \pm 0.13_{b}$	$2.93 \pm 0.15_{b}$
D (3-4m)	0.91 ± 0.04	$0.69 \pm 0.02_{ab}$	$8.22 \pm 0.42_{bc}$	$3.80 \pm 0.14_{b}$	$2.50 \pm 0.16_{abc}$	$0.88 \pm 0.04_{bc}$	0.73 ± 0.02	$8.58 \pm 0.30_{bc}$	$3.44 \pm 0.12_{bc}$	$2.37 \pm 0.14_{bc}$
E (4-5m)	0.89 ± 0.05	$0.71 \pm 0.03_{b}$	$7.35 \pm 0.51_{bc}$	$3.53 \pm 0.16_{b}$	$2.12 \pm 0.17_{c}$	$0.80 \pm 0.04_{bcd}$	0.67 ± 0.03	$7.25 \pm 0.31_{cd}$	$3.14 \pm 0.13_{cd}$	$2.17 \pm 0.12_{c}$
F (5-6m)	0.92 ± 0.05	$0.73 \pm 0.03_{b}$	$7.99 \pm 0.53_{bc}$	$3.70 \pm 0.21_{b}$	$2.22 \pm 0.17_{bc}$	$0.65 \pm 0.05_{cde}$	0.59 ± 0.04	$6.20 \pm 0.52_{d}$	$2.89 \pm 0.15_{cd}$	$1.85 \pm 0.15_{c}$
G (6-7m)	0.94 ± 0.09	$0.71 \pm 0.05_{b}$	$7.62 \pm 0.85_{bc}$	$3.81 \pm 0.33_{b}$	$2.61 \pm 0.27_{abc}$	$0.55 \pm 0.06_{e}$	0.61 ± 0.06	$4.33 \pm 0.72_{d}$	$2.37 \pm 0.15_{d}$	$1.44 \pm 0.16_{c}$
H (7m+)	0.78 ± 0.07	$0.63 \pm 0.05_{ab}$	$6.31 \pm 0.62_{c}$	$3.27 \pm 0.23_{b}$	$1.96 \pm 0.19_{c}$	$0.48 \pm 0.11_{de}$	0.45 ± 0.10	$4.11 \pm 0.81_{d}$	$2.29 \pm 0.30_{cd}$	$1.36 \pm 0.31_{c}$
P-value	***0.21***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***<0.01***	***0.02***	***<0.01***	***<0.01***	***<0.01***

FIGURES

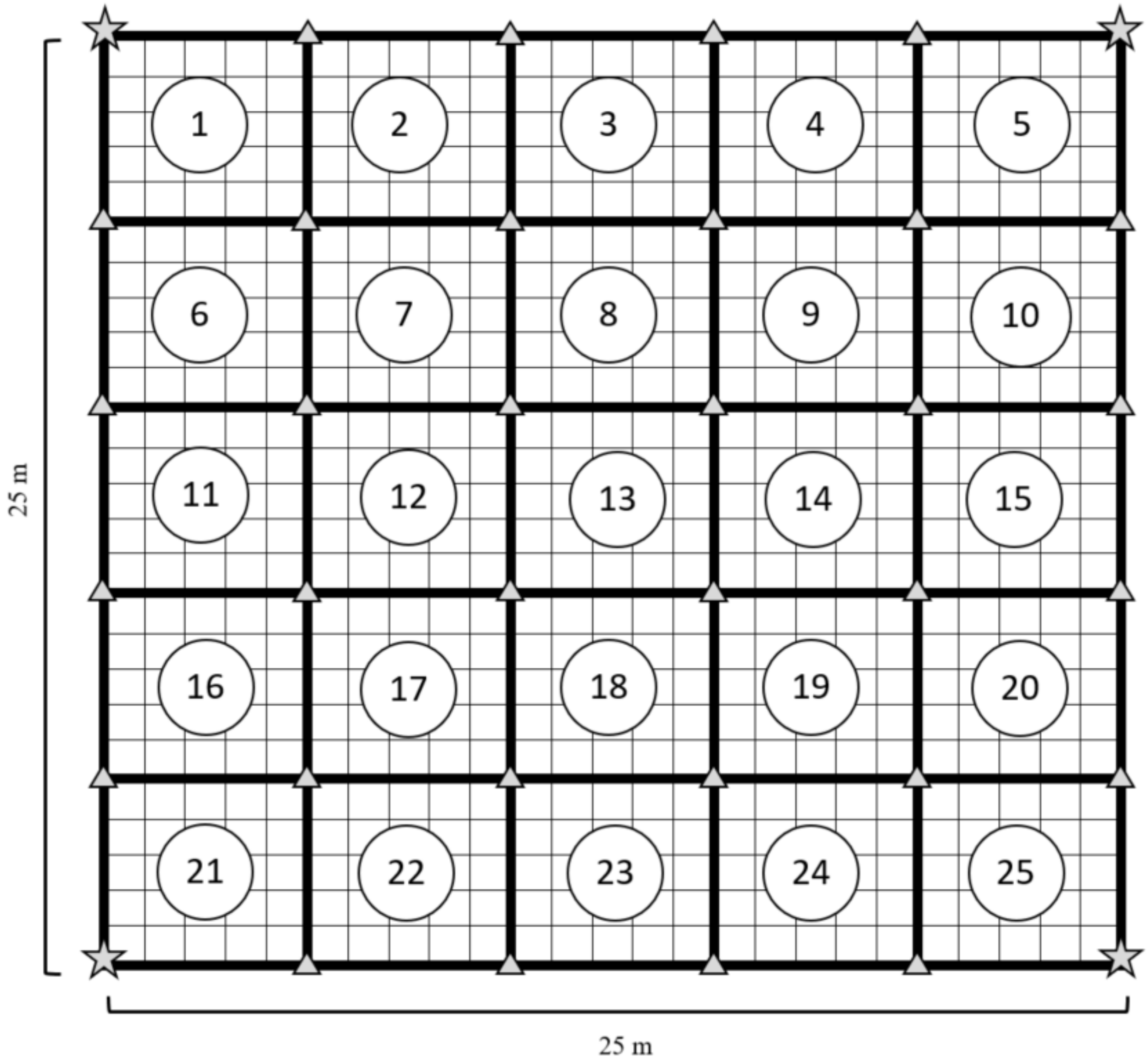

Figure 2.1 Aerial illustration of permanent plot design with stars representing metal T-posts at the four corners, triangles representing temporary step in posts placed every 5 m, and each 5 m x 5 m subplot labeled numerically 1-25.

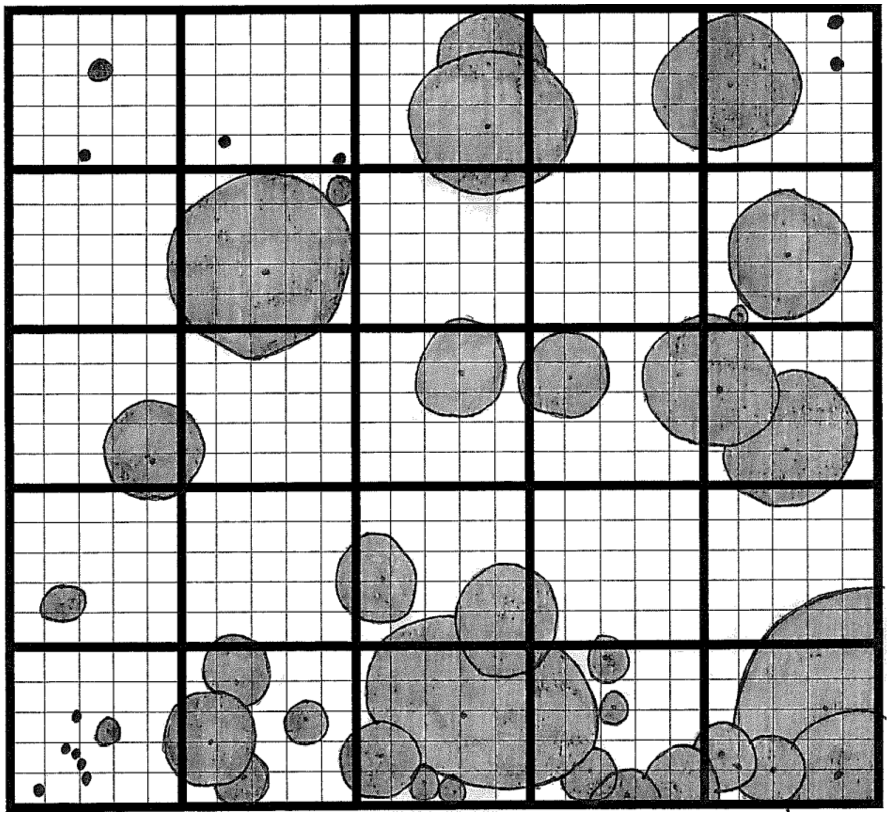

Figure 2.2 Aerial illustration of a finalized hand-drawn map where each "circle" represents the relative location and canopy size of an eastern redcedar tree which was used as a reference for future sampling and for calculating ERC stand canopy cover (%) within each plot.

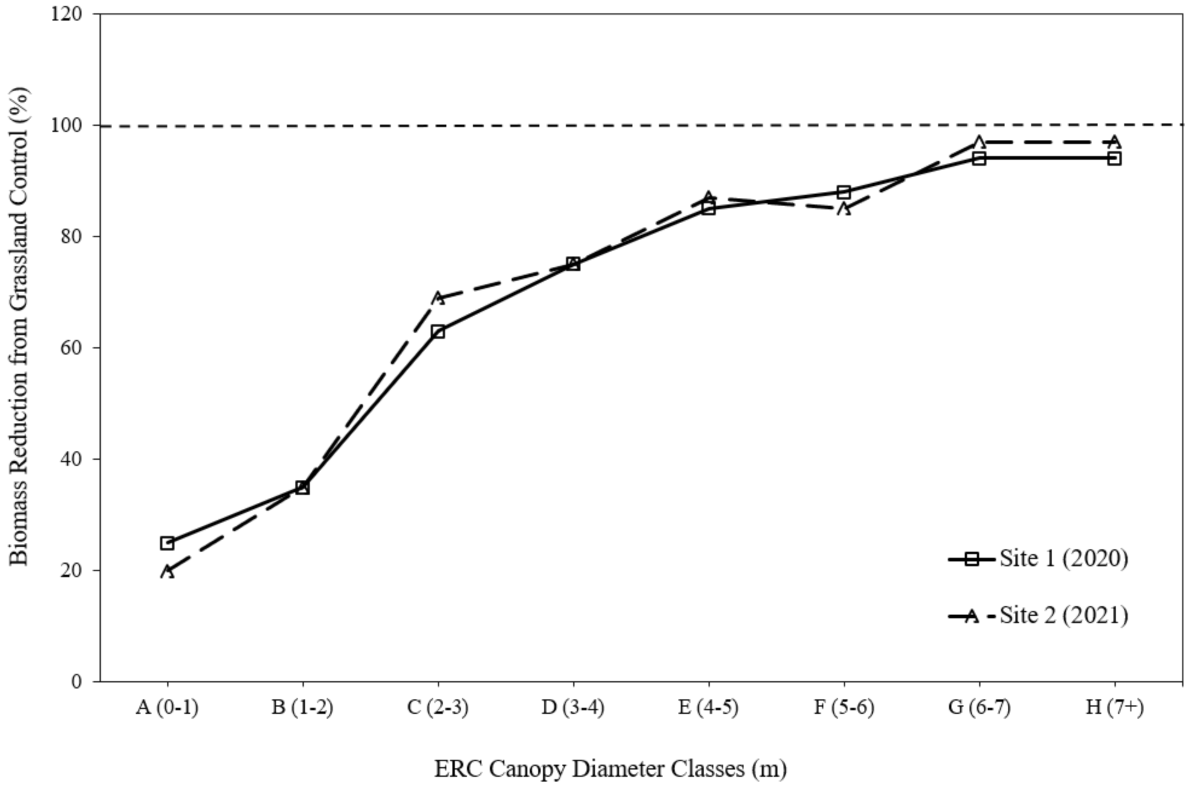

Figure 2.3 Mean biomass reduction (%) from grassland control samples in relation to tree classes of eastern redcedar canopy diameter (m) for Site 1 in 2020 and Site 2 in 2021. The horizontal dotted line on top represents 100% biomass reduction, or a herbaceous biomass value of 0.

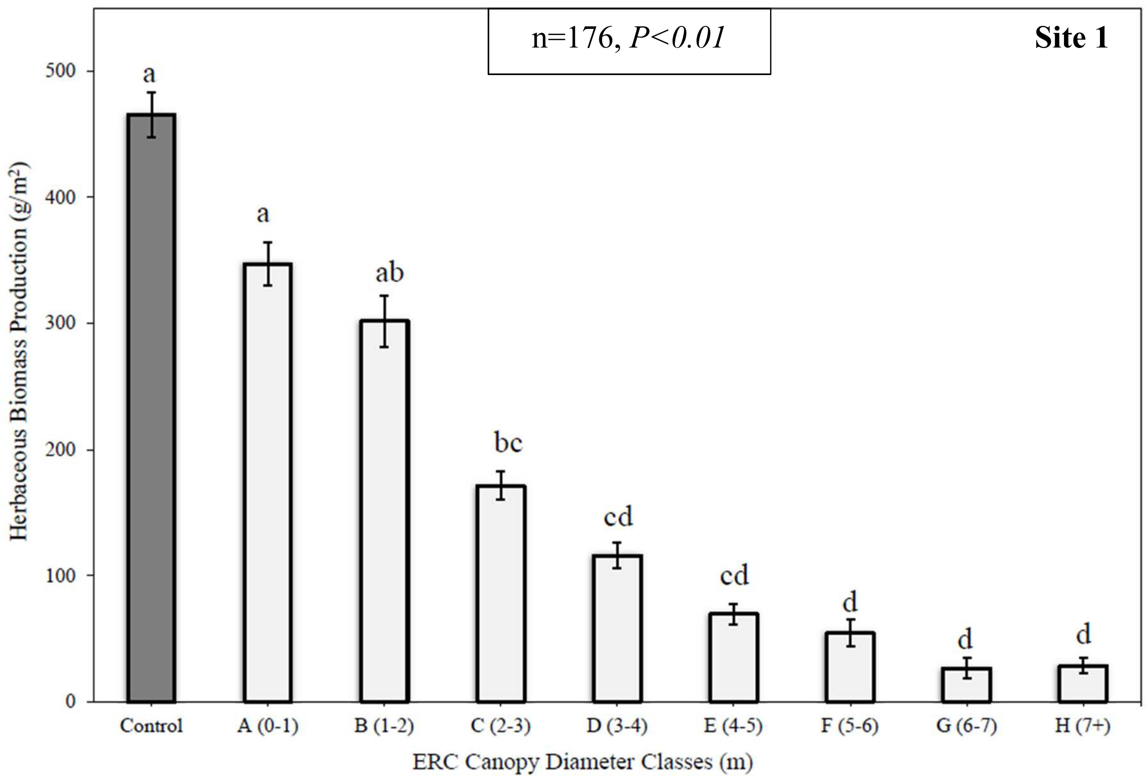

Figure 2.4 Mean herbaceous biomass production (g/m^2) with standard error bars by treatment at Site 1 in 2020, where Control represents biomass samples collected from open grassland locations without ERC encroachment and treatments A-H represent biomass samples collected underneath ERC canopies based on canopy diameter (m). Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using a Kruskal-Wallis test and Dunn's Post Hoc test with a Bonferroni p-value adjustment.

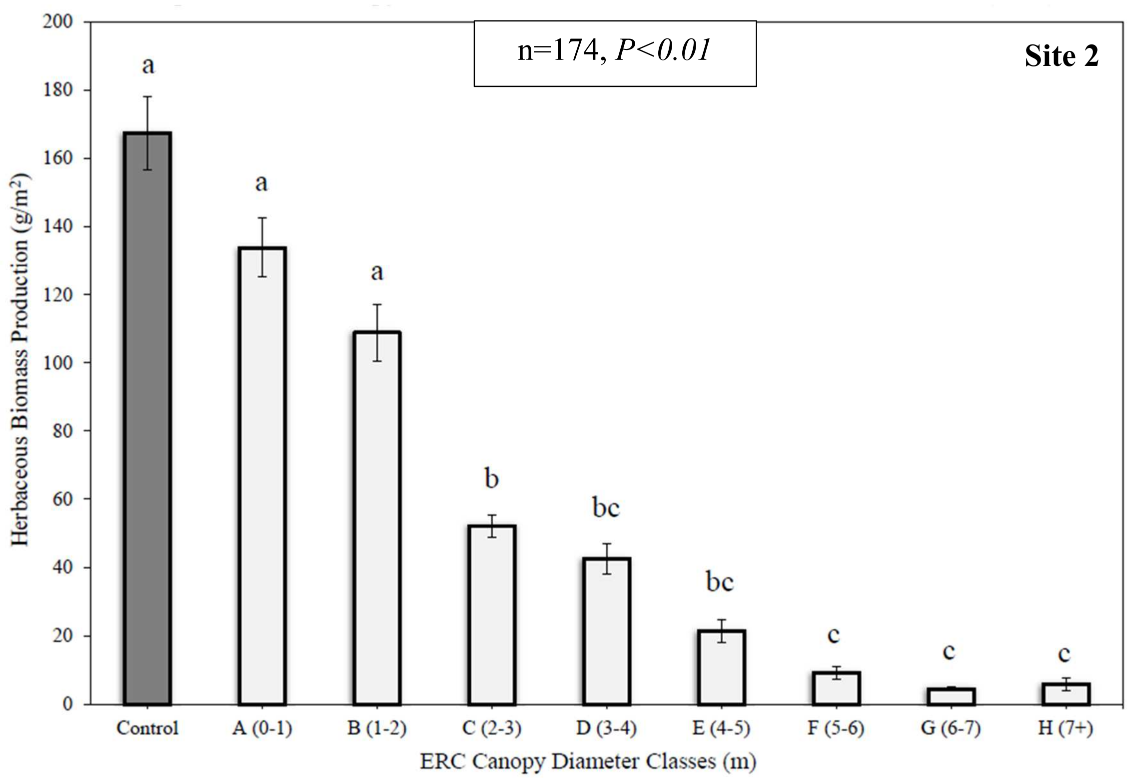

Figure 2.5 Mean herbaceous biomass production (g/m^2) with standard error bars by treatment at Site 2 in 2021, where Control represents biomass samples collected from open grassland locations without ERC encroachment and treatments A-H represent biomass samples collected underneath ERC canopies based on canopy diameter (m). Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using a Kruskal-Wallis test and Dunn's Post Hoc test with a Bonferroni p-value adjustment.

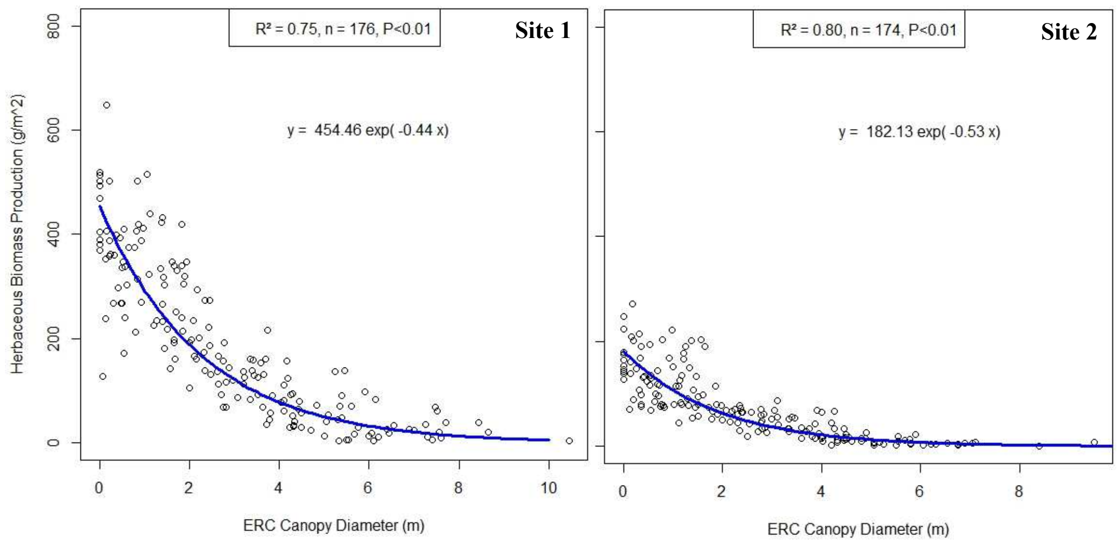

Figure 2.6 Exponential relationships between ERC canopy diameter (m) and herbaceous biomass production (g/m^2) for Site 1 in 2020 (n=164) and Site 2 in 2021 (n=162). Samples with x=0 represent grassland control samples with no ERC encroachment (n=12/site).

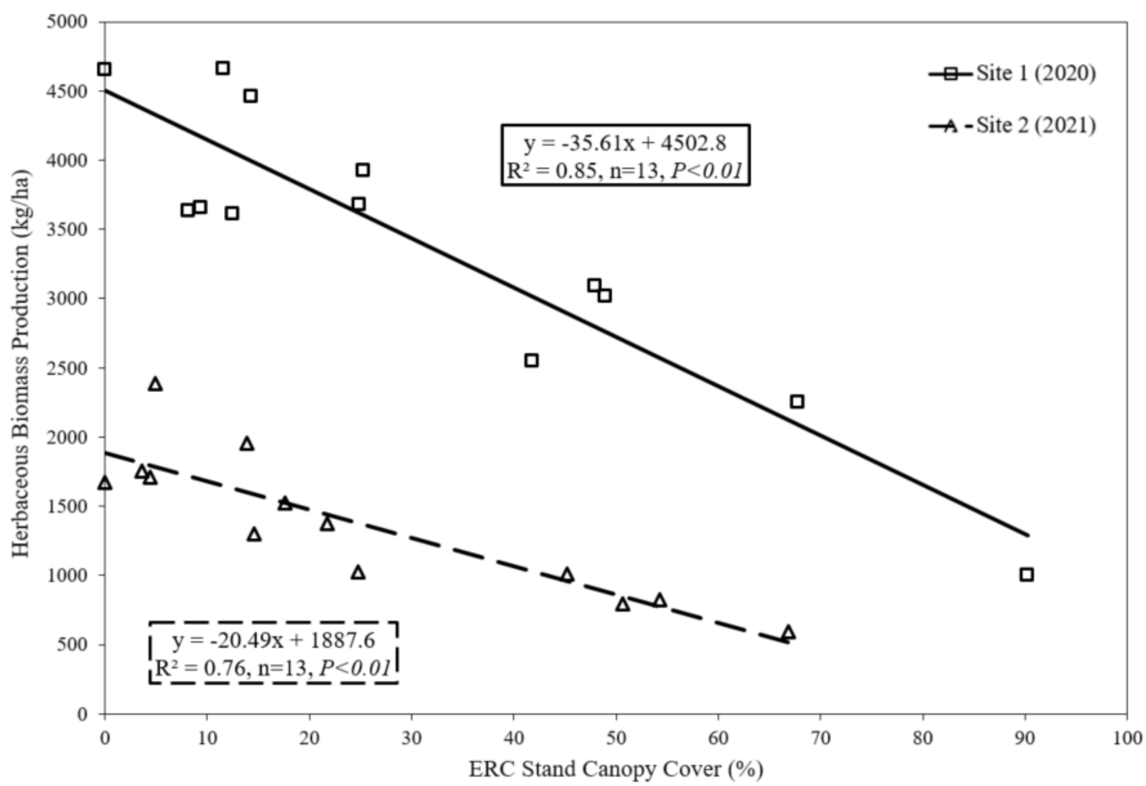

Figure 2.7 Relationship between eastern redcedar stand canopy cover (%) and herbaceous biomass production (kg/ha) by plot (n=24) ranging from 0-90% ERC stand

canopy cover at Site 1 in 2020 and Site 2 in 2021. Data points where x=0% represent mean biomass production assuming no ERC encroachment (grassland control).

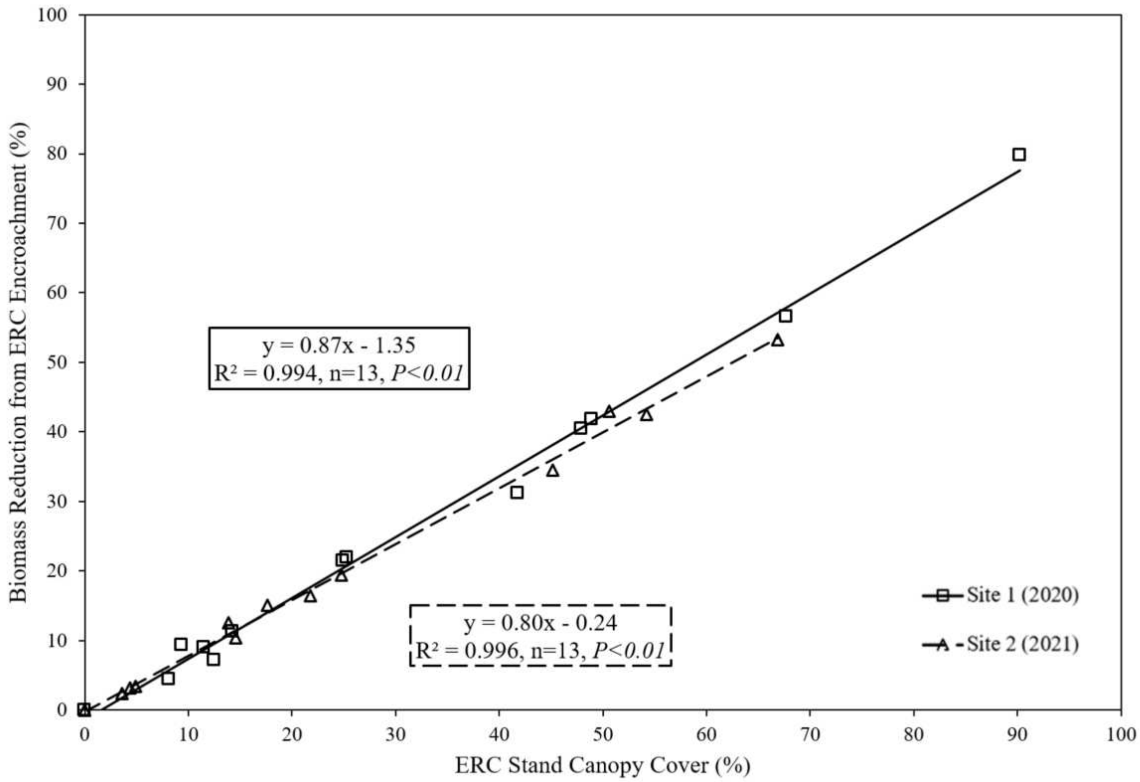

Figure 2.8 Relationship between eastern redcedar stand canopy cover (%) and herbaceous biomass reduction (%) with a linear trend line for Site 1 in 2020 and Site 2 in 2021. Trend line equations, sample sizes, and coefficients of determination are outlined by Site.

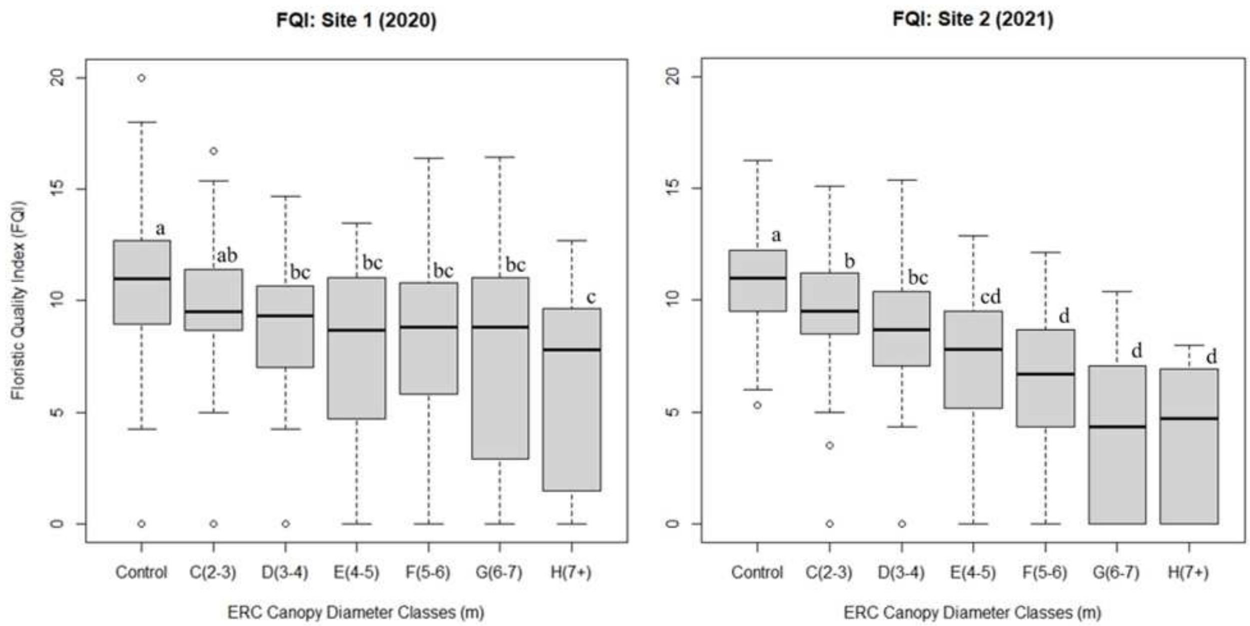

Figure 2.9 Floristic quality indexes (FQI) between treatment groups at Site 1 in 2020 and Site 2 in 2021. Median and interquartile range (IQR) represent treatments analyzed using a Kruskal-Wallis test. The line in the middle of the box represents the median, whiskers represent IQR, and small circles represent potential outliers. Different letters indicate significance (*P<0.05*) between treatment medians within the year.

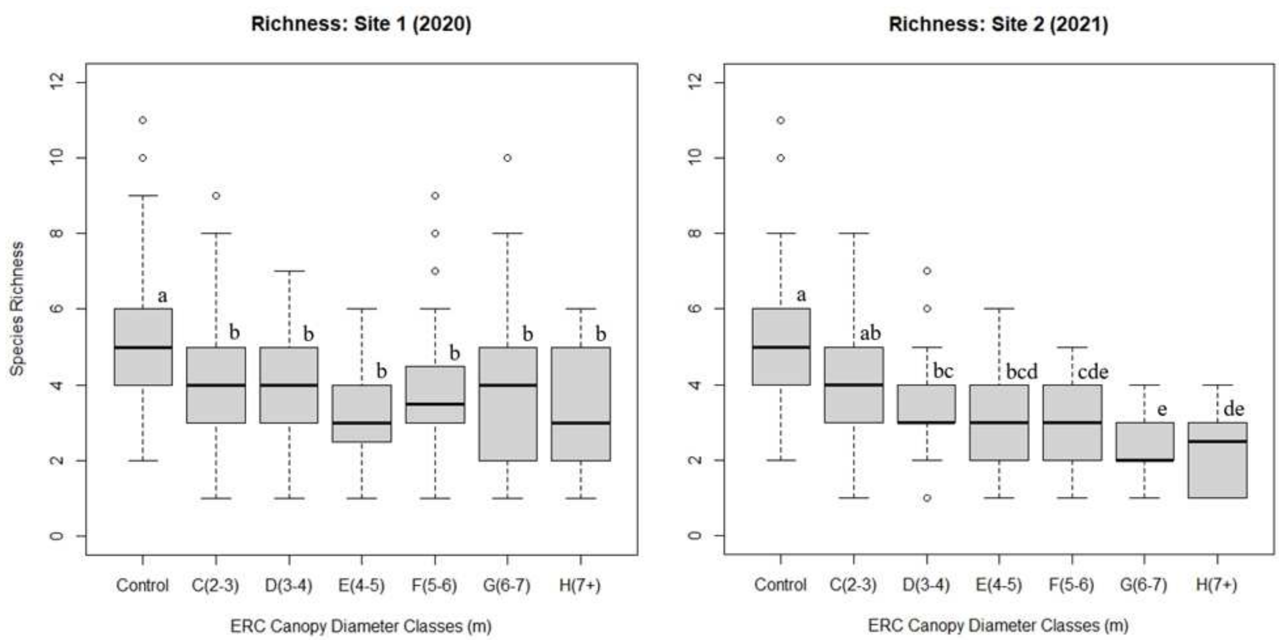

Figure 2.10 Total species richness between treatment groups at Site 1 in 2020 and Site 2 in 2021. Median and interquartile range (IQR) represent treatments analyzed using a Kruskal-Wallis test. The line in the middle of the box represents the median, whiskers represent IQR, and small circles represent potential outliers. Different letters indicate significance (*P<0.05*) between treatment medians within the year.

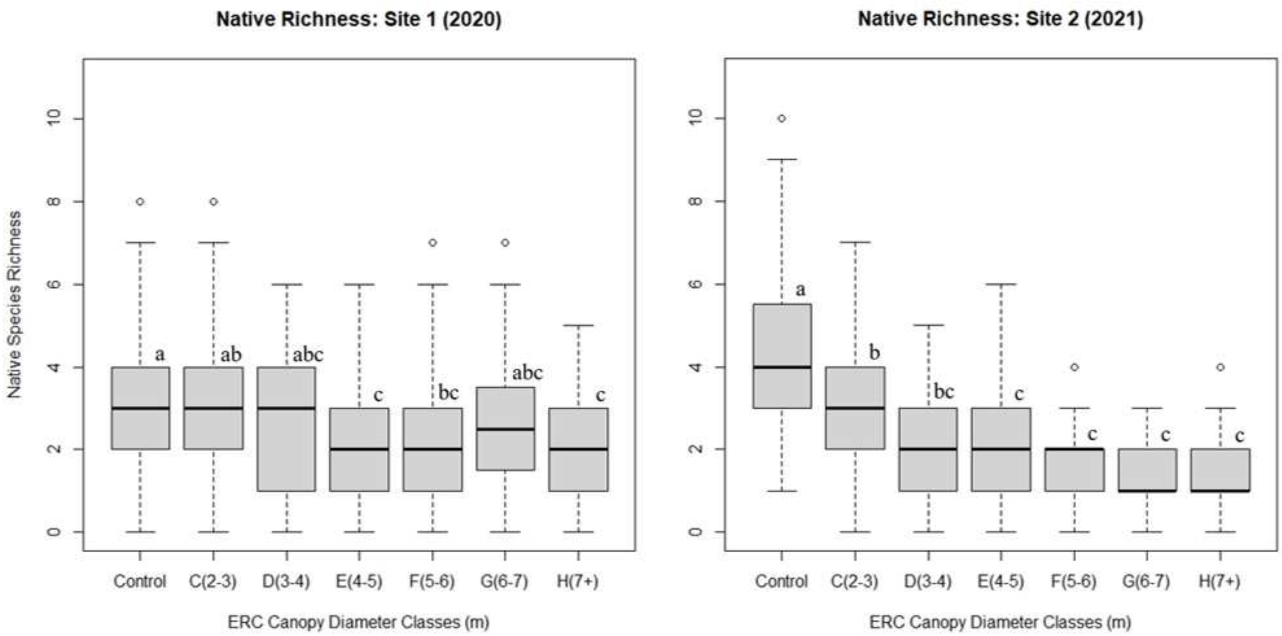

Figure 2.11 Native species richness between treatment groups at Site 1 in 2020 and Site 2 in 2021. Median and interquartile range (IQR) represent treatments analyzed using a Kruskal-Wallis test. The line in the middle of the box represents the median, whiskers represent IQR, and small circles represent potential outliers. Different letters indicate significance (*$P<0.05$*) between treatment medians within the year.

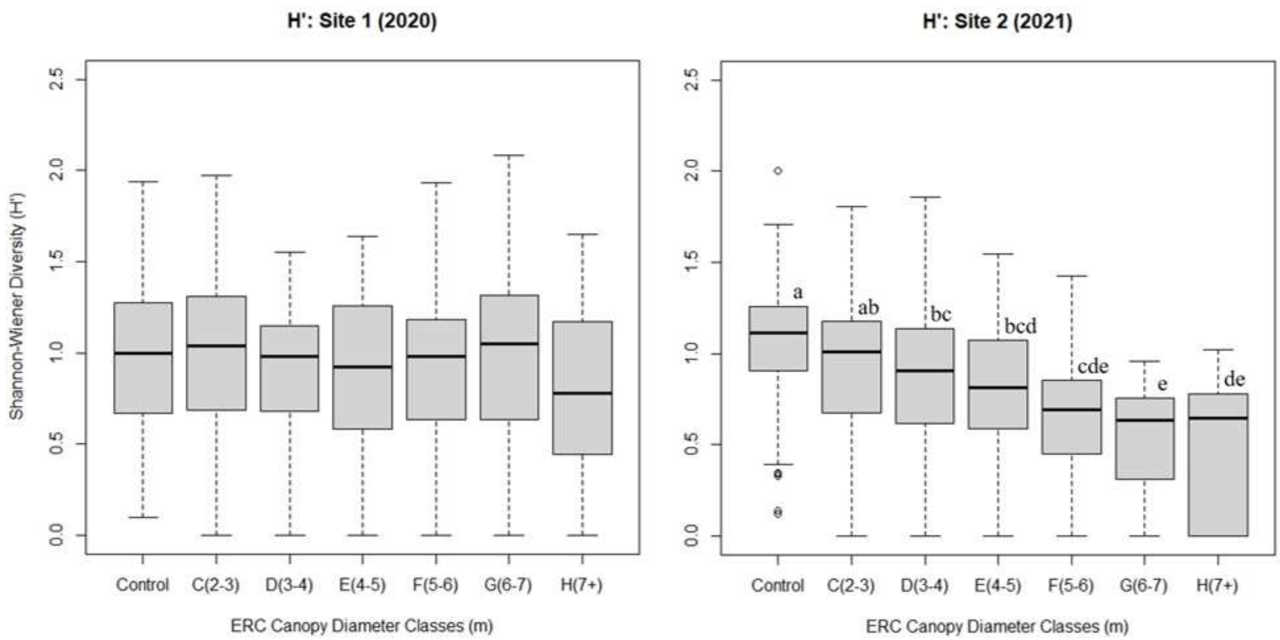

Figure 2.12 Shannon-Wiener Diversity (*H'*) between treatment groups at Site 1 in 2020 and Site 2 in 2021. Median and interquartile range (IQR) represent treatments analyzed using a Kruskal-Wallis test. The line in the middle of the box represents the median, whiskers represent IQR, and small circles represent potential outliers. Different letters indicate significance ($P<0.05$) between treatment medians within the year.

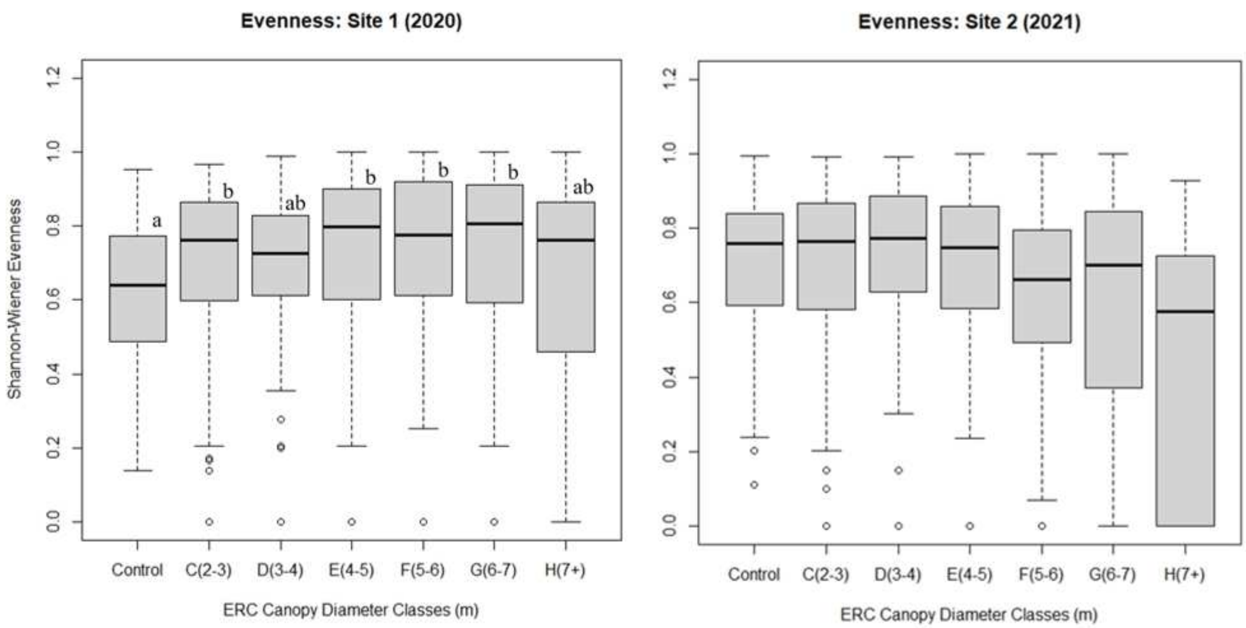

Figure 2.13 Shannon-Wiener Evenness between treatment groups at Site 1 in 2020 and Site 2 in 2021. Median and interquartile range (IQR) represent treatments analyzed using a Kruskal-Wallis test. The line in the middle of the box represents the median, whiskers represent IQR, and small circles represent potential outliers. Different letters indicate significance (*P<0.05*) between treatment medians within the year.

CHAPTER 3: LARGE EASTERN REDCEDAR IMPACT ON SOIL SEED BANK IN THE NORTHERN GREAT PLAINS MIXED-GRASS PRAIRIE

ABSTRACT

Eastern redcedar (ERC) (*Juniperus virginiana* L.) trees are invading prairies throughout the Great Plains due to fire suppression and escaping from planted ERC shelterbelts. This encroachment poses a threat to native plant communities in terms of their reproduction, regeneration, and diversity. It is unknown how ERC trees impact the vegetative soil seed bank mixed-grass prairie or the accumulation and longevity of ERC seed in the seed bank. The objective of this study was to evaluate how large female ERC trees impact the soil seed bank at varying distances from an ERC trunk. In addition, we classified, quantified, and weighed ERC seeds found in the soil seed bank. In October 2020 (Site 1) and 2021 (Site 2) in south-central South Dakota, ten female ERC trees with canopy diameters 5-10 m, similar environmental characteristics (ie. soil type, slope), and isolated from other large ERC trees were selected for soil sampling at four treatment distances: under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Four transects extended from each tree stem where a soil core (10 cm dia. x 10 cm depth) was sampled at the four treatment distances, totaling 16 cores per tree and 160 cores overall. Soil cores were broken down and sieved (2 mm) to remove rocks, litter, and plant materials. Soil processed through the sieve was planted in flat trays, watered, and grown in an environmentally controlled greenhouse. Emerged seedlings were identified and counted in each tray for 3 months. Remaining soil that did not fall through the 2 mm sieve was sorted for ERC seeds where they were classified, counted, and weighed. Seedling density was not different (*$P=0.27$, $P=0.74$*) among

treatments at both sites. Seed bank composition among treatments was variable within and between sites, but samples collected underneath ERC canopies tended to consist of more introduced forb and annual/biennial species. Little correlation existed between aboveground vegetation and emerged seedlings. Most ERC seeds found in the soil seed bank were damaged and found underneath ERC canopies. Our results suggest that large female ERC are impacting seed bank composition primarily underneath their canopies and regeneration following ERC removal from the seed bank will likely not reflect aboveground vegetation compared to non-encroached grasslands. We recommend further research of other restoration strategies to reestablish native perennial vegetation quickly underneath previous ERC canopies following ERC removal.

INTRODUCTION

Grasslands historically covered 46 million km^2 of Earth's surface representing nearly 42% of the living vegetation (Anderson 2006). Encroachment of woody species onto grasslands and savannas is a widely researched global phenomenon, with junipers (*Juniperus spp.* L.) and pines (*Pinus spp.* L.) being the most common woody encroachers in the United States (Miller et al. 2000). Historic grasslands in North America were maintained through a combination of wildfire and grazing, inhibiting the spread of forest from northern latitudes or riparian areas and resulting in a grass dominated landscape for the last 5000-8000 years (Higgins 1986, Twidwell et al. 2013). Since European settlement, dramatic changes have converted our grasslands from their historical state through fire suppression, cultivation, and woody encroachment (Engle et al. 2008). This woody encroachment in North America has led to grassland systems being one of the most endangered ecosystems (Engle et al. 2008). The increase of woody plants on grasslands alters nutrient cycling, forage production, flora and fauna species composition, landscape heterogeneity, and risk of wildfire (Belsky 1994, DeSantis et al. 2011, Knapp et al. 2008, Limb et al. 2010, Miller et al. 2000, Van Auken 2009, Van Els et al. 2010, Wang et al. 2018, Williams et al. 2017).

In the Great Plains, eastern redcedar (*Juniperus virginiana* L.) (hereafter ERC) is the most prominent woody encroacher (Meneguzzo and Liknes 2015, Schmidt and Leatherberry 1995). ERC are spreading at alarming rates and have been termed the "green glacier" by researchers, occupying up to seven million hectares of rangeland and increasing exponentially in some areas (Bidwell et al. 1996, Engle et al. 2008, McKinley et al. 2008). ERC is an early successional native conifer species in North America present in every state east of the 100th meridian, with higher densities in Oklahoma, Kansas, Nebraska, Missouri, and South Dakota (Meneguzzo and Liknes 2015, Twidwell et al. 2021). ERC have increased by nearly 125,000 hectares in an eight-state region in the Northern Great Plains (NGP) between 2007-2012, by 2.3% per year in portions of the Kansas Flint Hills, and at a rate of 8% per year in Oklahoma (Briggs et al. 2002, Meneguzzo and Liknes 2015, Wang et al. 2018). Fire historically controlled and confined this native conifer species primarily to riparian areas or steep, rocky slopes (Lawson 1990). Fire suppression, overgrazing, and planting ERC in shelterbelts has allowed ERC to successfully encroach and spread rapidly onto grasslands in the Great Plains due to its ability to compete for scarce resources and its high reproductive rate, with female trees producing up to 1.5 million berry-like cones on productive years (Briggs et al. 2002, Engle et al. 1987, Holthuijzen and Sharik 1985, Lawson 1990). Avian generalists, small mammals, and white-tailed deer are known to eat these fruit-like cones off ERC contributing to its seed dispersal and propagation on our grassland systems (Bidwell et al. 1996, Holthuijzen and Sharik 1985, Horncastle et al. 2004) resulting in a potential closed canopy in as little as 40 years (Briggs et al. 2002).

In these encroached grassland systems, much of the regeneration after woody removal is dependent on the belowground plant communities, including the soil seed bank (Everett and Ward 1984, Harper 1977). Soil seed banks provide temporal reserves of previous plant communities and can influence genetic heterogeneity within a population, species richness, and species diversity (Perkins et al. 2019, Plue and Cousins 2013), but often are only expressed in perennial systems following disturbance (Sternberg et al. 2003). Seed banks are composed of seeds produced from onsite sexual reproduction and offsite seed immigration by dispersal through wind, water, or other vectors (e.g. animals, humans, etc.) (Soons et al. 2004), although most seeds are dispersed short distances (< 30 m) from their parent source (Wilson 1993). Some seeds even possess adaptations to allow for further dispersal distances including fluffy pappas (Skarpaas et al. 2004), wing-like structures, or seed hooks (Nathan 2006). Seed production is variable among species and is usually dependent on life span, where some annual plants, such as *Bromus tectorum* (cheatgrass), can produce hundreds of seeds per individual (Pyke and Mack 1983). Annual plants rely heavily on seed production and success, being it's their sole method of reproduction (Burnside et al. 1996). Perennial plants may have minimal to high seed production, with some plants producing only a few seeds in their lifetime (Arizaga et al. 2000). Seed longevity is influenced by disturbance (fire, grazing, and seed predation) and varies among species, with forbs often outlasting grasses due to their hard seed coat (Baskin and Baskin 2001, Snyman 2010). Forbs can germinate after sown in the seed bank for up to 17 years (Burnside et al. 1996) whereas grasses can persist up to 5 years, but viability of most grasses doesn't last more than a year (Baskin and Baskin 2001, Burnside et al. 1996, Snyman 2010). These factors often skew seed bank composition in perennial dominated systems and express high annual and forb production when germinated (Perkins et al. 2019).

The effects of woody encroachment on the soil seed bank have yet to be studied in the NGP. Few seed bank studies exist that evaluate the impact of woody encroachment on grasslands and shrub lands, with varying results on its response pre- and post-removal of the woody invaders. In central Nevada on encroached sagebrush communities, pinyon-juniper tree cover did not impact seed density and species diversity in the soil seed bank as tree cover increased (Allen and Nowak 2008). The seed bank community based on seed density by species and by life form was not different between tree cover classes (Allen and Nowak 2008) and its common for the soil seed bank to be homogeneous among microhabitats (Allen and Nowak 2008, Torres et al. 2012). Low correspondence existed between aboveground vegetation and seed bank composition (Allen and Nowak 2008), indicating limited potential for restoration from seed bank alone following woody encroachment control (Görzen et al. 2019, Lang and Halpern 2007). Most of the species present in the seed bank that were not found in the aboveground vegetation were annual forbs (Allen and Nowak 2008, Koniak and Everett 1982). In contrast, *Leptospermum scoparium* (Manuka) trees in southern Australia were found to reduce seed bank species richness and abundance underneath their canopies (Price and Morgan 2008). Similar results were also found with reduced seed bank species richness in the western Cascade Range of Oregon from conifer encroachment on mountain meadows (Lang and Halpern 2007) and *Quercus suber* (cork oak) in central Spain (Torres et al. 2012). Understanding how woody encroachment impacts seed bank production and composition may shed light onto how plant communities will respond following woody control efforts via mechanical removal or prescribed fire (Torres et al. 2012), although the predictability of post-fire vegetation has proven in some studies to be extremely difficult (Everett and Ward 1984).

Propagation from the vegetative bud bank is responsible for a majority of plant regeneration in an undisturbed or disturbed perennial species dominated grassland and seedling establishment from the seed bank is rare (Benson et al. 2004, Benson and Hartnett 2006). However, seed banks are important and sometimes expressed following disturbance events in perennial dominated systems and can aid in adding diversity or genetic heterogeneity to the plant community (Egler 1954, Sternberg et al. 2003). Fire is a common disturbance in the NGP and has been used widely to control woody encroachment, especially ERC (Ortmann et al. 1998). A few studies have even evaluated the soil seed bank following woody encroachment control using prescribed fire in California (Everett and Ward 1984) and Nevada (Koniak and Everett 1982). In the White River Mountains of eastern Nevada, succession following pinyon-juniper control was evaluated through plant community comparisons pre- and post-fire (Everett and Ward 1984). Most initial colonizers following prescribed fire in pinyon-juniper woodlands were species found in the seed bank prior to the fire (Everett and Ward 1984), unlike previous studies where little correlation was found between aboveground and belowground plant communities (Abrams 1988, Allen and Nowak 2008, Görzen et al. 2019, Hopfensperger 2007). In the Sweetwater Mountains of California, species diversity and seedling emergence decreased in pinyon-juniper stands compared to open grass plots post-fire (Koniak and Everett 1982), suggesting woody encroachment does impact a plant community's response following control compared to non-encroached sites. These results suggest seed bank reserves may not provide rescue or resiliency after long term disturbance, such as species invasion through woody encroachment (Everett and Ward 1984, Godefriod et al. 2017, Koniak and Everett 1982).

Few seed bank studies exist that evaluated the impact of woody encroachment on grasslands and shrub lands (Allen and Nowak 2008). In addition, little research has been conducted on how ERC impact plant communities in the NGP (Zou et al. 2018), especially one that compares both aboveground and belowground plant communities. This research is the first of its kind to our knowledge evaluating how ERC encroachment may impact the belowground soil seed bank, which is an important factor to plant community regeneration following disturbance (Sternberg et al. 2003) and will be essential to understanding how these communities will respond following ERC control via mechanical removal or prescribed fire. The objectives of our study were to 1) determine the impact of large ERC trees on seed bank production and composition at varying distances and aspects from ERC trunks, 2) compare aboveground and belowground plant communities to predict potential regeneration following ERC removal, and 3) classify, categorize, and calculate the density of ERC seeds in the soil seed bank. Based on knowledge from previous studies, the alternative hypotheses for this study were:

1. Seed bank production will not be different among treatments of distance or aspect, specifically underneath ERC canopies in comparison to open grassland plots.
2. Seed bank composition will differ underneath ERC canopies in comparison to the canopy edge, two meters from the canopy edge, and grassland control locations due to an increase in abundance of introduced and annual/biennial species.
3. Seed bank diversity, evenness, and species richness will not differ underneath ERC canopies compared to open grassland plots. Floristic Quality Index (FQI) and native species richness will be different underneath ERC canopies compared to other treatments.
4. Aboveground plant communities will show little resemblance to emergent seedlings from the soil seed bank.

5. ERC seed density in the soil seed bank will be highest underneath ERC canopies and decrease in density as distance increases from ERC trunks.
6. Based on previous literature (Holthuijzen and Sharik 1984, Parker 1952), minimal seed predation will occur in ERC seeds accumulated in the soil seed bank.

MATERIALS AND METHODS

STUDY AREA

This study was conducted on two separate private ranches in south-central South Dakota in the Northern Great Plains mixed-grass prairie. Ranch 1, referred to as Site 1 (285 ha), is located in the Bijou Hills of Brule County along the east side of the Missouri River near Academy, South Dakota. This ecoregion contains a mixture of steep hills (15-40% slopes) surrounded by rolling mixed-grass prairie, cropland, and rangeland pastures. Soils primarily consist of Okaton bouldery silty clay (clayey residuum weathered from shale) where sampling was conducted (Soil Survey Staff 2022). Elevation ranges from 400 to 500 meters above sea level. Ranch 2, referred to as Site 2 (70 ha), is located in Charles Mix County along the east side of the Missouri River near Platte, South Dakota. This ecoregion contains a mixture of steep valleys and drainages (15-40% slopes) surrounded by rolling mixed-grass prairie, flat-topped ridges, cropland, and rangeland pastures. Soils primarily consist of Betts-Ethan loams (fine-loamy till) with abundant moraine at or near the soil surface (Soil Survey Staff 2022). Elevation ranges from 340 to 680 meters above sea level.

Sites 1 and 2 are close in geographic proximity (<40 km), therefore the same data was used to describe their climate. The landscape experiences a semiarid climate, consisting of hot, dry summers and cold, wet winters. The average annual temperature in

2020 was 8.5□C with a low of -24.4□C (February) and a high of 35.5□C (June). The total annual precipitation in 2020 was 445 mm with 86% of the precipitation occurring during the growing season (May – August), which was 15% higher than the 30-year average (1990-2019) during the growing season (HPRCC 2022, Mesonet 2022). The average annual temperature in 2021 was 9.1□C with a low of -31.7□C (February) and a high of 40.6□C (June). The total annual precipitation in 2021 was 399.8 mm with 53.7% of the precipitation occurring during the growing season (May – August), which was 36.4% lower than the 30-year average during the growing season indicating a drought (HPRCC 2022, Mesonet 2022). Deviations of monthly temperature and precipitation from the 30-year (1990-2019) average are shown in Appendix Tables A.1, A.2 and Figures A.1, A.2.

Site 1 consists of a disturbed mixed-grass prairie with ERC encroachment and no previous cattle grazing activity or prescribed fire within the past five years. The vegetation at this site is dominated by introduced graminoids including *Poa pratensis* L. (Kentucky bluegrass) and *Bromus inermis* Leyss. (smooth bromegrass) with native graminoids mixed throughout including *Nassella viridula* (Trin.) Barkworth (green needlegrass), *Andropogon gerardii* Vitman (big bluestem), *Sporobolus compositus* (Poir.) Merr. (composite dropseed), *Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould (Scribner's rosette grass), and *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass). Various forb species are present adding to the diversity of the site including *Solidago missouriensis* Nutt. (Missouri goldenrod), *Monarda fistulosa* L. (wild bergamot), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), and *Artemisia ludoviciana* Nutt. (white sagebrush).

Site 2 consists of a disturbed mixed-grass prairie with ERC encroachment and previous cattle grazing from April – June at a stocking rate of 75 cow-calf pairs on 70 hectares. However, this land was deferred (not grazed) in 2021 for sampling and to build fuel for a prescribed fire in spring 2022. The vegetation at this site is dominated by a mixture of native graminoids such as *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needle and thread), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama), *Bouteloua dactyloides* (Nutt.) J. T. Columbus (buffalograss), and *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama). Numerous forb species add to the diversity of the landscape, primarily dominated by natives, such as *Echinacea angustifolia* DC. (blacksamson echinacea), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), *Symphyotrichum ericoides* (L.) G.L. Nesom (white heath aster), *Verbena stricta* Vent. (hoary verbena), and some introduced species including *Cirsium arvense* (L.) Scop. (Canada thistle) and *Verbascum thapsus* L. (common mullein).

TREE SELECTION

Ten ERC trees were selected for sampling based on the following characteristics: (1) the tree is female, (2) average canopy diameter 5-10 m (mature and high reproductive rate), (3) at least five meters from other large (>3 m canopy diameter) ERC trees, and (4) environmental characteristics are similar (ie. slope, soil composition, aspect). For future reference, trees were tagged with aluminum tree tags at the base of their trunk and their approximate GPS location recorded.

TREATMENTS

Four treatments were compared in this study based on distance from mature, female ERC trees. The treatments were control (grassland locations >5 m from tree canopy, GL), under canopy (UC), canopy edge (CE), and two meters from the canopy edge (2M).

FIELD METHODS: SOIL SEED BANK CORE SAMPLING

Seed bank soil cores were collected during the last week of October following seed set of the current year's vegetation (Allen & Nowak 2008). One transect in each of the four cardinal directions extended from each ERC tree stem. Using a lever-action golf hole cutter (10 cm dia. x 10 cm depth), a soil core was collected on each transect line at the four treatment distances (UC, CE, 2M, and GL), totaling 4 cores per transect, 16 cores per tree, and 160 cores overall. UC cores were collected at half canopy radius and CE cores where the midline of the core was in line with the canopy edge. Soil cores were stored in plastic Ziploc bags in a cooler on ice while driven back to Brookings, SD from the field site near Chamberlain, SD. Once arriving at the lab, soil cores were stored in a walk-in refrigerator (4 °C) awaiting preparation and planting in the greenhouse.

LAB METHODS: PREPARATION AND GREENHOUSE GERMINAL SOIL SEED BANK EXPERIMENT

Soil core samples were broken down by hand, rolled to break up clumps, and sieved through a 2 mm wire sieve to eliminate roots, rhizomes, crowns, litter, and rocks. Excess material that was larger than 2 mm and therefore did not fall through the 2 mm wire sieve was placed in individual plastic Ziploc bags for future lab procedures. After sieving, 166 plastic seed flats (25 cm x 25 cm standard greenhouse trays with perforated bottoms) were prepared with a paper towel in the base to eliminate soil loss through drainage holes in the bottom. Two centimeters of Miracle Gro® potting soil was placed in each seed flat to help fertilize the samples and add soil content for adequate plant growth. One sieved sample (10 cm dia. x 10 cm depth) was placed in its own seed flat (25 cm x 25 cm) on top of the potting soil and grown in a controlled greenhouse with a photoperiod cycle of 16 hours light and 8 hours dark and at 23 ± 3 □C to ensure maximized germination. Seed flats were watered daily by misting mid-morning. During abnormally high evaporation periods, seed flats were monitored with toothpicks for dry soil and watered again in the afternoon if needed to ensure adequate soil moisture. A total of 166 seed flats consisted of 40 flats from each of the four treatments and six control flats with only Miracle Gro® potting soil were placed randomly around the greenhouse to monitor greenhouse weeds.

LAB METHODS: ERC SEED DENSITY

The excess material larger than 2 mm saved from previous sieving procedures was used to determine ERC seed density in the soil seed bank. ERC seeds are teardrop shaped, light orange to dark brown in color, and range between 2-4 mm in size (Van Haverbeke and Read 1976), which inhibits them from passing through the 2 mm sieve. As a result, we can assume a majority of ERC seeds present in our original soil core were collected in the excess material in the 2 mm sieve used in previous lab procedures. When sorting through the litter searching for ERC seeds, we found three common categories of ERC seeds: intact, broken, and seeds with a distinct hole in the seed coat (Figure B.1). Intact seeds were visibly in near perfect shape, consisting of no cracks, holes, or deformities in the seed coat. Broken seeds were a mixture of different shapes and sizes where visual interpretation of distinct shapes, physical characteristics, and seed coat thickness were used to identify and classify as pieces coming from previous ERC seeds. Seeds with a distinct hole in the seed coat were classified based on small circular holes at the tip, base, or side of the seed. These seeds with a hole were thought to represent seeds that had either been parasitized or attempted germination, since perfect holes in the seed coat are likely uncommon from normal weathering or decomposition procedures. ERC seeds were sorted into intact, broken, and hole for each sample where we counted the number of seeds (or pieces) and recorded their total mass in grams to the nearest hundredth for each sample.

DATA COLLECTION

Eastern Redcedar Trees Sampled

We collected the following data on the ten ERC trees sampled in this experiment: canopy diameter (m), slope (%), aspect, approximate GPS location, and notes on physical and microenvironment characteristics.

Greenhouse Germinal Soil Seed Bank Experiment

The seedling emergence method was used to determine seed density and viability (Espeland et al. 2010). Seed flats were checked daily and closely monitored for emerging seedlings. As seedlings emerged, plastic flags were placed next to species to ensure proper monitoring. Once a week for the duration of the experiment, seedlings were photographed, identified (if possible), counted, recorded, and removed. Unknown plants that were not identifiable during the seedling stage were transplanted into individual pots (12 cm dia. x 10 cm depth), filled with Miracle Gro® potting soil, and grown to maturity to aid in identification. After 4 months, we ceased watering the seed flats for one week and allowed them to fully dry. Then, we mixed the soil to aid in germination for seeds that may have been too deep or shallow in the tray. Wetting, drying, and mixing cycles have shown to increase seed germination and break dormancy in some species (Espeland et al. 2010). This drying and mixing process was repeated until no new seedlings emerged. After six months of growth in the greenhouse, no new seedlings emerged, and the experiment was terminated. Soil was removed from the seed flats, placed in plastic Ziploc bags, and stored in case future analysis is needed on seeds that did not germinate. Therefore, the soil seed bank in this study was only measured based on emergent seeds. Those seeds that did not emerge were not used in analysis.

DATA ANALYSIS

Site 1 (2020) and Site 2 (2021) were only sampled one year each (ie. spatial replication but no temporal replication), so analyses between years and between sites was not conducted. Analyses were conducted among and between treatments within each site for a given year based on a single soil core (10 cm dia. x 10 cm depth) as the sample unit where we blocked by tree and each treatment contained four subsamples per tree, totaling 40 samples per treatment at each site. Statistical analyses were conducted in program R, where normality tests were conducted required for analysis of variance (ANOVA) on all dependent variables including total soil seed bank density, seed bank composition by functional group, Shannon-Wiener diversity, Shannon-Wiener evenness, Floristic Quality Index (FQI), total species richness, native species richness, and ERC soil seed bank density (R Development Core Team 2015). Kruskal-Wallis (KW) tests were used when dependent variables failed to meet normality. If dependent variables met normality and ANOVA found significance ($P<0.05$) among treatments, Tukey's HSD was conducted to test for differences between treatments. For dependent variables that did not meet normality and KW found significance (*P<0.05*) among treatment medians, Dunn's post hoc test was used to test for differences between treatments.

Total seed production per soil core was determined using the following formula:

$$Seed\ Density = \frac{\sum_{i=1}^{n} Spp_n}{0.00785}$$

where Spp_n represents the total seedling emergence for each species per core for *n* species present and 0.00785 is the conversion to 0.1/m^3.

Species Composition and Functional Groups

To determine species composition within each sample, we identified individual species emerged from the seed bank and recorded their abundance. We used seedling emergence data by species to compare functional groups among and between treatments including: life form (Forb *vs.* Graminoid *vs.* Shrub *vs.* Tree), origin (Native *vs.* Introduced), and life span (Annual *vs.* Perennial). Frequency and relative density by species were calculated within each treatment with the following formulas:

$$Frequency\ (\%)\ (Spp_x) = \left(\frac{\#\ of\ samples\ in\ which\ Spp_x\ occurs}{Total\ \#\ of samples\ examined}\right) * 100$$

$$Relative\ Density\ (\%)\ (Spp_x) = \left(\frac{Total\ seed\ density\ of\ Spp_x}{Total\ seed\ density\ per\ soil\ core}\right) * 100$$

Floristic Quality Index (FQI) is a metric used to express the tolerance and resiliency of species in relation to disturbance, degradation, and conservation concern.

FQI relies on coefficients of conservatism (C values) and species richness. Each species is given a numerical score (C value) that ranges between 0-10. Species with little conservation concern and that are well adapted to degraded habitats, such as annual or "weedy" species, are given a score of zero. Species of high conservation concern that require unchanged natural conditions, such as rare native species, are given a score of at most ten. Introduced species are given a non-numerical value of a star (*) and are excluded when calculating the mean C value for each sample. We calculated FQI using the following formula:

$$FQI = \bar{C}\sqrt{n}$$

where $\bar{C}$ is the mean C value per sample and n is the number of species per sample (Northern Great Plains Floristic Quality Assessment Panel 2001).

Diversity

Species richness, native species richness, Shannon-Wiener diversity, and Shannon-Wiener evenness were calculated at the sample level for analysis. Shannon's Diversity based on soil seed bank emergence by species was calculated with the formula:

$$H' = -\sum_{i=1}^{s} P_i \ln P_i$$

where s is the number of species, P_i is the proportion of individuals in the *i*th species, and ln is the natural logarithm (Magurran 2004). Shannon's diversity assumes all species are randomly sampled within a study area and incorporates species richness and evenness (Magurran 2004).

Shannon's evenness was calculated with the formula:

$$Evenness = \frac{H'}{\ln S}$$

where *H'* is Shannon's diversity, ln is the natural logarithm, and *S* is the species richness. Shannon's evenness quantifies how the relative abundance of species is distributed throughout a sample and ranges between 0-1. Low evenness will result from samples dominated by one or two species, whereas high evenness will result from samples with an even distribution of species (Magurran 2004, Moore 2013).

Rank abundance curves were created by ranking species in order of relative abundance within each treatment. These curves are used for visual interpretation of plant communities in terms of their species richness and evenness rather than as a statistical method (Magurran 2004). The slope and length of each curve are used to interpret the plant community. Steep sloped curves illustrate a plant community dominated by few species with low evenness. A curve with a gradual slope represents a plant community that has an even distribution of species. The species richness of a treatment is represented by the length of the curve, where longer curves indicate a higher species richness.

Total soil seed bank density and seed bank composition by functional group failed to meet normality assumptions required for ANOVA even after transformations for both sites. As a result, KW non-parametric tests were used to test for differences in the dependent variable medians among treatments within each year. If the KW test found significance ($P<0.05$) among treatment medians, Dunn's post-hoc test with a Bonferroni p-value adjustment was used for comparisons between treatment medians. Shannon-Wiener diversity (H'), Shannon-Wiener evenness, floristic quality index (FQI), total species richness, and native species richness met normality assumptions required for

ANOVA for both Sites following dependent variable transformations. As a result, we used ANOVA to test for differences among treatments and Tukey's HSD to test for differences between treatments if ANOVA tests found significance (*P<0.05*).

Soil Seed Bank Community Analysis

PC-Ord software was used for overall seed bank community analysis between the treatments UC, CE, 2M, and GL. Non-metric multidimensional scaling (NMS) ordination was performed using PC-Ord version 7.09 on seed bank composition at the sample level (seeds emerged/785cm^3) within 2020 at Site 1 and within 2021 at Site 2 (McCune and Mefford 2018). To compare all treatments, we created a main matrix composed of 159 samples with 89 total species for Site 1 and 160 samples with 74 total species for Site 2. NMS was run using the relative Sorenson distance measure at both sites with 2 axes at Site 1 and 3 axes at Site 2, a maximum of 500 iterations, 249 runs with randomized data, and 200 runs with real data. The second matrix was used for treatment (UC, CE, 2M, GL), aspect (N, E, S, W), and hill (uphill, side hill, downhill) comparison where we used MRPP on all NMS ordinations. MRPP was used to test for differences among and between treatments.

Aboveground Species Composition vs. Belowground Species Composition

PC-Ord software was used to compare aboveground vegetation composition to soil seed bank composition. We used relative foliar cover (%) for aboveground vegetation composition (aboveground) (Chapter 2) and relative seed density (%) for soil seed bank composition (belowground) for comparisons. We did not record foliar cover estimates at the sampled trees for soil seed bank. Therefore, we used foliar cover estimates from under canopies of similar sized ERC (5-10 m canopy diameter) and grassland control locations from our companion biomass sampling study (see Chapter 2). These foliar cover estimates were recorded in the same general locations at each site where the soil seed bank ERC trees were located. Therefore, we compared aboveground and belowground composition for under canopy (UC) and grassland control (GL). NMS was performed using PC-Ord version 7.09 at the sample level within 2020 at Site 1 and within 2021 at Site 2 (McCune and Mefford 2018). To compare treatments aboveground and belowground we created four main matrixes: UC Site 1, UC Site 2, GL Site 1, and GL Site 2. For UC Site 1, we created a main matrix composed of 188 samples (148 aboveground and 40 belowground) with 84 total species. For UC Site 2, we created a main matrix composed of 128 samples (88 aboveground and 40 belowground) with 60 total species. For GL Site 1, we created a main matrix composed of 160 samples (120 aboveground and 40 belowground) and 80 total species. NMS was run using the relative Sorenson distance measure with three axes. For GL Site 2, we created a main matrix composed of 160 samples (120 aboveground and 40 belowground) and 78 total species. NMS was run using the correlation distance measure with three axes for UC Site 1, UC Site 2, and GL Site 2. For all four NMS runs (UC Site 1, UC Site 2, GL Site 1, and GL Site 2) we used a maximum of 500 iterations, 249 runs with randomized data, and 200 runs with real data. The second matrix was used for treatment comparison between aboveground and belowground communities where we used MRPP on all NMS ordinations.

ERC Seed Density

ERC seed abundance and mass of our three categories (intact, broken, and hole) allowed us to calculate total ERC seed potential assuming no decomposition, predation, or germination had taken place. We used the total abundance and total mass of intact ERC seeds among all samples to determine the average mass of an ERC seed. Then in each sample, we took the total mass of broken seeds and divided it by the average mass of an ERC seed to construct an estimate of how many seeds our broken seeds could create if they were reassembled together. This estimate is likely low since this calculation does not include the embryo mass. Total ERC seed bank potential was calculated with the following formula:

$$Total\ ERC\ Seed\ Bank\ Potential = \ Intact + Broken + Hole$$

where *Intact* is the abundance of undamaged seeds in each sample, *Broken* is the hypothetical seed abundance if broken seeds were reassembled, and *Hole* is the abundance of predated or potentially germinated seeds in the soil.

ERC seed density of intact, broken, hole, and total potential seeds did not meet assumptions required for ANOVA even after dependent variable transformations for both sites. As a result, KW non-parametric tests were used to test for differences in the dependent variable medians among treatments within each year. If the KW test found significance ($P<0.05$) among treatment medians, Dunn's post-hoc test with a Bonferroni p-value adjustment was used for comparisons between treatment medians.

RESULTS

Weather

Site 1 (2020) and Site 2 (2021) experienced very different weather regimes during their respective growing seasons. Average temperature was higher than the 30-year (1990-2019) average for both Sites, especially during the growing season with an average increase of 0.40˚C and 0.57˚C at Site 1 and Site 2, respectively (Table A.1). Total precipitation was lower than the 30-year average at both Sites consisting of 445 mm (25.2% reduction) and 400 mm (36.6% reduction) at Site 1 and Site 2, respectively (Table A.2). However, Site 1 had 15% more precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 1, monthly precipitation was lower in May and higher during the months of June, July, and August compared to the 30-year average (Table A.2, Figure A.2). Site 2 had 36.5% less precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 2, monthly precipitation was lower in May, June, and August and higher in July than the 30-year average (Table A.2, Figure A.2).

Sampled Trees

The ERC trees selected for sampling were found on NW, N, and NE facing hillsides with slopes ranging from 16-31% and 13-22% at Site 1 and Site 2, respectively. The canopy diameters ranged from 5.25-8.35 m and 5.80-8.00 m with an average canopy diameter of 6.0 m and 6.8 m at Site 1 and Site 2, respectively (Table 3.1). The average hillside slope was higher at Site 1 (23.5%) compared to Site 2 (17.1%) (Table 3.1).

Seed Bank Production, Species Composition, and Functional Groups

Site 1 produced more emerged seedlings (12,591) than Site 2 (5,826) among treatments. Treatment did not have a significant effect on seedling production at Site 1 ($P=0.24$) and at Site 2 ($P=0.74$) (Figure 3.3). A total of 26 families, 75 genera, and 89 species emerged from the soil seed bank among treatments at Site 1. Site 2 had fewer species emerged consisting of 19 families, 58 genera, and 74 species (Table 3.2). Treatment UC contained the most species (63) at Site 1 whereas treatment 2M contained the most species (52) at Site 2. No seedlings emerged from the greenhouse control seed flats which indicated there was no seed dispersal present within the greenhouse and the potting soil was not contaminated.

The most abundant graminoid found in the soil seed bank was *Poa pratensis* L. (Kentucky bluegrass) with a frequency of 100% and greater than 85% at Site 1 and Site 2, respectively (Tables 3.3, 3.4). Treatment did not seem to impact frequency or relative density of *Poa pratensis* L. at either Site. *Oxalis stricta* L. (common yellow oxalis) was the most abundant forb at Site 1 with frequencies 82.5-95% and relative densities of 13.4-20.1% (Table 3.3). *Androsace occidentalis* Pursh (western rockjasmine) was the most abundant forb at Site 2 with frequencies 70-82.5% and relative densities 16-22.5% (Table 3.4).

Some species displayed a trend between treatments at both Sites. At Site 1, a few graminoids including *Sporobolus compositus* (Poir.) Merr (composite dropseed), *Hesperostipa spartea* (Trin.) Barkworth (porcupinegrass), *Muhlenbergia racemosa* (Michx.) Britton, Sterns & Poggen (Marsh muhly), *Koeleria macrantha* (Ledeb.) Schult. (prairie junegrass), and *Schizachyrium scoparium* (Michx.) Nash (little bluestem) increased in frequency and relative density in treatments 2M and GL compared to treatments UC and CE (Table 3.3). In contrast, *Sporobolus cryptandrus* (Torr.) A. Gray (sand dropseed), *Elymus repens* (L.) Gould (quackgrass), and *Juncus torreyi* Coville (torrey’s rush) increased in frequency and relative density in treatments UC and CE compared to treatments 2M and GL (Table 3.3). We found trends in some forb species where *Conyza Canadensis* (L.) Cronquist (Canadian horseweed), *Typha spp.* L. (cattail), *Sisymbrium altissimum* L. (tall tumblemustard), *Cirsium arvense* (L.) Scop. (Canada thistle), *Solanum ptycanthum* Dunal (west Indian nightshade), *Chenopodium album* L. (lambsquarters), *Amaranthus spp.* L. (pigweed), and *Cirsium flodmanii* (Rydb.) Arthur (flodman's thistle) increased in frequency and relative density in treatments UC and CE compared to treatments 2M and GL (Table 3.3). In contrast, forb species *Melilotus officinalis* (L.) Lam. (sweetclover), *Monarda fistulosa* L. (wild bergamot), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), *Heliopsis helianthoides* (L.) Sweet (smooth oxeye), and *Lotus unifolioatus* (Hook.) Benth. (American bird's-foot trefoil) increased in frequency and relative density in treatments 2M and GL compared to treatments UC and CE. One introduced tree species, *Morus alba* L. (white mulberry), was present only in treatment UC with 25% and 0.3% frequency and relative density, respectively. At Site 2, some graminoids including *Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould (scribner's rosette grass), composite dropseed, *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama), little bluestem, *Bouteloua gracillis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama), *Andropogon gerardii* Vitman (big bluestem), *Boutleloua dactyloides* (Nutt.) J.T. Columbus (buffalograss), *Calamovilfa longifolia* (Hook.) Scribn (prairie sandreed), and *Hesperostipa comata* (Trin. & Rupr) Barkworth (needle and thread) increased in frequency and relative density in treatments 2M and GL compared to treatments UC and CE (Table 3.4). In contrast, graminoids *Eragrostis cilianensis* (All.) Vign Ex Janchen (stinkgrass), *Panicum miliaceum* L. (proso millet), and *Setaria viridis* (L.) P. Beauv (green bristlegrass) increased in frequency and relative density in treatments UC and CE compared to treatments 2M and GL (Table 3.4). In addition, some forb species increased in frequency and relative density in treatments UC and CE compared to treatments 2M and GL including Canadian horseweed, *Physalis hispida* (Waterf.) Cronquist (prairie groundcherry), *Amaranthus albus* L. (prostrate pigweed), *Lactuca serriola* L. (prickly lettuce), and Canada thistle. Other forb species increased in frequency and relative density in treatments 2M and GL compared to treatments UC and CE including upright prairie coneflower, *Erigeron strigosus* Muhl. Ex Willd. (prairie fleabane), *Echinacea angustifolia* DC. (blacksamson echinacea), and *Artemisia ludoviciana* Nutt. (white sagebrush).

Treatment had a significant ($P<0.05$) effect on select functional groups among treatments at Site 1 and Site 2. At Site 1, we found significance ($P<0.05$) among treatment medians in functional groups forb introduced perennial (FIP), forb native annual/biennial (FNA/B), graminoid introduced annual (GIA), graminoid native perennial (GNP), and tree introduced perennial (TIP) (Table 3.5). FIP composition was significantly ($P<0.01$) higher in treatment UC (2.17%) than treatments 2M (0.33%) and GL (0.60%). FNA/B composition was significantly higher ($P=0.05$) in treatment UC (20.4%) compared to GL (9.1%). In contrast, GIA composition was significantly ($P=0.01$) higher in treatment GL (2.25%) compared to UC (0.11%). Treatments UC and 2M were significantly different ($P=0.01$) in GNP composition with 6.66% and 4.28% in treatments 2M and UC, respectively. TIP composition was zero in all treatments except UC (0.35%) resulting in significance ($P<0.01$) between UC and treatments CE, 2M, and GL. At Site 2, we found significance ($P<0.05$) among treatment medians in functional groups forb introduced annual/biennial (FIA/B), GIA, GNP, total introduced, total native, total annual/biennial (A/B), and total perennial (Table 3.6). FIA/B composition was significantly ($P<0.01$) higher in treatment UC (0.90%) than GL (0.04%). In contrast to

Site 1, GIA composition at Site 2 was significantly (*P<0.01*) higher in treatment UC (7.58%) compared to treatments CE, 2M, and GL at 2.29%, 1.29%, and 2.89%, respectively. Treatment UC and treatments 2M and GL significantly differed (*P<0.01*) in GNP composition where the highest composition was found in treatment 2M (15.5%) and the lowest in treatment UC (4.06%). Total introduced and native composition differed between treatments UC and 2M where higher native composition was present in treatment 2M compared to UC. In addition, A/B composition was significantly (*P<0.01*) higher in treatments UC (44.9%) and CE (43%) compared to treatment 2M (31.2%). Total perennial composition was highest in treatment 2M (67.8%) and significantly (*P<0.01*) differed from treatment UC (54.9%). Tables 3.5 and 3.6 consist of average seed bank composition (mean ± standard error) and p-values found from KW tests on treatment medians. Table A.3 contains treatment medians used for KW analyses that produced significance values for Tables 3.5 and 3.6.

Diversity

Treatment had a significant (*P<0.05*) effect on floristic quality index (FQI) at both sites and native species richness at Site 2 (Table 3.7). In contrast, treatment did not have a significant effect on the other dependent variables including Shannon-Wiener diversity (H'), Shannon-Wiener evenness, total species richness, and Site 1 native species richness (Table 3.7). In general, ERC canopies (UC) tended to decrease FQI values in comparison to CE, 2M, and GL. The lowest mean FQI at both sites was found in treatment UC with 6.5 and 5.98 at Site 1 and Site 2, respectively. The highest mean FQI at both sites was found in treatment 2M with 8.39 and 9.23 at Site 1 and Site 2, respectively. At Site 1, mean FQI significantly differed (*P=0.05*) between treatments UC and 2M, whereas treatments CE and GL were similar to the other treatments (Table 3.7). At Site 2, mean FQI significantly differed (*P<0.01*) between treatment UC and treatments CE, 2M, and GL (Table 3.7). Treatment had a significant (*P<0.01*) effect on native species richness at Site 2 where ERC canopies (UC) decreased native species richness in comparison to treatments 2M and GL (Table 3.7). The lowest mean richness for native species was found in treatment UC (5.48) with the highest in GL (7.15) (Table 3.7).

Rank abundance curves illustrated that seed bank composition at Site 1 was primarily dominated by a couple of species (Kentucky bluegrass and common yellow oxalis) due to its initial steep slope among all four treatments. Following the initial descent, curves were gradual and similar among treatments indicating reasonable evenness among remaining species (Figure 3.4). Rank abundance curves at Site 2 indicated dominance by a few species (Kentucky bluegrass, hoary verbena, and western rockjasmine), but the initial curves were not as steep as those from Site 1, suggesting a higher evenness among species at Site 2 (Figure 3.4). Overall rank abundance curves were similar among treatments at Site 2 with no apparent outlier in terms of total species. In addition, the curves at Site 1 were longer than those at Site 2 indicating a higher species richness among treatments at Site 1, which is supported by Table 3.2 (Figure 3.4).

Soil Seed Bank Community Analysis

A 2-dimensoinal solution using a relative Sorenson distance measure was used to interpret plant communities among our treatments UC, CE, 2M, and GL at Site 1 and Site 2. At Site 1, our solution found a final stress of 17.32 and a cumulative variation of

87.2% among the axes, with axis one explaining 51.9% and axis two explaining 35.3% of the variation. Species correlations with the main matrix axes are summarized in Table 3.8 Axis one was positively driven by *Oxalis stricta* (r=0.705) and negatively driven by Kentucky bluegrass (r=-0.561). *Juncus interior* had the highest positive correlation (r=0.402) with axis two and *Nepeta cataria* had the highest negative correlation (r=-0.267). A-values among MRPP comparisons were very low (-0.0005-0.01147), indicating heterogeneity within treatments did not occur by random chance (Table 3.10). MRPP comparisons found significance (*P=0.01*) among treatments but not among aspect (*P=0.13*) or hillside slope (*P=1.00*) (Table 3.10). MRPP pairwise comparisons found seed bank composition in UC samples different from 2M and GL. Other pairwise comparisons between other treatments were not significant (*P>0.05*).

At Site 2, our solution resulted in a final stress of 14.25 where axis one explained 39.1% of the variation and axis two explained 39.6% of the variation (78.6% cumulative). Axis one was positively driven by *Verbena stricta* (r=0.431) and negatively driven by Kentucky bluegrass (r=-0.598) (Table 3.9). Axis two was positively driven by *Androsace occidentalis* (r=0.635) and negatively driven by *Unknown Forb 12* (r=-0.302) (Table 3.9). MRPP comparisons resulted in A-values near zero (-0.0044-0.0147), indicating heterogeneity within treatments did not occur by random chance. MRPP comparisons among groups found significance in treatment (*P<0.01*), but not with aspect (*P=0.95*) or hillside slope (*P=0.99*) (Table 3.10). Pairwise comparisons between treatments found seed bank composition in treatment UC to differ from CE, 2M, and GL. Other comparisons between treatments were not significant (*P>0.05*).

Plotted NMS ordinations for Site 1 and Site 2 are illustrated in Figure 3.5. Distinct differences in convex hulls between treatments are not apparent for either Site (Figure 3.5). However, grouping does appear to occur in treatment centroids (multivariate averages) but are not consistent among Sites (Figure 3.5). Treatment centroids UC/DL and 2M/GL are grouped at Site 1. In contrast, treatment centroids UC/GL and DL/2M are grouped at Site 2, which is not expected (Figure 3.5).

Aboveground Species Composition vs. Belowground Species Composition

A 3-dimensional solution was used to interpret the relationship between our aboveground and belowground plant communities in all four comparisons where UC Site 1, UC Site 2, and GL Site 2 used a correlation distance measure and GL Site 1 used a relative Sorenson distance measure. Final stresses ranged between (10.96-19.36) among our four NMS ordinations with a cumulative variation of 62.8%, 89.5%, 89.3%, and 83.7% for ordinations UC Site 1, UC Site 2, GL Site 1, and GL Site 2, respectively (Table 3.11). MRPP comparisons, using the same distance measure as its NMS ordination compliment, between aboveground and belowground plant communities were significant (*P<0.01*) for all four ordinations indicating a difference in plant community structure (Table 3.11). A-values from MRPP comparisons were close to zero (0.1098-0.1839) for all four ordinations, indicating heterogeneity within the treatments did not occur by random chance (Table 3.11). Supporting our results from MRPP comparisons, we can see clear separation between aboveground and belowground plant communities in all four NMS ordinations in terms of their plotted convex hulls and centroids (Figures 3.6, 3.7).

ERC Seed Density

A total of 307 samples were sorted in search of ERC seeds, consisting of 147 and 160 from Site 1 and Site 2, respectively (Table 3.12). Treatment had a significant ($P<0.05$) effect on ERC seed density of intact, broken, hole, and total seeds (Table 3.12). ERC seed density was significantly ($P<0.01$) higher underneath ERC canopies in all seed categories. Treatment UC differed in seed density compared to treatments CE, 2M, and GL for all seed categories. Treatment CE differed in seed density compared to treatments UC, 2M, and GL in all seed categories. Treatments 2M and GL were similar in seed density among all categories. A majority of ERC seeds found in the soil seed bank were damaged (either broken or contain a hole) and can be deemed unviable.

At Site 1, we found the density of intact seeds underneath ERC canopies to be over four times greater than at the CE and over 60 times greater than in treatments 2M and GL (Table 3.12). Damaged seeds (broken + hole) represented 77%, 70%, 50%, and 40% of the total seed potential in treatments UC, CE, 2M, and GL, respectively (Table 3.12). Total potential of ERC seed density in the soil seed bank was over five times greater underneath ERC canopies in comparison to their canopy edge and over 125 times greater than 2M and GL. At Site 2, similar to Site 1 we also found intact ERC seed density to be four times greater in treatment UC compared to CE and over 150 times higher than treatments 2M and GL (Table 3.12). Damaged seeds (broken + hole) represented 53%, 43%, 39%, and 50% of the total seed potential in treatments UC, CE, 2M, and GL, respectively (Table 3.12).

ERC seed density in the soil seed bank was higher at Site 2 than at Site 1 (Table 3.12, Figure 3.8). The total potential of ERC seeds in the soil seed bank directly underneath ERC canopies was about 36,000 seeds/0.1m^3 at Site 1 and nearly 53,000 seeds/0.1m^3 at Site 2 (Figure 3.8). However, most of these seeds were damaged and we found intact ERC seed density was much lower in the soil seed bank underneath ERC canopies at 8,000 seeds/0.1m^3 and 25,000 seeds/0.1m^3 at Site 1 and Site 2, respectively (Figure 3.8). Total ERC seed density at the canopy edge was much lower at both sites compared to UC, consisting of about 6,500 seeds/0.1m^3 and 10,000 seeds/0.1m^3 at Site 1 and Site 2, respectively. ERC seed density was minimal in treatments 2M and GL with 61-288 seeds/0.1m^3 at both Sites (Figure 3.8).

DISCUSSION

Grasslands in the NGP are experiencing afforestation through ERC encroachment resulting in an altered soil seed bank beneath ERC canopies compared to open grassland plots. The lack of prescribed fire, overgrazing, and planting ERC in shelterbelts has contributed to its prolific expansion onto our rangelands and raises concern of future rangeland sustainability and resiliency (Limb et al. 2010). Our study evaluated the impact of large (5-10 m canopy diameter) individual ERC trees on soil seed bank production and composition in the NGP mixed-grass prairie of South Dakota. Some researchers have evaluated the impact of woody encroachment on the soil seed bank in the Pacific Northwest (Lang and Halpern 2007), Australia (Price and Morgan 2008), and central Nevada (Allen and Nowak 2008), but this research is the first of its kind in the Great Plains, especially with ERC as the focal species. The results of this study are critical for land managers in understanding the potential regeneration of aboveground vegetation following the control of ERC via mechanical removal or prescribed fire.

Soil Seed Bank Production

Seedling density was not different among treatment distance, aspect, and hill in relation to an individual ERC tree. These findings support our first hypothesis concluding that an individual ERC canopy does not influence the seed density in the soil seed bank. Similar findings were also found in pinyon-juniper woodlands in central Nevada (Allen and Nowak 2008) and in conifer encroached meadows in the western Cascade Range of Oregon (Lang and Halpern 2007) where seed density did not change as tree cover increased. In contrast to our results, other researchers have found a correlation between tree cover and seedling abundance where *Pinus monophylla* (Single-leaf pinyon) trees in California (Koniak and Everett 1982), *Leptospermum scoparium* (Manuka) trees in southern Australia (Price and Morgan 2008), and *Quercus suber* (cork oak) trees in central Spain (Torres et al. 2012) have shown to decrease seedling abundance as tree cover increases. Conflicting results among research suggests the influence of canopy cover on seed density might vary depending on the focal species (*Juniperus spp., Pinus spp., Quercus spp.*), the ecosystem (shrub land, grassland, forest meadow), or microsite characteristics.

Although we found no difference in seedling density among treatments, we did find a difference in total seedling emergence between our two sampling sites. Seedling emergence was nearly two times greater at Site 1 than Site 2 with an average of 10,000 emergent seedlings per 0.1 m^3 at Site 1. However, these results at Site 1 are likely skewed from an overwhelming seedling emergence of Kentucky bluegrass with an average relative density of 48% among treatments. In addition, Site 1 and Site 2 were sampled in different years which experienced very different weather patterns (HPRCC 2022, Mesonet 2022). Site 1 received 15% more precipitation during the growing season than the 30-year average, whereas Site 2 received 36.5% less precipitation (HPRCC 2022, Mesonet 2022). Precipitation during the growing season impacts plant maturity and seed production (Baskin and Baskin 1998). Seed bank soil cores were collected in late October in attempt to include seed rain from the current year's growing season (Allen and Nowak 2008). As a result, we expected to see more seed production at Site 1 compared to Site 2 due to the higher precipitation during the growing season at Site 1.

Soil Seed Bank Composition

Seed bank composition among treatments was variable within and between sites. However, samples collected underneath ERC canopies tended to have a higher percentage of introduced forb species, total annual/biennial species, total introduced species, and a lower percentage of native graminoid species. These findings support our second hypothesis where individual ERC canopies did impact the soil seed bank composition. This contradicts findings in Allen and Nowak (2008) where the seed bank community was not different between tree cover classes when analyzed based on seed density by species and by life form. In addition, we found an average annual/biennial percentage among treatments to be much lower (17% and 38%) than previously reported where other studies found up to 89% of emerged seedlings to be annuals (Allen and Nowak 2008). This low percentage of annual/biennial species expressed in our study is surprising in a perennial dominated grassland where seed banks often reflect high forb and annual/biennial species (Perkins et al. 2019).

We found ERC canopies did not impact soil seed bank species diversity, evenness, and richness, which contradicts findings from previous literature (Lang and

Halpern 2007, Price and Morgain 2008, Torres et al. 2012). However, we also observed floristic quality index (FQI) and native species richness in our study, which was not assessed in previous woody encroachment seed bank studies. ERC canopies reduced FQI and native species richness in emergent seedlings from the soil seed bank. These findings suggest a shift towards a more degraded seed bank community underneath ERC canopies compared to open grassland plots, which supports our third hypothesis.

Soil Seed Bank Community Analyses

Multivariate analysis using PC-Ord software suggest ERC canopies are indeed altering belowground seed bank composition in comparison to open grassland plots and supports findings in previous seed bank literature with pinyon-juniper stands in California (Koniak and Everett 1982). In addition, we found little correlation between aboveground vegetation species composition and the soil seed bank composition which supports our fourth hypothesis. Aboveground and belowground communities at both sites clearly displayed separation between convex hulls from NMS output (Figures 3.6, 3.7). This trend was apparent both underneath ERC canopies and in open grassland plots, suggesting that ERC canopies do not lead to similarity between aboveground and belowground communities. These findings are widely supported in previous literature where aboveground vegetation and seed bank composition are often very different (Abrams 1988, Benson and Hartnett 2006, Görzen et al. 2019, Hopfensperger 2007) with up to 63% of the species in the seed bank not represented in the aboveground vegetation (Allen and Nowak 2008). However, one study found different results in early-succession California pinyon-juniper woodlands where initial colonizers post-fire were those found in the soil seed bank (Everett and Ward 1984). In most cases, these differences between aboveground and belowground communities indicate a limited restoration potential from the seed bank alone (Godefroid et al. 2017, Görzen et al. 2019).

ERC Seed Density

We found a large quantity (up to 52,000 seeds/0.1 m^3) of ERC seeds accumulated in the soil seed bank underneath ERC canopies and decreased in density with increasing distance from ERC stems, supporting our fifth hypothesis. Similar results were found in the Nebraska mixed-grass prairie where ERC seeds did not accumulate outside the canopy (Tunnell et al. 2004). Although seed accumulation was rare outside ERC canopies, it was present in some samples. We can assume these seeds were dispersed via birds or small mammals and will contribute to ERC expansion into new territory (Holthuijzen and Sharik 1985, Holthuijzen et al. 1986, Horncastle et al. 2004). However, contradicting our sixth hypothesis, most of the accumulated ERC seeds were unviable due to being damaged, parasitized, or showed signs of potential previous germination. This supports findings from previous studies suggesting accumulation of ERC seeds does not occur in the soil seed bank due to rapid loss in viability (Holthuijzen and Sharik 1984, Tunnell et al. 2004). Signs of potential predation were found among ERC seeds in the soil seed bank which is supported by Livingston (1972) where over 50% predation was reported. Contrasting findings were found in other studies where ERC seed predation in the soil seed bank was minimal or not observed (Holthuijzen and Sharik 1984, Parker 1952). We did not conduct tetrazolium tests on intact ERC seeds found in the soil seed bank, but previous literature suggests viability is expected to be low with 5-10% viability when collected directly from the seed bank (Tunnell et al. 2004) and just 5.5% viable after sown for 14 months (Holthuijzen and Sharik 1984). Our results from categorizing and calculating the density of ERC seeds in the soil seed bank indicate most accumulation occurs underneath ERC canopies and a majority of seeds are damaged. However, we found seed dispersal up to five meters from the ERC canopy indicating potential for encroachment to nearby sites (Holthuijzen et al. 1986, Horncastle et al. 2004).

ERC seed accumulation and dispersal found in this study raises concern for land managers throughout the Great Plains. With seed dispersal away from ERC exceeding 65% in some cases (Hothuijzen and Sharik 1985), land managers may struggle to combat woody encroachment if nearby parcels are not being properly managed (Horncastle et al. 2004). The dispersal of ERC seeds coupled with the lack of prescribed fire, planting ERC in shelterbelts, and limited public resources (prescribed burn associations) for conducting prescribed fires on private lands could lead to widespread juniper dominated forests on our grasslands in the near future (DeSantis et al. 2011, Twidwell et al. 2013). Since large, female ERC trees bearing up to 1.5 million berry-like cones per year (Holthuijzen and Sharik 1985) should be targeted for mechanical removal to reduce seed dispersal and establishment. In addition, this research highlights how ERC canopies are changing the soil seed bank composition including reducing FQI, native species richness, and native graminoid composition. Long-term chronic encroachment of ERC may reduce the sustainability and resiliency of our grasslands and alter aboveground native diversity with the soil seed bank unable to provide ecological rescue (Godefroid et al. 2017). Findings among studies in other ecosystems support the need for woody control due to its negative impact on the soil seed bank (Lang and Halpern 2007, Price and Morgan 2008, Torres et al. 2012). Following the control of ERC via mechanical removal or prescribed fire, the results from this research suggest that plant communities directly underneath previous ERC canopies will express a higher composition of annual/biennial and introduced species as it naturally begins secondary succession. Restoration back to healthy native grasslands may rely on vegetative expansion through rhizomes, seed dispersal from nearby populations, or manually spread seed.

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TABLES

Table 3.1 Summary of ERC trees selected for seed bank sampling at Site 1 in 2020 and Site 2 in 2021.

	Site 1 (2020)		Site 2 (2021)	
ERC Tree	Canopy Diameter (m)	Slope (%)	Canopy Diameter (m)	Slope (%)
1	5.25	16	6.60	18
2	5.65	24	7.65	19
3	6.55	28	6.00	16
4	6.00	28	8.00	17
5	7.10	31	6.00	22
6	8.35	17	6.90	14
7	5.25	25	6.20	13
8	5.60	23	5.80	23
9	5.35	24	6.30	15
10	5.30	19	8.00	14
Mean	***6.04***	***23.5***	***6.75***	***17.1***

Table 3.2 Soil seed bank species composition for Site 1 in 2020 and Site 2 in 2021 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

Species Composition								
	Site 1 (2020)				Site 2 (2021)			
Treatment	Total Seeds Emerged[a]	Families	Genera	Species	Total Seeds Emerged[a]	Families	Genera	Species
UC	3,328	23	54	63	1,551	16	35	42
CE	2,653	23	49	57	1,317	17	43	50
2M	3,160	23	51	56	1,378	17	45	52
GL	3,450	20	52	57	1,580	14	39	48
Total	12,591[b]	26	75	89	5,826[b]	19	58	74

[a] Total seeds emerged per treatment or 40 soil cores (0.0314 m^3).

[b] Total seeds emerged per site or 160 soil cores (0.1256 m^3)

Table 3.3 Soil seed bank species list with frequency (%) and relative density (%) for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020. Species are sorted numerically by decreasing average relative density (%) among treatments.

USDA Common Name	Scientific Name	Family	Origin	Life Span	C- Value	Site 1 (2020)							
						UC		CE		2M		GL	
						Freq.	Den.	Freq.	Den.	Freq.	Den.	Freq.	Den.
Grasses													
Kentucky bluegrass	*Poa pratensis* L.	*Po*	I	P	*	100.0	50.5	100.0	50.8	100.0	49.2	100.0	42.9
Smooth brome	*Bromus inermis* Leyss.	*Po*	I	P	*	52.5	1.7	76.9	5.1	55.0	2.9	60.0	1.9
Scribner's rosette grass	*Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould	*Po*	N	P	6	47.5	1.3	53.8	1.7	75.0	4.0	62.5	2.3
Canada bluegrass	*Poa compressa* L.	*Po*	I	P	*	25.0	0.8	46.2	2.5	22.5	2.0	32.5	2.2
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	*Po*	I	A	*	5.0	0.1	23.1	1.1	10.0	0.2	27.5	1.8
Green needlegrass	*Nassella viridula* (Trin.) Barkworth	*Po*	N	P	5	12.5	0.2	17.9	0.7	12.5	0.2	15.0	0.3
Composite dropseed	*Sporobolus compositus (*Poir.) Merr.	*Po*	N	P	4	5.0	0.1	5.1	0.1	15.0	0.3	12.5	0.2
Cheatgrass	*Bromus tectorum* L.	*Po*	I	A	*			10.3	0.2	2.5	0.1	5.0	0.1
Porcupinegrass	*Hesperostipa spartea* (Trin.) Barkworth	*Po*	N	P	8			2.6	<0.1	7.5	0.1	7.5	0.1
Rough bentgrass	*Agrostis scabra* Willd.	*Po*	N	P	1	5.0	0.1	5.1	0.1			5.0	0.1
Sand dropseed	*Sporobolus cryptandrus* (Torr.) A. Gray	*Po*	N	P	6	7.5	0.1	2.6	<0.1				
Annual bluegrass	*Poa annua* L.	*Po*	I	A	*					7.5	0.1		
Marsh muhly	*Muhlenbergia racemosa* (Michx.) Britton, Sterns & Poggen	*Po*	N	P	4					2.5	0.1	2.5	<0.1
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	*Po*	N	P	5	2.5	<0.1			2.5	<0.1	2.5	<0.1
Prairie junegrass	*Koeleria macrantha* (Ledeb.) Schult.	*Po*	N	P	7					2.5	<0.1	2.5	<0.1
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	*Po*	N	P	6					2.5	<0.1		
Quackgrass	*Elymus repens* (L.) Gould	*Po*	I	P	*	2.5	<0.1						

Western wheatgrass	*Pascopyrum smithii* (Rydb.) Á. Löve	*Po*	N	P	4	2.5	<0.1						
Grass-likes													
Inland rush	*Juncus interior* Wiegand	*Ju*	N	P	5	10.0	4.4	17.9	1.5	40.0	0.9	20.0	0.5
Needleleaf sedge	*Carex duriuscula* C. A. Mey.	*Cy*	N	P	10	12.5	0.3	17.9	0.6	25.0	0.5	10.0	0.4
Torrey's rush	*Juncus torreyi* Coville	*Ju*	N	P	2	2.5	<0.1						
Forbs													
Common yellow oxalis	*Oxalis stricta* L.	*Ox*	N	P	0	82.5	13.4	84.6	16.6	95.0	20.1	92.5	15.1
Western rockjasmine	*Androsace occidentalis* Pursh	*Pr*	N	A	5	57.5	5.3	43.6	2.8	52.5	3.6	32.5	14.4
Canadian horseweed	*Conyza canadensis* (L.) Cronquist	*As*	N	A/B	0	65.0	3.0	61.5	3.4	57.5	3.4	45.0	2.9
Sweetclover	*Melilotus officinalis* (L.) Lam.	*Fa*	I	A/B	*	20.0	0.7	25.6	1.5	45.0	2.6	50.0	2.4
Hoary verbena	*Verbena stricta* Vent.	*Ve*	N	P	2	47.5	2.0	41.0	1.4	52.5	1.6	52.5	1.4
Cattail	*Typha spp.* L.	*Ty*	N	P	2	65.0	3.7	25.6	1.0	25.0	0.3	22.5	0.3
Prairie groundcherry	*Physalis hispida* (Waterf.) Cronquist	*So*	N	P	8	5.0	0.2	7.7	0.2	5.0	0.1	10.0	4.4
Tall tumblemustard	*Sisymbrium altissimum* L.	*Br*	I	A/B	*	17.5	3.2	12.8	1.0	7.5	0.1		
Hairy rockcress	*Arabis hirsuta* (L.) Scop.	*Br*	N	A/B/P	7	15.0	0.8	23.1	0.4	15.0	2.1	7.5	0.2
Black medick	*Medicago lupulina* L.	*Fa*	I	A/P	*	2.5	<0.1	17.9	0.5	40.0	1.6	37.5	1.1
Canada thistle	*Cirsium arvense* (L.) Scop.	*As*	I	P	*	62.5	1.5	35.9	0.9	7.5	0.2	12.5	0.3
Wild bergamot	*Monarda fistulosa* L.	*La*	N	P	5	12.5	0.2	10.3	0.3	25.0	0.8	30.0	1.5
West Indian Nightshade	*Solanum ptycanthum* Dunal	*So*	N	A	0	42.5	1.9	17.9	0.4	7.5	0.1	5.0	0.1
Prickly lettuce	*Lactuca serriola* L.	*As*	I	A/B	*	30.0	0.4	20.5	0.6	20.0	0.4	27.5	0.6
Sleepy silene	*Silene antirrhina* (L.)	*Ca*	N	A	3	12.5	0.3	15.4	1.2	7.5	0.2	2.5	<0.1
Bull Thistle	*Cirsium vulgare* (Savi) Ten.	*As*	I	B	*	37.5	0.8	2.6	0.1	17.5	0.5	12.5	0.1
Clasping Venus' looking-glass	*Triodanis perfoliata* (L.) Nieuwl.	*Cm*	N	A	6			10.3	0.7	2.5	0.2	2.5	0.1
Lambsquarters	*Chenopodium album* L.	*Ch*	I	A	*	22.5	0.4			2.5	0.2	2.5	0.3

Prostrate pigweed	*Amaranthus albus* L.	*Am*	I	A	*	7.5	0.6	2.6	0.1	2.5	<0.1	2.5	<0.1
Catnip	*Nepeta cataria* (L.)	*La*	I	P	*	10.0	0.5	2.6	<0.1	2.5	<0.1	7.5	0.1
Shortstalk chickweed	*Cerastium brachypodum* (Engelm. Ex A. Gray) B.L. Rob.	*Ca*	N	P	4	2.5	<0.1	5.1	0.6	2.5	0.1		
Cuman ragweed	*Ambrosia psilostachya* DC.	*As*	N	P	2	2.5	<0.1	15.4	0.2	10.0	0.2	10.0	0.1
Common mullien	*Verbascum thapsus* L.	*Sc*	I	B	*	2.5	<0.1	5.1	0.1			7.5	0.4
Tall cinquefoil	*Potentilla arguta* Pursh	*Ro*	N	P	8	2.5	0.1	7.7	0.1	5.0	0.1	12.5	0.2
Nodding plumeless thistle	*Carduus nutans* L.	*As*	I	B/P	*	7.5	0.1	10.3	0.2	2.5	<0.1	5.0	0.1
White sagebrush	*Artemisia ludoviciana* Nutt.	*As*	N	P	3			5.1	0.2	2.5	<0.1	7.5	0.1
Flodman's thistle	*Cirsium flodmanii* (Rydb.) Arthur	*As*	N	P	5	7.5	0.2	5.1	0.1				
Annual ragweed	*Ambrosia artemisifolia* L.	*As*	N	A	0	2.5	<0.1	2.6	<0.1	10.0	0.1	5.0	0.1
Stiff goldenrod	*Oligoneuron rigidum* (L.) Small var. *humile* (Porter) G.L. Nesom	*As*	N	P	4	7.5	0.1			5.0	0.1	5.0	0.1
Prairie fleabane	*Erigeron strigosus* Muhl. Ex Willd.	*As*	N	A/B/P	3	2.5	<0.1	7.7	0.1	7.5	0.1	2.5	<0.1
Common evening primrose	*Oenothera biennis* L.	*On*	N	P	0	5.0	0.1			2.5	0.1		
Showy goldenrod	*Solidago speciosa* Nutt.	*As*	N	P	10	2.5	<0.1	5.1	0.1	5.0	0.1	5.0	0.1
Yellow salsify	*Tragopogon dubius* Scop.	*As*	I	B	*	7.5	0.1			2.5	<0.1	5.0	0.1
Drummond's false pennyroyal	*Hedeoma drummondii* Benth.	*La*	N	P	4	2.5	<0.1	2.6	0.1	2.5	<0.1		
Upright prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.	*As*	N	P	3							5.0	0.2
Black bindweed	*Polygonum convolvulus* L.	*Py*	I	A	*	2.5	<0.1	2.6	<0.1	5.0	0.1	2.5	<0.1
Stinging nettle	*Urtica dioica* L.	*Ur*	I	P	*			2.6	0.1	2.5	<0.1		
Common dandelion	*Taraxacum officinale* F.H. Wigg.	*As*	I	P	*	2.5	<0.1	7.7	0.1				
Small-leaf pussytoes	*Antennaria parvifolia* Nutt.	*As*	N	P	6	5.0	0.1	2.6	<0.1				
White heath aster	*Symphyotrichum ericoides* (L.) G.L. Nesom	*As*	N	P	2	2.5	<0.1					7.5	0.1
Curly dock	*Rumex crispus* L.	*Py*	I	P	*	2.5	<0.1	5.1	0.1				
Field sowthistle	*Sonchus arvensis* L.	*As*	I	P	*	2.5	<0.1					2.5	0.1

Smooth oxeye	*Heliopsis helianthoides* (L.) Sweet	*As*	N	P	5							2.5	0.1
Prairie violet	*Viola pedatifida* G. Don	*Vi*	N	P	8			2.6	<0.1	2.5	<0.1		
Candle anemone	*Anemone cylindrica* A. Gray	*Ra*	N	P	7	2.5	<0.1	2.6	<0.1				
Fringed willowherb	*Epilobium ciliatum* Raf.	*On*	N	P	3	2.5	<0.1	2.6	<0.1				
Ribseed sandmat	*Chamaesyce glyptosperma* (Engelm.) Small	*Eu*	N	A	0			2.6	<0.1			2.5	<0.1
Field pennycress	*Thlaspi arvense* L.	*Br*	I	A	*			2.6	<0.1			2.5	<0.1
Purple prairie clover	*Dalea purpurea* Vent.	*Fa*	N	P	8					2.5	0.1		
Dotted blazing star	*Liatris punctata* Hook.	*As*	N	P	7					2.5	0.1		
Leafy spurge	*Euphorbia esula* L.	*Eu*	I	P	*					2.5	<0.1	2.5	<0.1
Neckweed	*Veronica peregrina* L.	*Sc*	N	A	0					2.5	<0.1	2.5	<0.1
Redroot amaranth	*Amaranthus retroflexus* L.	*Am*	N	A	0	5.0	0.1						
Common pepperweed	*Lepidium densiflorum* Schrad.	*Br*	N	A	0							2.5	0.1
Great ragweed	*Ambrosia trifida* L.	*As*	N	A	0			2.6	<0.1				
Fireweed	*Chamerion angustifolium* (L.) Holub	*On*	N	P	5			2.6	<0.1				
Annual mercury	*Mercurialis annua* L.	*Eu*	I	A	*			2.6	<0.1				
Norwegian cinquefoil	*Potentilla norvegica* L.	*Ro*	N	A/B/P	0			2.6	<0.1				
White panicle aster	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom	*As*	N	P	4					2.5	<0.1		
Oakleaf goosefoot	*Chenopodium glaucum* L.	*Ch*	I	A	*	2.5	<0.1						
Stickywilly	*Galium aparine* (L.)	*Ru*	N	A	0	2.5	<0.1						
Pennsylvania pellitory	*Parietaria pensylvanica* Muhl. Ex Willd.	*Ur*	N	A	3	2.5	<0.1						
Whorled milkwort	*Polygala verticillata* L.	*Py*	N	A	8	2.5	<0.1						
Unknown forb		-	-	-	-	2.5	<0.1						
Carolina draba	*Draba reptans* (Lam.) Fernald	*Br*	N	A	1							2.5	<0.1
American bird's-foot trefoil	*Lotus unifoliolatus* (Hook.) Benth.	*Fa*	N	A	3							2.5	<0.1

Sub-shrub and Shrubs													
Prairie sagewort	*Artemisia frigida* Willd.	*As*	N	P	4	2.5	<0.1					2.5	<0.1
Trees													
White mulberry	*Morus alba* L.	*Mo*	I	P	*	25.0	0.3						

Notes: families include = Am, Amaranthaceae; As, Asteraceae; Br, Brassicaceae; Ca, Caryophyllaceae; Ch, Chenopodiaceae; Cm, Campanulaceae; Cy, Cyperaceae; Eu, Euphorbiaceae; Fa, Fabaceae; Ju, Juncaceae; La, Lamiaceae; Mo, Moraceae; On, Onagraceae; Ox, Oxalidaceae; Po, Poaceae; Pr, Primulaceae; Py, Polygonaceae; Ra, Ranunculaceae; Ro, Rosaceae; Ru, Rubiaceae; Sc, Scrophulariaceae; So, Solanaceae; Ty, Typhaceae; Ur, Urticaceae; Ve, Verbenaceae; Vi, Violaceae
Origin = I, introduced; N, native | Life Span = A, annual; B, biennial; P, perennial | C-value = *, introduced species

Table 3.4 Soil seed bank species list with frequency (%) and relative density (%) for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 2 in 2021. Species are sorted numerically by decreasing average relative density (%) among treatments.

USDA Common Name	Scientific Name	Family	Origin	Life Span	C-Value	Site 2 (2021)							
						UC		CE		2M		GL	
						Freq.	Den.	Freq.	Den.	Freq.	Den.	Freq.	Den.
Grasses													
Kentucky bluegrass	*Poa pratensis* L.	*Po*	I	P	*	90	29.5	87.5	27.6	85	22.4	92.5	25.8
Scribner's rosette grass	*Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould	*Po*	N	P	6	22.5	1.4	52.5	3.3	60	4.5	57.5	4.9
Composite dropseed	*Sporobolus compositus (*Poir.) Merr.	*Po*	N	P	4	15	0.4	27.5	2.0	30	1.4	32.5	3.4
UG14	-	-	-	-	-			5	0.2	2.5	<0.1	10	6.7
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	*Po*	I	A	*	30	2.8	7.5	0.3			12.5	2.4
Stinkgrass	*Eragrostis cilianensis* (All.) Vign. Ex Janchen	*Po*	I	A	*	42.5	2.0	20	1.1	17.5	0.8	17.5	1.1
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	*Po*	N	P	5	2.5	<0.1	2.5	<0.1	15	0.9	20	1.2
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	*Po*	N	P	6	2.5	0.4	10	0.4	17.5	0.9	15	0.4

Proso millet	*Panicum miliaceum* L.	*Po*	I	A	*	17.5	1.0	10	0.4	2.5	<0.1	7.5	0.2
Prairie junegrass	*Koeleria macrantha* (Ledeb.) Schult.	*Po*	N	P	7	5	0.3	15	0.7	12.5	0.6		
Blue grama	*Bouteloua gracillis* (Willd. ex Kunth) Lag. Ex Griffiths	*Po*	N	P	7			7.5	0.2	12.5	0.8	12.5	0.5
Big bluestem	*Andropogon gerardii* Vitman	*Po*	N	P	5			2.5	0.2	20	0.7	17.5	0.6
Sand dropseed	*Sporobolus cryptandrus* (Torr.) A. Gray	*Po*	N	P	6			2.5	0.2	5	1.0		
Canada bluegrass	*Poa compressa* L.	*Po*	I	P	*	5	0.5	5	0.2	2.5	0.1	5	0.4
Green bristlegrass	*Setaria viridis* (L.) P. Beauv.	*Po*	I	A	*	7.5	0.3	5	0.4			2.5	<0.1
Buffalograss	*Bouteloua dactyloides* (Nutt.) J.T. Columbus	*Po*	N	P	4					7.5	0.2	10	0.4
Cheatgrass	*Bromus tectorum* L.	*Po*	I	A	*			5	0.2	7.5	0.2	5	0.1
Prairie sandreed	*Calamovilfa longifolia* (Hook.) Scribn.	*Po*	N	P	5					2.5	<0.1	5	0.3
Foxtail barley	*Hordeum jubatum* L.	*Po*	N	P	0					5	0.2	5	0.1
Porcupinegrass	*Hesperostipa spartea* (Trin.) Barkworth	*Po*	N	P	8					2.5	0.2		
Sixweeks fescue	*Vulpia octoflora* (Walter) Rydb.	*Po*	N	A	0			2.5	<0.1			2.5	<0.1
UG5	-	-	-	-	-					2.5	<0.1		
Green needlegrass	*Nassella viridula* (Trin.) Barkworth	*Po*	N	P	5	2.5	<0.1						
Needle and thread	*Hesperostipa comata* (Trin. & Rupr.) Barkworth	*Po*	N	P	6							2.5	<0.1
Smooth brome	*Bromus inermis* Leyss.	*Po*	I	P	*							2.5	<0.1
Grass-likes													
Needleleaf sedge	*Carex duriuscula* C. A. Mey.	*Cy*	N	P	10	12.5	0.7	15	0.6	22.5	1.2	10	0.25
Forbs													
Western rockjasmine	*Androsace occidentalis* Pursh	*Pr*	N	A	5	70	18.4	82.5	22.5	80	21.9	70	16.0
Hoary verbena	*Verbena stricta* Vent.	*Ve*	N	P	2	82.5	16.9	97.5	19.3	90	25.1	97.5	14.0
Canadian horseweed	*Conyza canadensis* (L.) Cronquist	*As*	N	A/B	0	97.5	11.8	82.5	6.5	65	3.4	80	5.7
Carolina draba	*Draba reptans* (Lam.) Fernald	*Br*	N	A	1	35	1.6	37.5	3.3	30	1.9	45	2.9

Clasping Venus' looking-glass	*Triodanis perfoliata* (L.) Nieuwl.	*Cm*	N	A	6	15	3.3	15	1.5	10	0.9	10	1.3
Common yellow oxalis	*Oxalis stricta* L.	*Ox*	N	P	0	32.5	1.7	15	1.0	27.5	1.8	27.5	1.6
Ribseed sandmat	*Chamaesyce glyptosperma* (Engelm.) Small	*Eu*	N	A	0	10	0.5	30	2.5	22.5	0.8	42.5	1.7
Wild bergamot	*Monarda fistulosa* L.	*La*	N	P	5	12.5	0.5	22.5	1.4	17.5	0.7	32.5	1.3
Upright prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.	*As*	N	P	3	10	0.6	7.5	0.4	25	1.7	20	1.0
Prairie fleabane	*Erigeron strigosus* Muhl. ex Willd.	*As*	N	A/B/P	3	7.5	0.2	10	0.4	15	1.0	25	1.7
Bigbract verbena	*Verbena bracteata* Cav. ex Lag. & Rodr.	*Ve*	N	A/B/P	0	10	0.4	7.5	0.3	12.5	0.4	12.5	2.0
West Indian Nightshade	*Solanum ptycanthum* Dunal	*So*	N	A	0	35	1.6	5	0.2			7.5	0.2
Warty Spurge	*Euphorbia spathulata* Lam.	*Eu*	N	A/P	5	12.5	0.3	12.5	0.5	20	0.8	10	0.3
Shortstalk chickweed	*Cerastium brachypodum* (Engelm. Ex A. Gray) B.L. Rob.	*Ca*	N	P	4	7.5	0.2	7.5	0.2	10	0.7	12.5	0.4
Prairie groundcherry	*Physalis hispida* (Waterf.) Cronquist	*So*	N	P	8	15	0.6	2.5	<0.1	2.5	0.2		
Prostrate pigweed	*Amaranthus albus* L.	*Am*	I	A	*	7.5	0.2	10	0.4	2.5	0.2	2.5	<0.1
Drummond's false pennyroyal	*Hedeoma drummondii* Benth.	*La*	N	P	4	15	0.8						
Blacksamson echinacea	*Echinacea angustifolia* DC.	*As*	N	P	7			2.5	<0.1	7.5	0.4	5	0.2
Spotted sandmat	*Chamaesyce maculata* (L.) Small	*Eu*	N	A	0			5	0.2	7.5	0.3	2.5	<0.1
Fireweed	*Chamerion angustifolium* (L.) Holub	*On*	N	P	5	2.5	<0.1	2.5	<0.1	10	0.3	2.5	<0.1
Hairy rockcress	*Arabis hirsuta* (L.) Scop.	*Br*	N	A/B/P	7			2.5	0.2	2.5	<0.1	5	0.2
UF6	-	-	-	-	-			2.5	<0.1	5	0.3		
Prickly lettuce	*Lactuca serriola* L.	*As*	I	A/B	*	7.5	0.2	2.5	0.2				
Woolly plantain	*Plantago patagonica* Jacq.	*Pl*	N	A	1			2.5	<0.1	5	0.2	2.5	<0.1
Canada thistle	*Cirsium arvense* (L.) Scop.	*As*	I	P	*	7.5	0.2	2.5	<0.1				
Lambsquarters	*Chenopodium album* L.	*Ch*	I	A	*	2.5	<0.1	2.5	<0.1	2.5	<0.1		
Rough false pennyroyal	*Hedeoma hispida* Pursh	*La*	N	A	4	2.5	<0.1	2.5	<0.1	2.5	<0.1		
UF12	-	-	-	-	-	2.5	<0.1			2.5	0.2		

Bull Thistle	*Cirsium vulgare* (Savi) Ten.	*As*	I	B	*	5	0.1			2.5	<0.1		
Common pepperweed	*Lepidium densiflorum* Schrad.	*Br*	N	A	0	5	0.1			2.5	<0.1		
Yellow salsify	*Tragopogon dubius* Scop.	*As*	I	B	*			5	0.2				
Stiff goldenrod	*Oligoneuron rigidum* (L.) Small var. *humile* (Porter) G.L. Nesom	*As*	N	P	4			2.5	<0.1	2.5	<0.1		
White panicle aster	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom	*As*	N	P	4			2.5	<0.1			2.5	<0.1
Nodding plumeless thistle	*Carduus nutans* L.	*As*	I	B/P	*	2.5	<0.1			2.5	<0.1		
Redroot amaranth	*Amaranthus retroflexus* L.	*Am*	N	A	0	2.5	<0.1			2.5	<0.1		
White sagebrush	*Artemisia ludoviciana* Nutt.	*As*	N	P	3					2.5	<0.1	2.5	<0.1
Alfalfa	*Medicago sativa* L.	*Fa*	I	A/P	*			2.5	<0.1				
American bird's-foot trefoil	*Lotus unifoliolatus* (Hook.) Benth.	*Fa*	N	A	3			2.5	<0.1				
Slimpod Venus' looking-glass	*Triodanis leptocarpa* (Nutt.) Nieuwl.	*Cm*	N	A	8			2.5	<0.1				
Common yarrow	*Achillea millefolium* L.	*As*	N	P	3					2.5	<0.1		
False gromwell	*Onosmodium bejariense* DC. ex A. DC.	*Bo*	N	P	7					2.5	<0.1		
Cattail	*Typha spp.* L.	*Ty*	N	P		2.5	<0.1						
Tall blue lettuce	*Lactuca biennis* (Moench) Fernald	*As*	N	A/B	6	2.5	<0.1						
Buffalobur nightshade	*Solanum rostratum* Dunal	*So*	N	A	0							2.5	<0.1
Fringed willowherb	*Epilobium ciliatum* Raf.	*On*	N	P	3							2.5	<0.1
Smooth oxeye	*Heliopsis helianthoides* (L.) Sweet	*As*	N	P	5							2.5	<0.1
Western Wallflower	*Erysimum asperum* (Nutt.) DC.	*Br*	N	B/P	3							2.5	<0.1
Sub-shrubs and Shrubs													<0.1
Prairie sagewort	*Artemisia frigida* Willd.	*As*	N	P	4							2.5	<0.1

Notes: families include = Am, Amaranthaceae; As, Asteraceae; Bo, Boraginaceae; Br, Brassicaceae; Ca, Caryophyllaceae; Ch, Chenopodiaceae; Cm, Campanulaceae; Cy, Cyperaceae; Eu, Euphorbiaceae; Fa, Fabaceae; La, Lamiaceae; On, Onagraceae; Ox, Oxalidaceae; Pl, Plantaginaceae; Po, Poaceae; Pr, Primulaceae; So, Solanaceae; Ty, Typhaceae; Ve, Verbenaceae
Origin = I, introduced; N, native | Life Span = A, annual; B, biennial; P, perennial | C-value = *, introduced species

Table 3.5 Soil seed bank composition by functional group for Site 1 in 2020 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Mean composition with standard error (mean ± standard error) is represented for each functional group by treatment. Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using Kruskal-Wallis test and Dunn's Post Hoc test.

Treatment	Mean Seed Bank Composition (%/sample) by Functional Group at Site 1 (2020)															
	Forb	FIA/B	FIP	FNA/B	FNP	Unk. Forb	Graminoid	GIA	GIP	GNP	ShNP	TIP	Intro	Native	A/B	Peren.
UC	41.7 ± 2.88	6.17 ± 1.52	$2.17 \pm 0.44_{a}$	$12.9 \pm 2.00_{a}$	20.4 ± 2.47	0.03 ± 0.03	57.9 ± 2.89	$0.11 \pm 0.08_{a}$	53.5 ± 3.16	$4.28 \pm 1.79_{a}$	0.03 ± 0.03	$0.35 \pm 0.12_{a}$	62.3 ± 3.19	37.7 ± 3.18	19.2 ± 2.44	80.8 ± 2.44
CE	35.4 ± 2.93	4.75 ± 0.90	$1.99 \pm 0.78_{ab}$	$10.0 \pm 1.98_{ab}$	18.6 ± 2.48	0.0	64.7 ± 2.93	$1.12 \pm 0.39_{ab}$	58.8 ± 3.07	$4.72 \pm 1.01_{ab}$	0.00	0.0_{b}	66.7 ± 3.30	33.3 ± 3.30	15.9 ± 1.98	84.1 ± 1.98
2M	38.7 ± 3.12	5.97 ± 0.84	$0.33 \pm 0.15_{c}$	$8.97 \pm 1.72_{ab}$	23.4 ± 2.57	0.0	61.3 ± 3.12	$0.32 \pm 0.13_{ab}$	54.3 ± 3.27	$6.66 \pm 0.94_{b}$	0.00	0.0_{b}	61.0 ± 3.59	39.0 ± 3.59	15.3 ± 1.73	84.7 ± 1.73
GL	39.8 ± 3.31	6.12 ± 0.89	$0.60 \pm 0.24_{bc}$	$9.10 \pm 2.42_{b}$	24.0 ± 2.57	0.0	60.1 ± 3.32	$2.25 \pm 0.78_{b}$	53.0 ± 3.59	$4.86 \pm 0.90_{ab}$	0.07 ± 0.07	0.0_{b}	62.0 ± 3.56	38.0 ± 3.56	17.5 ± 2.55	82.5 ± 2.55
P-Value	***0.48***	***0.30***	***<0.01***	***0.05***	***0.26***	***0.40***	***0.45***	***0.01***	***0.71***	***0.01***	***0.58***	***<0.01***	***0.74***	***0.74***	***0.80***	***0.80***

Notes: Life Form = F, forb; G, graaminoid, Sh, shrub; T, tree | Origin: I, introduced; N, native | Life Span = A/B, annual/biennial; P, perennial | Other = A/B, total annual/biennial; Forb, total forb; Graminoid, total graminoid; Peren., total perennial; Unk, unknown.

Table 3.6 Soil seed bank composition by functional group for Site 2 in 2021 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Mean composition with standard error (mean ± standard error) is represented for each functional group by treatment. Different letters indicate significance (*P<0.05*) between treatment medians within the year analyzed using Kruskal-Wallis test and Dunn's Post Hoc test.

Treatment	Mean Seed Bank Composition (%/sample) by Functional Group at Site 2 (2021)																
	Forb	FIA/B	FIP	FNA/B	FNP	Unk. Forb	Graminoid	GIA	GIP	GNA	GNP	Unk. Graminoid	ShNP	Intro	Native	A/B	Peren.
UC	58.7 ± 3.26	$0.90 \pm 0.33_{a}$	0.37 ± 0.23	$36.4 \pm 2.80_{a}$	20.8 ± 2.66	0.21 ± 0.21	41.3 ± 3.26	$7.58 \pm 1.58_{a}$	29.7 ± 3.37	0.00	$4.06 \pm 1.19_{a}$	0.00	0.00	$38.5 \pm 3.03_{a}$	$61.3 \pm 3.04_{a}$	$44.9 \pm 3.30_{a}$	$54.9 \pm 3.36_{a}$
CE	64.2 ± 2.98	$0.67 \pm 0.30_{ab}$	0.24 ± 0.17	$40.0 \pm 3.02_{a}$	23.2 ± 2.61	0.16 ± 0.11	35.8 ± 2.98	$2.29 \pm 0.51_{b}$	25.5 ± 2.76	0.08 ± 0.08	$7.81 \pm 1.21_{ab}$	0.10 ± 0.07	0.00	$28.7 \pm 2.88_{ab}$	$71.0 \pm 2.86_{ab}$	$43.0 \pm 2.98_{a}$	$56.7 \pm 3.01_{ab}$
2M	61.2 ± 3.28	$0.48 \pm 0.28_{ab}$	0.00	$29.4 \pm 2.67_{a}$	30.7 ± 3.04	0.58 ± 0.38	38.8 ± 3.28	$1.29 \pm 0.39_{b}$	21.6 ± 2.51	0.00	$15.5 \pm 2.00_{c}$	0.41 ± 0.33	0.00	$23.4 \pm 2.47_{b}$	$75.7 \pm 2.37_{b}$	$31.2 \pm 2.75_{b}$	$67.8 \pm 2.75_{b}$
GL	54.4 ± 3.56	$0.04 \pm 0.04_{b}$	0.00	$30.4 \pm 2.81_{a}$	23.9 ± 2.17	0.07 ± 0.07	45.5 ± 3.58	$2.89 \pm 0.84_{b}$	27.6 ± 3.08	0.23 ± 0.23	$13.0 \pm 1.74_{bc}$	1.76 ± 1.46	0.07 ± 0.07	$30.6 \pm 3.06_{ab}$	$67.6 \pm 3.25_{ab}$	$33.6 \pm 2.69_{ab}$	$64.6 \pm 2.89_{ab}$
P-Value	***0.29***	***<0.01***	***0.14***	***0.05***	***0.06***	***0.64***	***0.29***	***<0.01***	***0.34***	***0.57***	***<0.01***	***0.23***	***0.39***	***0.01***	***0.01***	***<0.01***	***<0.01***

Notes: Life Form = F, forb; G, graminoid, Sh, shrub; T, tree | Origin: I, introduced; N, native | Life Span = A/B, annual/biennial; P, perennial | Other = A/B, total annual/biennial; Forb, total forb; Graminoid, total graminoid; Peren., total perennial; Unk, unknown.

Table 3.7 Shannon-Wiener diversity (H'), Shannon-Wiener evenness, Floristic Quality Index (FQI), species richness, and native species richness for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Mean and standard error is represented by treatment. Different letters indicate significance (*P<0.05*) between treatment within the year analyzed using ANOVA and Tukey's HSD.

Treatment	Site 1 (2020)					Site 2 (2021)				
	H'	Evenness	FQI	Total Rich	Nat Rich	H'	Evenness	FQI	Total Rich	Nat Rich
UC	1.41 ± 0.06	0.61 ± 0.02	$6.50 \pm 0.40_{a}$	10.30 ± 0.40	5.78 ± 0.32	1.54 ± 0.05	0.78 ± 0.02	$5.98 \pm 0.44_{a}$	7.75 ± 0.40	$5.48 \pm 0.32_{a}$
CE	1.42 ± 0.06	0.64 ± 0.02	$7.00 \pm 0.66_{ab}$	9.56 ± 0.45	5.41 ± 0.43	1.59 ± 0.05	0.80 ± 0.02	$7.78 \pm 0.43_{b}$	7.73 ± 0.38	$5.98 \pm 0.31_{ab}$
2M	1.40 ± 0.06	0.62 ± 0.02	$8.39 \pm 0.45_{b}$	9.53 ± 0.36	5.95 ± 0.33	1.67 ± 0.05	0.81 ± 0.02	$9.23 \pm 0.39_{b}$	8.28 ± 0.36	$6.90 \pm 0.31_{b}$
GL	1.38 ± 0.07	0.63 ± 0.03	$7.19 \pm 0.44_{ab}$	9.25 ± 0.38	5.20 ± 0.30	1.71 ± 0.05	0.80 ± 0.02	$7.99 \pm 0.44_{b}$	8.75 ± 0.40	$7.15 \pm 0.36_{b}$
P-Value	***0.98***	***0.87***	***0.05***	***0.29***	***0.41***	***0.11***	***0.56***	***<0.01***	***0.18***	***<0.01***

Table 3.8 Soil seed bank correlation between NMS main matrix and species for Site 1 in 2020 comparing treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). All species present in table are significant (*P<0.01*) based on Pearson correlation coefficients and sample size.

Species Correlations with NMS Axes (Site 1)			
Axis 1			
Species	r	r-squared	tau
Oxalis stricta	0.705	0.496	0.615
Conyza canadensis	0.500	0.250	0.430
Medicago lupulina	0.331	0.109	0.250
Bromus inermis	0.289	0.084	0.181
Euphorbia esula	0.277	0.077	0.156
Verbascum thapsus	0.222	0.049	0.109
Bromus tectorum	0.213	0.045	0.093
Dichanthelium oligosanthes	0.213	0.045	0.210
Agrostis scabra	0.211	0.045	0.151
Typha spp.	-0.221	0.049	-0.184
Melilotus officinalis	-0.374	0.140	-0.341
Poa pratensis	-0.561	0.314	-0.438
Axis 2			
Juncus interior	0.402	0.161	0.095
Androsace occidentalis	0.394	0.155	0.462
Silene antirrhina	0.329	0.108	0.258
Amaranthus retroflexus	0.254	0.065	0.049
Liatris punctata	0.231	0.054	0.109
Triodanis perfoliata	0.213	0.045	0.076
Lotus unifoliolatus	-0.217	0.047	-0.109
Physalis hispida	-0.217	0.047	-0.008
Bromus inermis	-0.219	0.048	-0.264
Sisymbrium altissimum	-0.259	0.067	0.012
Nepeta cataria	-0.267	0.071	-0.092

Table 3.9 Soil seed bank correlation between NMS main matrix and species for Site 2 in 2021 comparing treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). All species present in table are significant (*P<0.01*) based on Pearson correlation coefficients and sample size.

Species Correlations with NMS Axes (Site 2)			
Axis 1			
Species	r	r-squared	tau
Verbena stricta	0.431	0.186	0.401
Ratibida columnifera	0.235	0.055	0.094
UG5	0.232	0.054	0.112

Sporobolus compositus	-0.214	0.046	-0.127
Poa pratensis	-0.598	0.358	-0.497
Axis 2			
Androsace occidentalis	0.635	0.403	0.672
Unknown Graminoid 14	0.223	0.05	0.089
Calamovilfa longifolia	0.215	0.046	0.082
Dichanthelium oligosanthes	0.207	0.043	0.149
Onosmodium bejariense	-0.204	0.042	-0.11
Cirsium vulgare	-0.225	0.051	-0.138
Lepidium densiflorum	-0.237	0.056	-0.167
Draba reptans	-0.3	0.09	-0.213
Unknown Forb 12	-0.302	0.091	-0.157

Table 3.10 Community analyses on seed bank composition within Site 1 and Site 2 by treatment (UC, CE, 2M, GL), aspect (N, E, S, W), and hill orientation to the sampled ERC (uphill, side hill, and downhill) using MRPP with a relative Sorenson distance measure.

	A-value	P-value
Site 1		
Treatment	0.0115	0.0111
Aspect	0.0041	0.1333
Hill	-0.0047	1.0000
Site 2		
Treatment	0.0147	0.0003
Aspect	-0.0044	0.9548
Hill	-0.0040	0.9881

Table 3.11 Community analyses comparing aboveground foliar cover composition to soil seed bank composition for under ERC canopies (UC) and grassland control locations (GL) using NMS and MRPP for Site 1 and Site 2.

	Distance Measure	Min. Stress	Axis 1	Axis 2	Axis 3	A-value (MRPP)	P-value (MRPP)
Site 1 (2020)							
UC	Correlation	19.36	21.9	21.8	19.1	0.1625	<0.01
GL	Relative Sorenson	10.96	49.4	24.8	15.2	0.1839	<0.01
Site 2 (2021)							
UC	Correlation	14.15	48.3	25.6	15.6	0.1098	<0.01
GL	Correlation	14.15	38	23	22.7	0.1682	<0.01

Table 3.12 ERC seed density in soil seed bank (seeds/$0.1m^3$) categorized by Intact, Broken, Hole, and Total Potential for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) at Site 1 in 2020 and Site 2 in 2021. Mean and standard error is represented by treatment. Different letters indicate significance ($P<0.05$) between treatment medians within the site and seed category analyzed using a Kruskal-Wallis test and Dunn's Post Hoc test.

Treatment	Site 1 (2020)					Site 2 (2021)				
	Intact	Broken	Hole	Total[1]	N	Intact	Broken	Hole	Total[1]	N
UC	$8362 \pm 925_{a}$	$21162 \pm 2569_{a}$	$6868 \pm 1100_{a}$	$36392 \pm 4047_{a}$	36	$24605 \pm 2228_{a}$	$19009 \pm 3006_{a}$	$9070 \pm 1434_{a}$	$52684 \pm 8330_{a}$	40
CE	$1933 \pm 340_{b}$	$3501 \pm 410_{b}$	$1090 \pm 209_{b}$	$6525 \pm 790_{b}$	34	$5755 \pm 761_{b}$	$3633 \pm 574_{b}$	$894.9 \pm 141_{b}$	$10283 \pm 1626_{b}$	40
2M	$144 \pm 34_{c}$	$120 \pm 34_{c}$	$23 \pm 11_{c}$	$287 \pm 59_{c}$	38	$159 \pm 45_{c}$	$66 \pm 10_{c}$	$38 \pm 6_{c}$	$263 \pm 42_{c}$	40
GL	$75 \pm 22_{c}$	$29 \pm 13_{c}$	$23 \pm 10_{c}$	$127 \pm 29_{c}$	39	$32 \pm 11_{c}$	$7.2 \pm 1.1_{c}$	$22 \pm 4_{c}$	$61 \pm 10_{c}$	40
P-Value	***<0.01***	***<0.01***	***<0.01***	***<0.01***		***<0.01***	***<0.01***	***<0.01***	***<0.01***	

[1]Total potential of ERC seed density in the soil seed bank assuming no decomposition, predation, or damage to Broken and Hole seeds.

FIGURES

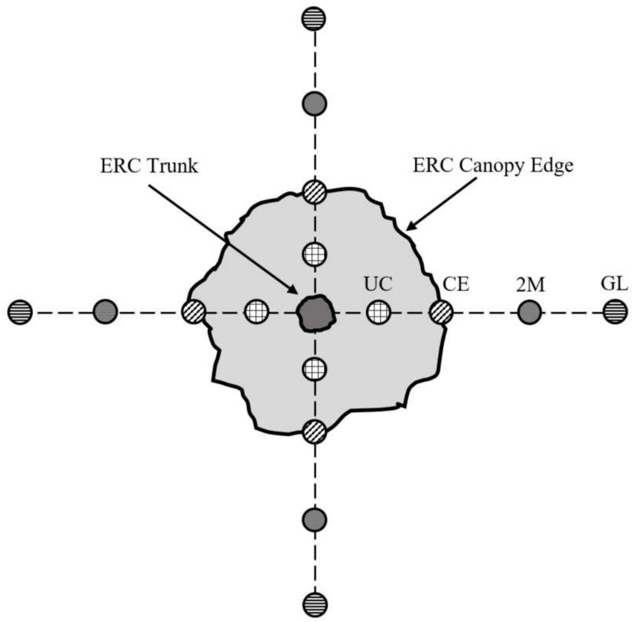


Figure 3.1 Aerial illustration of soil seed bank core sampling design with four transects extending from an ERC trunk where treatments contain under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Each soil core was 785 cm^3 (10 cm diameter x 10 cm depth).

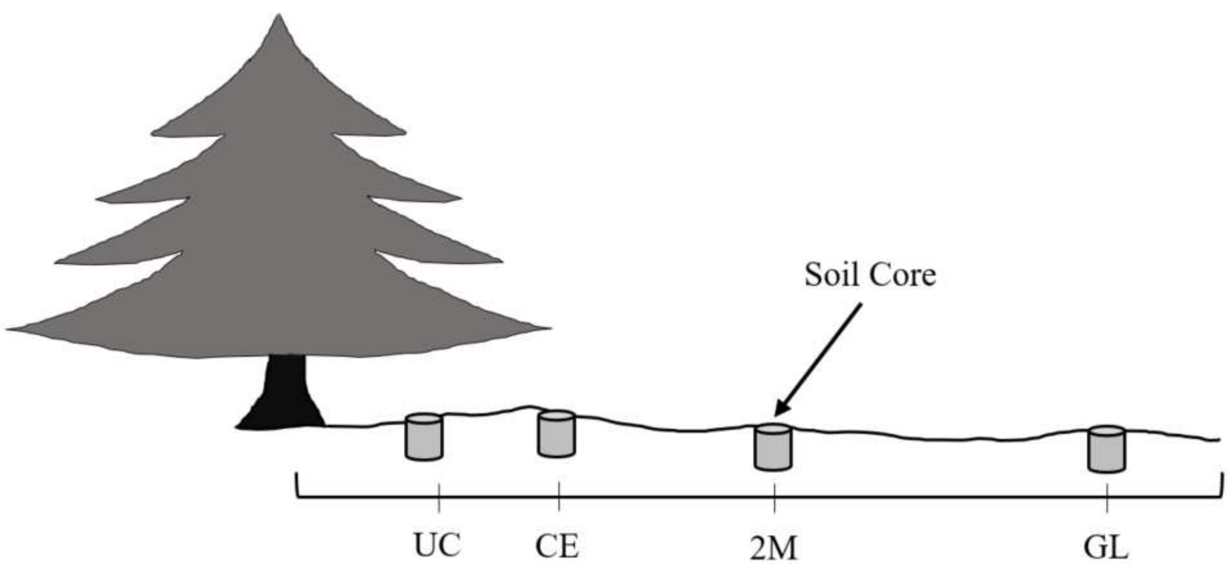


Figure 3.2 Lateral illustration of soil core sampling design of one transect out of four total per ERC, with treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Each soil core was 785 cm^3 (10 cm diameter x 10 cm depth).

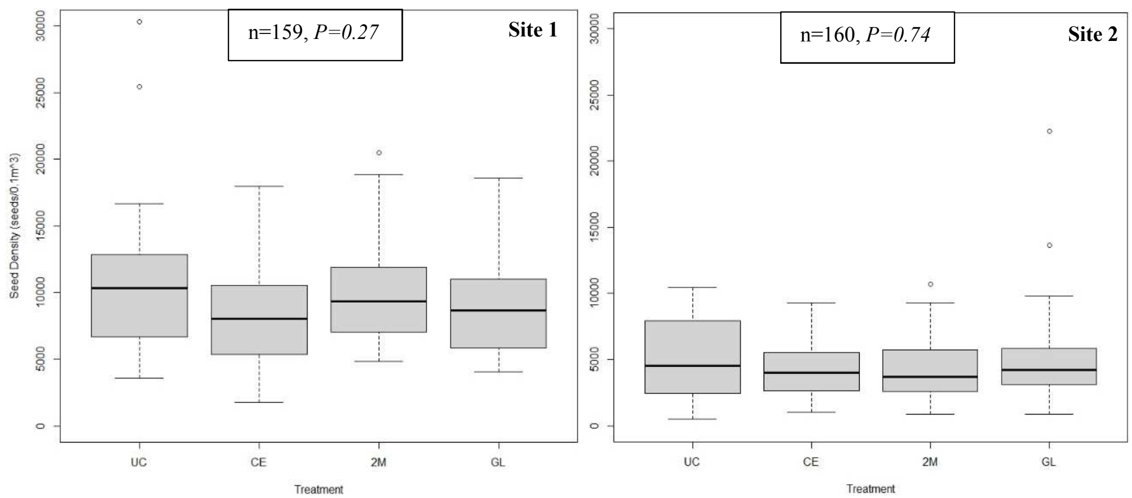
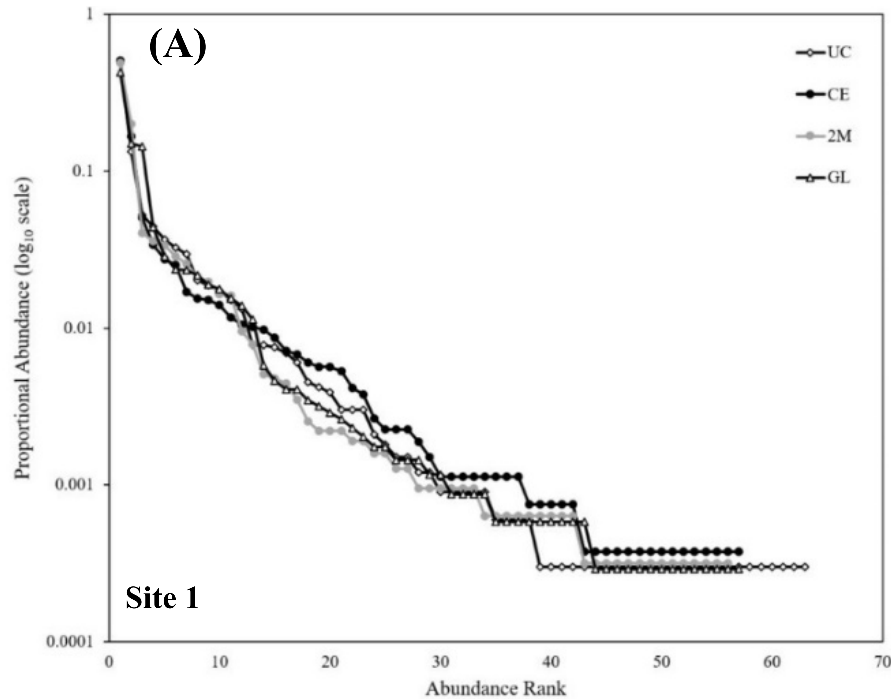


Figure 3.3 Total emerged seedlings (seeds/0.1m^3) at Site 1 in 2020 and Site 2 in 2021 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Sample size (n) and p-value (*P*) from Kruskal-Wallis tests among treatment medians are displayed at the top of each figure.



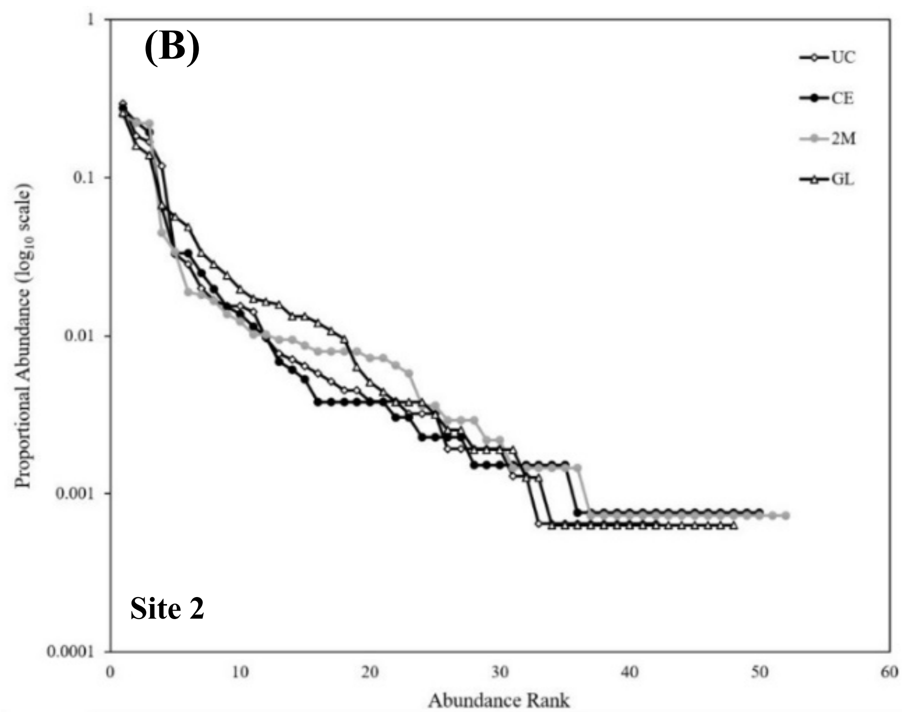


Figure 3.4 Rank abundance curves on soil seed bank composition for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 (A) and Site 2 in 2021 (B). Proportional abundance ($\log_{10}$ scale) of relative seed density (%) is displayed on the *y*-axis and species abundance are ranked in order of largest to smallest on the *x*-axis.

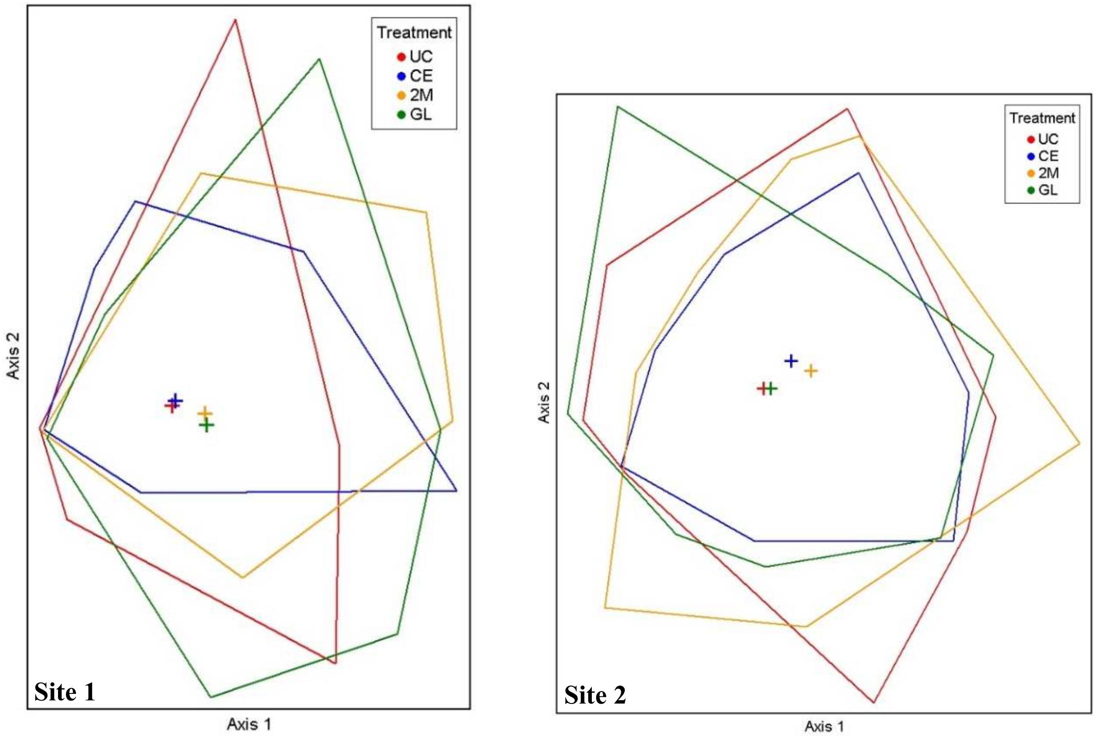


Figure 3.5 NMS ordination plots using a relative Sorenson distance measure of seed bank composition among treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Convex hulls and centroids (cross symbol) by treatment are displayed, where convex hulls encompass each treatment and centroids are the multivariate average.

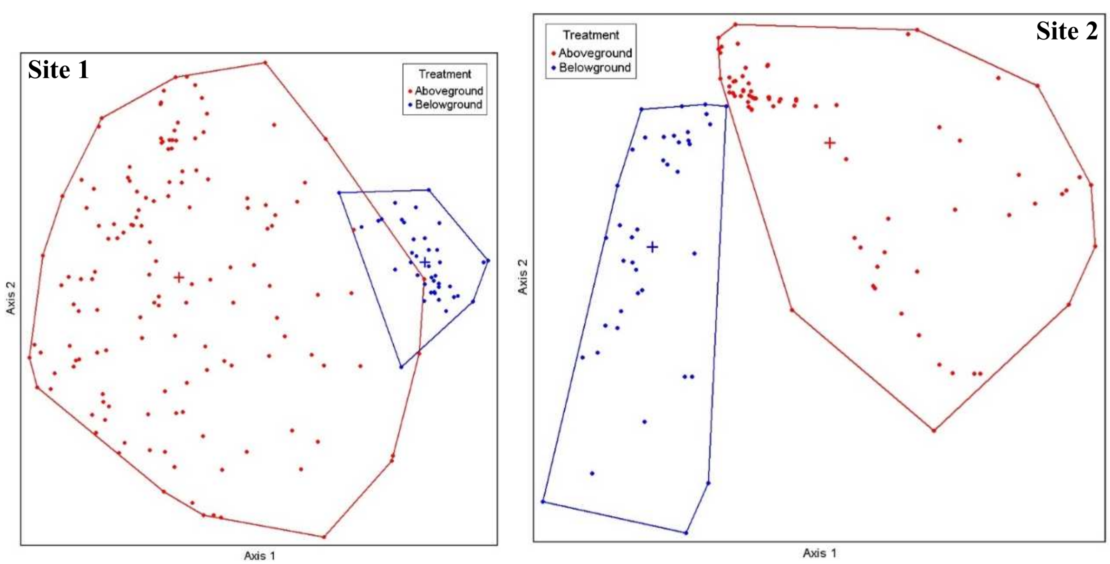


Figure 3.6 NMS ordination plots comparing aboveground relative foliar cover to belowground relative seed bank composition under ERC canopies (UC) at Site 1 and Site 2. Samples are represented by dots where n=188 and n=128 for Site 1 and Site 2, respectively. Convex hulls and centroids (cross symbol) by treatment are displayed, where convex hulls encompass each treatment and centroids are the multivariate average.

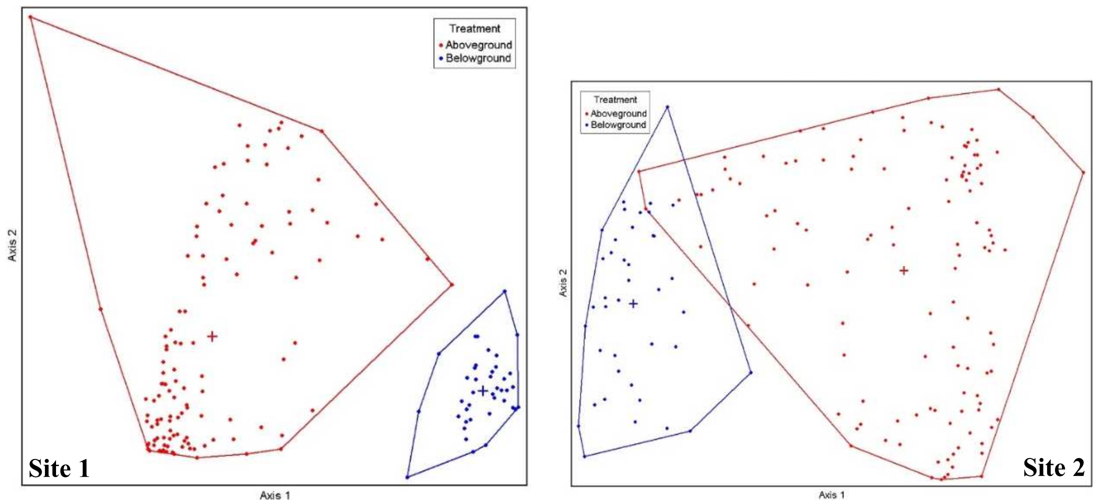


Figure 3.7 NMS ordination plots comparing aboveground relative foliar cover to belowground relative seed bank composition on grassland control (GL) sites at Site 1 and Site 2. Samples are represented by dots where n=160 for Site 1 and Site 2, respectively. Convex hulls and centroids (cross symbol) by treatment are displayed, where convex hulls encompass each treatment and centroids are the multivariate average.

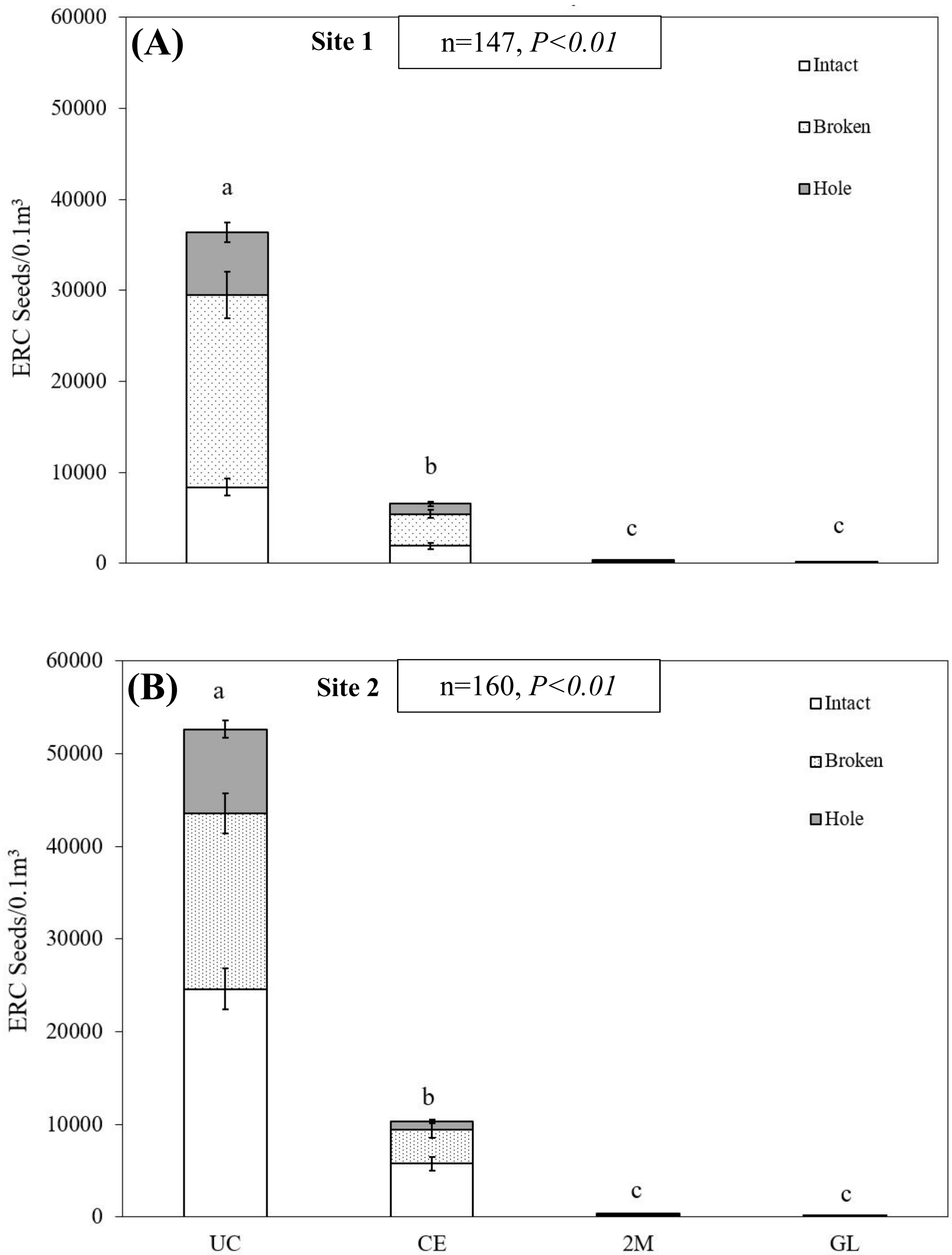


Figure 3.8 ERC seed density in the soil seed bank (seeds/0.1m^3) categorized by Intact (open), Broken (dotted), and Hole (solid) at Site 1 in 2020 (A) and Site 2 in 2021 (B) for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Bars represent mean seed density (seeds/0.1m^3) with standard error bars. Letters indicate significance (*P<0.05*) between treatment medians using a Kruskal-Wallis test followed by Dunn's post hoc comparison.

CHAPTER 4: LARGE EASTERN REDCEDAR IMPACT ON BUD BANK PRODUCTION AND COMPOSITION IN THE NORTHERN GREAT PLAINS MIXED-GRASS PRAIRIE

ABSTRACT

Eastern redcedar (ERC) (*Juniperus virginiana* L.) trees are invading prairies throughout the Great Plains due to fire suppression and escaping from planted ERC shelterbelts. This encroachment poses a threat to native plant communities in terms of their reproduction, regeneration, and diversity. It is unknown how ERC trees impact belowground vegetative propagules in the mixed-grass prairie, such as crown and rhizome bud production and composition, also known as the “bud bank”. The objective of this study was to evaluate how large ERC trees impact the bud bank at varying distances from an ERC trunk. In October 2020 (Site 1) and 2021 (Site 2) in south-central South Dakota, ten female ERC trees with canopy diameters 5-10 m, similar environmental characteristics (ie. soil type, slope), and isolated from other large ERC trees were selected for soil sampling at four treatment distances: under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Four transects extended from each tree stem where a soil core (10 cm dia. x 10 cm depth) was sampled at the four treatment distances, totaling 16 cores per tree and 160 cores overall. Soil cores were washed with high-pressure water to remove debris and soil to expose vegetative propagules. Roots were removed from individual plants and their propagules were separated into crowns and rhizomes. A dissecting microscope (10-67.5x magnification) was used to classify and count crown and rhizome buds per soil core by the functional group. Soil cores were combined per ERC tree for analyses purposes with

an ERC tree as the sample unit. A total of 64,015 live buds were counted among treatments at both sites, consisting of 25,906 and 38,109 total buds at Site 1 and Site 2, respectively. Bud production was minimal underneath ERC canopies and did not differ between other treatments. We found nearly a three-fold decrease in native bud composition underneath ERC canopies compared to grassland control locations. In addition, nonmetric multidimensional scaling and perMANOVA comparisons found samples collected underneath ERC canopies to be significantly different ($P<0.01$) than all other treatments. Our results suggest that mature female ERC are drastically negatively impacting bud production and composition underneath their canopies and regeneration following ERC removal from the bud bank will likely be minimal. Other restoration strategies are recommended to reestablish native perennial vegetation underneath previous ERC canopies.

INTRODUCTION

Grasslands historically covered 46 million km^2 of Earth's surface representing nearly 42% of the living vegetation (Anderson 2006). Encroachment of woody species onto grasslands and savannas is a widely researched global phenomenon, with junipers (*Juniperus spp.* L.) and pines (*Pinus spp.* L.) being the most common woody encroachers in the United States (Miller et al. 2000). Historic grasslands in North America were maintained through a combination of wildfire and grazing, inhibiting the spread of forest from northern latitudes or riparian areas and resulting in a grass dominated landscape for the last 5000-8000 years (Higgins 1986, Twidwell et al. 2013). Since European settlement, dramatic changes have converted our grasslands from their historical state through fire suppression, cultivation, and woody encroachment (Engle et al. 2008). This woody encroachment in North America has led to grassland systems being the most endangered ecosystems (Engle et al. 2008). The increase of woody plants on grasslands alters nutrient cycling, forage production, flora and fauna species composition, landscape heterogeneity, and risk of wildfire (Belsky 1994, DeSantis et al. 2011, Knapp et al. 2008, Limb et al. 2010, Miller et al. 200, Van Auken 2009, Van Els et al. 2010, Wang et al. 2018, Williams et al. 2017).

In the Great Plains, eastern redcedar (*Juniperus virginiana* L.) (hereafter ERC) is the most prominent woody encroacher (Meneguzzo and Liknes 2015, Schmidt and Leatherberry 1995). ERC are spreading at alarming rates and have been termed the "green glacier" by researchers, occupying up to seven million hectares of grassland and increasing exponentially in some areas (Bidwell et al. 1996, Engle et al. 2008, McKinley et al. 2008). ERC is an early successional native conifer species in North America present in every state east of the 100th meridian, with higher densities in Oklahoma, Kansas, Nebraska, Missouri, and South Dakota (Meneguzzo and Liknes 2015, Twidwell et al. 2021). ERC have increased by nearly 125,000 hectares in an eight-state region in the Northern Great Plains (NGP) between 2007-2012, by 2.3% per year in portions of the Kansas Flint Hills, and at a rate of 8% per year in Oklahoma (Briggs et al. 2002, Meneguzzo and Liknes 2015, Wang et al. 2018). Fire historically controlled and confined this native conifer species primarily to riparian areas or steep, rocky slopes (Lawson 1990). Fire suppression, overgrazing, and planting ERC in shelterbelts has allowed ERC to successfully encroach and spread rapidly on grasslands in the Great Plains due to its ability to compete for scarce resources and its high reproductive rate, with female trees producing up to 1.5 million berry-like cones on productive years (Briggs et al. 2002, Engle et al. 1987, Holthuijzen and Sharik 1985, Lawson 1990). Avian generalists, small mammals, and white-tailed deer are known to eat these fruit-like cones off ERC contributing to its seed dispersal and propagation on our grassland systems (Bidwell et al. 1996, Holthuijzen and Sharik 1985, Horncastle et al. 2004) resulting in a potential closed canopy in as little as 40 years (Briggs et al. 2002).

In the NGP mixed-grass prairie, a perennial species dominated ecosystem, the regeneration of aboveground vegetation relies heavily on tillering or vegetative reproduction via rhizomes or stolons (Ott and Hartnett 2015). These clonal plants produce vegetative propagules that consist of belowground reserves of meristems (such as rhizomes) and other perennating organs which was deemed the term "bud bank" by Harper (1977). The bud bank is produced asexually by perennial plants and is their primary method of regeneration in the NGP mixed-grass prairie (Ott and Hartnett 2015), where researchers have found bud banks contributing up to 99% of new vegetation in undisturbed systems (Benson and Hartnett 2006) and 80% in a disturbed system in the tallgrass prairie (Rogers and Hartnett 2001). Parent individuals (genets) produce genetically identical offspring (ramets) asexually through vegetative propagation from dormant buds including rhizomes/stolons, tillers, bulbs, tubers, and corms (Harper 1977). These independent ramets are commonly connected through horizontal stems, such as rhizomes (below soil surface) and stolons (at soil surface) to the parent plants where they can access and store nutrients, water, and carbohydrates (Alpert and Mooney 1986). Rhizomes and stolons provide the genet with the ability to spread horizontally to find new resource pockets including increased growing space, light availability, and soil moisture (Harper 1977). This colonizing approach of clonal plants allows connected individuals to prevent the invasion of neighboring plants (Hartnett and Bazzaz 1985) and persist during extreme weather events (Bam et al. 2022). Bud bank production varies within and among species (Lehtilä 2000, Ott and Hartnett 2012) in perennial ecosystems and can be impacted by competition, climate, resource availability, or disturbance regime (Ott and Hartnett 2015) including grazing, fire, and drought (Klimešová and Klimeš 2007). Healthy perennial grassland populations rely on the bud bank for population persistence and resiliency to climate change, disturbance, and extreme weather events (Harper 1977, Ott and Hartnett 2015).

Previous research on bud bank production has focused on the effect of grazing (Dalgleish and Hartnett 2009), prescribed fire (Benson et al. 2004, Russel et al. 2015), species invasion (Bam et al. 2022, Collier et al. 2002), or climate change (Chelli et al. 2019, Dalgleish and Hartnett 2006), with only one article to our knowledge on the effect of woody encroachment (Ferraro et al. 2020) and one that assesses its response post-fire in a boreal forest (Lee 2004). Grazing and prescribed fire tend to increase bud bank densities due to reducing litter build-up, increasing light and nutrient availability, and reducing competition with aboveground plants and foliar canopies (Benson et al. 2004, Ott et al. 2019). In the savannas of Brazil, Ferraro et al. (2020) found that *Pinus elliottii* (slash pine) cover reduced bud bank abundance and the number of bud bearing organs compared to open non-encroached savannas, resulting in a loss of resiliency in savannas and restoration difficulty following woody removal. Prescribed fire through *Populus tremuloides* (aspen) stands proved that lightly burned patches rely on the vegetative bud bank whereas heavily burned patches rely on the seed bank for post-disturbance above ground regeneration (Lee 2004). Therefore, since fire is commonly used for woody encroachment control in the NGP, we would expect to see a dramatic increase in bud bank density post-fire on lands heavily encroached by ERC due to the altered resource availability (Ott and Hartnett 2011).

Seed banks have been widely studied, including some research on the impacts of woody encroachment on seed bank resiliency and longevity (Allen and Nowak 2008). However, minimal research exists on bud banks in general and the impact woody encroachment on bud bank production, composition, and resiliency has only been studied in Brazilian savannas (Ferraro et al. 2020, Klimesova and Klimes 2007). Therefore, this research is the first of its kind in the Great Plains examining the impact of woody encroachment, in this case *Juniperus virginiana* L., on the belowground bud bank. The objective of our study was to determine the impact of large individual ERC trees on bud bank production and composition at varying distances from the ERC tree stem. The alternative hypotheses for this study were:

1. Bud production will be significantly reduced underneath ERC canopies with negligible production in some cases, and bud production will not differ between the other treatments of canopy edge, two meters from the canopy edge, and grassland control.
2. ERC canopies will reduce native graminoid bud composition in comparison to non-encroached grassland control samples.

MATERIALS AND METHODS

STUDY AREA

This study was conducted on two separate private ranches in south-central South Dakota in the Northern Great Plains mixed-grass prairie. Ranch 1, referred to as Site 1 (285 ha), is located in the Bijou Hills of Brule County along the east side of the Missouri River near Academy, South Dakota. This ecoregion contains a mixture of steep hills (15-40% slopes) surrounded by rolling mixed-grass prairie, cropland, and rangeland pastures. Soils primarily consist of Okaton bouldery silty clay (clayey residuum weathered from shale) where sampling was conducted (Soil Survey Staff 2022). Elevation ranges from 400 to 500 meters above sea level. Ranch 2, referred to as Site 2 (70 ha), is located in Charles Mix County along the east side of the Missouri River near Platte, South Dakota. This ecoregion contains a mixture of steep valleys and drainages (15-40% slopes) surrounded by rolling mixed-grass prairie, flat-topped ridges, cropland, and rangeland pastures. Soils primarily consist of Betts-Ethan loams (fine-loamy till) with abundant moraine at or near the soil surface (Soil Survey Staff 2022). Elevation ranges from 340 to 680 meters above sea level.

Sites 1 and 2 are close in geographic proximity (<40 km), therefore the same data was used to describe their climate. The landscape experiences a semiarid climate, consisting of hot, dry summers and cold, wet winters. The average annual temperature in 2020 was 8.5°C with a low of -24.4°C (February) and a high of 35.5°C (June). The total annual precipitation in 2020 was 445 mm with 86% of the precipitation occurring during the growing season (May – August), which was 15% higher than the 30-year average (1990-2019) during the growing season (HPRCC 2022, Mesonet 2022). The average annual temperature in 2021 was 9.1°C with a low of -31.7°C (February) and a high of 40.6°C (June). The total annual precipitation in 2021 was 399.8 mm with 53.7% of the precipitation occurring during the growing season (May – August), which was 36.4% lower than the 30-year average during the growing season indicating a drought (HPRCC 2022, Mesonet 2022). Deviations of monthly temperature and precipitation from the 30-year (1990-2019) average are shown in Appendix Tables A.1, A.2 and Figures A.1, A.2.

Site 1 consists of a disturbed mixed-grass prairie with ERC encroachment and no previous cattle grazing activity or prescribed fire within the past five years. The vegetation at this site is dominated by introduced graminoids including *Poa pratensis* L. (Kentucky bluegrass) and *Bromus inermis* Leyss. (smooth bromegrass) with native graminoids mixed throughout including *Nassella viridula* (Trin.) Barkworth (green needlegrass), *Andropogon gerardii* Vitman (big bluestem), *Sporobolus compositus* (Poir.) Merr. (composite dropseed), *Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould (Scribner's rosette grass), and *Pascopyrum smithii* (Rydb.) Á. Löve (western wheatgrass). Various forb species are present adding to the diversity of the site including *Solidago missouriensis* Nutt. (Missouri goldenrod), *Monarda fistulosa* L. (wild bergamot), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), and *Artemisia ludoviciana* Nutt. (white sagebrush).

Site 2 consists of a disturbed mixed-grass prairie with ERC encroachment and previous cattle grazing from April – June at a stocking rate of 75 cow-calf pairs on 70 hectares. However, this land was deferred (not grazed) in 2021 for sampling and to build fuel for a prescribed fire in spring 2022. The vegetation at this site is dominated by a mixture of native graminoids such as *Hesperostipa comata* (Trin. & Rupr.) Barkworth (needle and thread), *Schizachyrium scoparium* (Michx.) Nash (little bluestem), *Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths (blue grama), *Bouteloua dactyloides* (Nutt.) J. T. Columbus (buffalograss), and *Bouteloua curtipendula* (Michx.) Torr. (sideoats grama). Numerous forb species add to the diversity of the landscape, primarily dominated by natives, such as *Echinacea angustifolia* DC. (blacksamson echinacea), *Ratibida columnifera* (Nutt.) Wooton & Standl. (upright prairie coneflower), *Symphyotrichum ericoides* (L.) G.L. Nesom (white heath aster), *Verbena stricta* Vent. (hoary verbena), and some introduced species including *Cirsium arvense* (L.) Scop. (Canada thistle) and *Verbascum thapsus* L. (common mullein).

TREE SELECTION

Ten individual ERC were selected for sampling based on the following characteristics: (1) the tree is female, (2) average canopy diameter 5-10 m (mature and high reproductive rate), (3) at least five meters from other large (>3 m canopy diameter) ERC trees, and (4) environmental characteristics are similar (ie. slope, soil composition, aspect). For future reference, trees were tagged with aluminum tree tags at the base of their trunk and their approximate GPS location recorded.

EXPERIMENTAL DESIGN

This experiment consisted of a complete block design. Our blocking variable in this experiment was an individual ERC tree that consisted of four treatments and four samples per treatment. The four samples were per treatment were combined for analyses, totaling 4 combined samples per tree, 10 replicates per treatment, and 40 replicates overall per site (4 treatments x 10 trees).

TREATMENTS

Four treatments were compared in this study based on distance from an ERC stem. The treatments were control (grassland locations >5m from tree canopy, GL), under canopy (UC), canopy edge (CE), and two meters from the canopy edge (2M).

FIELD METHODS: SOIL BUD BANK CORE SAMPLING

Bud bank soil cores were collected along with seed bank cores during the last week of October in 2020 (Site 1) and 2021 (Site 2) following seed set of the current year's vegetation (Allen & Nowak 2008). One transect in each of the four cardinal directions extended from each ERC tree stem. Using a lever-action golf hole cutter (10 cm dia. x 10 cm depth), a soil core was collected on each transect line at the four treatment distances (UC, CE, 2M, and GL), totaling 4 cores per transect, 16 cores per tree, and 160 cores overall at each Site (Figures 4.1 & 4.2). UC cores were collected at half canopy radius and CE cores where the midline of the core was in line with the canopy edge. Soil cores were stored in plastic Ziploc bags in a cooler on ice during sampling and while driven back to Brookings, SD from the field sites near Chamberlain, SD and Platte, SD. Once arriving at the lab, soil cores were stored in a walk-in refrigerator (4ºC) awaiting laboratory procedures.

LAB METHODS: PREPARATION, IDENTIFICATION, AND BUD COUNTING

Bud bank soil cores were placed in a wire-mesh 2 mm sieve and washed with high pressure water to remove soil, unwanted material (ie. rocks and litter), and to expose plant belowground structures including crown and rhizome buds. The wire-mesh 2 mm sieve aided in catching and containing the plant propagules during the washing procedure. Roots were removed from individual plants and their propagules were separated into crowns and rhizomes by functional groups: Introduced Graminoid Crown (IGC), Introduced Graminoid Rhizome (IGR), Native Graminoid Crown (NGC), Native Graminoid Rhizome (NGR), Forb Crown (FC), Forb Rhizome (FR), Shrub (SHR), Unknown Graminoid Crown (UGC), and Unknown Graminoid Rhizome (UGR). Bud morphology between graminoid and forb buds are distinct, which aided in proper identification. To maintain bud health while awaiting the dissecting scope data collection procedures, crowns and rhizomes were moistened, rolled up in wet paper towels, and placed in the refrigerator at 4 ºC.

DATA COLLECTION

Eastern Redcedar Trees Sampled

We collected the following data on the ERC trees sampled in this experiment at each Site: canopy diameter (m), slope (%), aspect, GPS location, and notes on physical and microenvironment characteristics.

Dissecting Scope Bud Identification and Counting

A dissecting microscope with magnification ranging from 10-67.5x was used for bud identification and counting. Live buds were identified and counted on crowns and rhizomes to the functional group level. Crown, rhizome, and total (crown + rhizome) buds were recorded within each soil sample. With this data, we calculated live bud densities (buds/0.1 m^3) for each functional group.

DATA ANALYSIS

Site 1 and Site 2 were only sampled one year each (ie. spatial replication but no temporal replication), so analyses between years and between sites was not conducted.

Analyses were conducted among and between treatments within each site for a given year. Some UC soil cores contained zero live buds which caused issues in analysis, so cores were combined by treatment for each tree, resulting in 10 replicates per treatment and 40 samples overall per site. Since the samples were combined, analysis on aspect was not conducted. Statistical analyses were conducted in program R, where normality tests were conducted required for analysis of variance (ANOVA) on all dependent variables including: total bud density and bud composition by functional group (R Development Core Team 2015). Kruskal-Wallis (KW) tests were used when dependent variables failed to meet normality. If dependent variables met normality and ANOVA found significance ($P<0.05$) among treatments, Tukey's HSD was conducted to test for differences between treatments. For dependent variables that did not meet normality and KW found significance ($P<0.05$) among treatment medians, Dunn's post hoc test was used to test for differences between treatments.

Total bud production per soil core was determined using the following formula:

$$Bud\ Production = \sum_{i=1}^{8} C_i + R_i$$

where C_i is the total number of crown buds of all functional groups, R_i is the total number of rhizome buds of all functional groups, and i represents the different functional groups observed (introduced/native, graminoid/forb/shrub/unknown).

Analyses on bud production and bud composition were conducted in program R. Total bud production met normality tests for both Sites, so ANOVA and Tukey's HSD were used for analysis among and between treatment means. Bud composition by functional group did not meet normality for both Sites, so Kruskal-Wallis and Dunn's test were used for analysis among and between treatment medians.

Bud Bank Community Analysis

PC-Ord software was used for overall bud community analysis between the treatments UC, CE, 2M, and GL. Non-metric multidimensional scaling (NMS) ordination was performed using PC-Ord version 7.09 on bud production data by functional group with tree as the sample unit by combining the four cores within each treatment per tree (live buds/3142cm^3) within 2020 at Site 1 and within 2021 at Site 2 (McCune and Mefford 2018). To compare all treatments, we created a main matrix composed of 40 samples with 8 total functional groups for Site 1 and 40 samples with 9 total functional groups for Site 2. NMS was run using the Euclidean distance measure for both Sites with 2 axes, a maximum of 500 iterations, 249 runs with randomized data, and 200 runs with real data. The second matrix was used for treatment comparison, where we used perMANOVA on all NMS ordinations. PerMANOVA was used to test for differences among and between treatments.

RESULTS

Weather

Site 1 (2020) and Site 2 (2021) experienced very different weather regimes during their respective growing seasons. Average temperature was higher than the 30-year (1990-2019) average for both Sites, especially during the growing season with an average increase of 0.40˚C and 0.57˚C at Site 1 and Site 2, respectively (Table A.1). Total precipitation was lower than the 30-year average at both Sites consisting of 445 mm (25.2% reduction) and 400 mm (36.6% reduction) at Site 1 and Site 2, respectively

(Table A.2). However, Site 1 had 15% more precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 1, monthly precipitation was lower in May and higher during the months of June, July, and August compared to the 30-year average (Table A.2, Figure A.2). Site 2 had 36.5% less precipitation during the growing season than the 30-year average (HPRCC 2022, Mesonet 2022). At Site 2, monthly precipitation was lower in May, June, and August and higher in July than the 30-year average (Table A.2, Figure A.2).

Trees Selected

The ERC trees selected for sampling were found on NW, N, and NE facing hillsides with slopes ranging from 16-31% and 13-22% at Site 1 and Site 2, respectively. The average hillside slope was higher at Site 1 (23.5%) compared to Site 2 (17.1%) (Table 3.1). The canopy diameters ranged from 5.25-8.35 m and 5.80-8.00 m with an average canopy diameter of 6.0 m and 6.8 m at Site 1 and Site 2, respectively (Table 3.1).

Bud Production

A total of 64,015 live buds were counted among both sites, consisting of 25,906 and 38,109 total buds at Site 1 and Site 2, respectively. The 25,906 buds counted within samples collected from Site 1 consisted of 745, 6,848, 9,149, and 9,164 from treatments UC, CE, 2M, and GL. The 38,109 buds counted within samples collected from Site 2 consisted of 969, 11,644, 11,635, and 13,861 from treatments UC, CE, 2M, and GL (Table 4.2). Treatment had a significant effect ($P<0.05$) on bud production at both Sites (Figure 4.3). Bud production in treatment UC was significantly different from treatments CE, 2M, and GL. Soil cores collected in treatment UC produced between 70-99% fewer buds than treatments CE, 2M, and GL. Bud production in treatments CE, 2M, and GL were not statistically different at both Sites.

Bud Composition

Treatment had a significant effect (*$P<0.05$*) on bud composition of select functional groups at both Sites (Tables 4.3, 4.4). Bud composition significantly differed (*$P<0.01$*) in functional groups native graminoid crown (NGC), native graminoid rhizome (NGR), and total native at both Sites. NGC composition was highest in treatment 2M at 19.4% and 36.4% and lowest in treatment UC at 5% and 8.5% at Site 1 and Site 2, respectively. NGC composition significantly differed between treatment UC and 2M at Site 1, whereas treatments CE and GL were not different in composition from treatments UC and 2M. At Site 2, NGC composition differed between UC and treatments 2M and GL, whereas treatment CE did not differ from UC, 2M, and GL composition. At Site 1, NGR composition was highest in treatment 2M at 13.9% and lowest in treatment UC at 2.8%. At Site 2, NGR composition was highest in treatment GL at 17.1% and lowest in treatment UC at 4.9%. NGR composition significantly differed between treatment UC and treatments 2M and GL at both Sites. Treatment CE was did not differ in NGR composition in comparison to treatments UC, 2M, and GL at both Sites. Total native composition at both sites was highest in treatment 2M at 33.3% and 53.2% and lowest in treatment UC at 7.8% and 13.4% for Site 1 and Site 2, respectively. Treatment UC significantly decreased in total native composition from treatments 2M and GL, whereas treatment CE did not differ from treatments UC, 2M, and GL. At Site 1, treatment also had a significant effect on introduced graminoid crown (IGC) and total introduced composition from KW tests among treatment medians, but no differences were found between treatments using Dunn's test following p-value adjustment. At Site 2, shrub and total graminoid composition significantly differed among treatments. However, after p-value adjustment and Dunn's test, no significance was found between treatments for shrub composition. Total graminoid bud composition was highest in treatment 2M at 97.5% and lowest in treatment UC at 75.8%. Treatment UC significantly differed from treatments CE, 2M, and GL, whereas treatments CE, 2M, and GL were similar in total graminoid composition.

Community Analysis Between Treatments

A 2-dimensional NMS solution using a Euclidean distance measure was used to interpret bud bank communities in each treatment at Site 1 and Site 2 (Figures 4.4, 4.5). At Site 1, the minimum stress was 6.13 with axis one explaining 91.7% and axis two explaining 6.5% of the variation with a cumulative of 98.2% among both axes. Axis one was driven by IGC (r=-0.971) and IGR (r=-0.942), which both had a negative correlation. Axis two was negatively driven by NGR (r=-0.524) and NGC (r=-0.360), with the highest positive correlation occurring with shrub (r=0.178) (Table 4.4). At Site 2, the minimum stress was 2.26 with axis one explaining 77.3% and axis two explaining 22.4% of the variation with a cumulative of 99.7% among both axes. Axis one was driven negatively by IGR (r=-0.924) and positively driven by shrub (r=0.303). Axis two was positively driven by NGC (r=0.741) along with IGC (r=-0.380) having the lowest correlation. PerMANOVA comparisons found a significant difference (*P<0.01*) among treatments and between UC and treatments CE, 2M, and GL at both Sites (Table 4.6). All other comparisons between treatments were not significant at both Sites (Table 4.6).

DISCUSSION

Our results show grasslands in the NGP are experiencing afforestation through ERC encroachment resulting in an altered belowground plant community underneath ERC canopies compared to open grassland plots. The lack of prescribed fire, overgrazing, and planting ERC in shelterbelts has contributed to its prolific expansion onto our rangelands and raises concern of future rangeland sustainability and resiliency (Limb et al. 2010). Our study evaluated the impact of large (5-10 m canopy diameter) individual ERC trees on bud bank production and composition in the NGP mixed-grass prairie of South Dakota. Previous studies that evaluated the bud bank in grassland or savanna ecosystems have focused on the effects of grazing (Dalgleish and Hartnett 2009), prescribed fire (Benson et al. 2004, Russel et al. 2015), species invasion (Bam et al. 2022, Collier et al. 2002), or climate change (Chelli et al. 2019, Dalgleish and Hartnett 2009). To our knowledge, only two studies assessed the bud bank on woody encroached landscapes including an evaluation on bud production below *Pinus elliottii* (slash pine) canopies in Brazilian savannas (Ferraro et al. 2020) and the bud bank response following prescribed fire through *Populus tremuloides* (aspen) stands (Lee 2004). As a result, our study in the NGP is the first of its kind evaluating the impact of woody canopy cover on bud bank production and composition, especially with ERC as the focal species. The results of this study will aid in future management on ERC encroached grasslands and provide land managers with critical information useful for understanding the potential regeneration of aboveground vegetation following the control of ERC via mechanical removal or prescribed fire.

ERC Canopies Reduce Bud Bank Production

ERC canopies significantly reduced bud production, resulting in over 70% less buds produced compared to other treatments outside ERC canopies. This reduced bud production directly underneath ERC canopies supports our first hypothesis in this study. Interestingly, we did not see any differences in bud production at the edge of ERC canopies compared to open grassland plots, although shading and reduced soil moisture was observed by other researchers at the edge of *Juniperus* canopies (Owens et al. 2006). Overall bud production was higher at Site 2, which was surprising due to the drought like conditions during the 2021 growing season (Mesonet 2022). However, consecutive years of grazing during the spring growing season coupled with reduced precipitation may have increased bud densities. Grazing has shown to increase belowground bud production (Benson et al. 2004, Ott et al. 2019) and bud densities can persist especially during drought years in grazing systems (Vanderweide and Hartnett 2015). The consecutive years of grazing at Site 2 may have stimulated bud production and created an environment more dependent on vegetative tillering due to a decrease in litter buildup and foliar plant cover (Benson et al. 2004). In contrast, Site 1 has not experienced any disturbance (ie. grazing or fire) in at least 10 years, which may have contributed to a depleted bud bank despite experiencing higher precipitation levels compared to Site 2 which in turn should increase bud production (Dalgleish and Hartnett 2006).

ERC Canopies Alter Bud Bank Composition

ERC canopies have shown to alter aboveground plant communities through limited light, litter accumulation, and reduced soil moisture (Engle et al. 1987, McKinley et al. 2008, Starks et al. 2014). As a result, we hypothesized the altered aboveground plant community in turn would modify belowground bud bank composition by reducing the percentage of native graminoid buds. Supporting our second hypothesis, ERC canopies reduced bud composition of native graminoids at both of our sampling sites. We found a three-fold decrease in native bud composition underneath ERC canopies compared to our grassland control locations. This decrease in native bud composition was not unusual since aboveground and belowground plant communities tend to be very similar in perennial dominated grasslands (Carter et al. 2012) and previous studies on aboveground plant composition underneath ERC canopies found a shift from native C4 grasses to introduced C3 grasses (Briggs et al. 2002, Gehring and Bragg 1992), which was also supported by findings in our complimentary study (Chapter 2). In addition, we should note overall native bud composition among treatments was higher at Site 2 compared to Site 1. This difference was likely due to previous grazing management conducted during when cool-season grasses are dominant and the bud bank tillering of native grasses often outcompeting non-natives in grazed systems in the NGP mixed-grass prairie (Bam et al. 2022).

Understanding the belowground bud bank communities underneath ERC canopies will help land managers predict how the aboveground vegetation responds following the removal of ERC on the NGP mixed-grass prairie. Vegetative bud banks have shown to play a key role in regeneration following disturbance (Benson and Hartnett 2006, Latzel et al. 2008), especially after low intensity fires (Lee 2004). Following the removal of ERC, we initially expect to see minimal regeneration dependent on the bud bank in areas previously shaded by ERC canopies. Bud production was insignificant underneath ERC canopies, so we expect to see initial colonizers emerging from the soil seed bank. Although we may see some immigration of nearby rhizomes or stolons from plants outside the previous ERC canopy pursuing new territory and nutrients (Alpert and Mooney 1986, Hutchings and Wijesinghe 1997, Liu et al. 2016), most initial colonizers will likely not establish from the vegetative bud bank. Therefore, to restore native grassland plant communities in these patches left from ERC canopies we may need to incorporate more strenuous restoration strategies, such as broadcast seeding or transplanting.

Prescribed fire historically controlled ERC in the Great Plains and limited its establishment to rocky slopes and riparian areas (Lawson 1990). Its prolific expansion has shown to alter aboveground plant communities and result in degraded grassland systems (Bidwell et al. 1996, Limb et al. 2010), resulting in reduced forage biomass (Limb et al. 2010), a shift from C4 to C3 grasses (Gehring and Bragg 1992), reduced soil moisture underneath ERC canopies (Adane and Gates 2015), and increased expenses for cattle operations (Twidwell et al. 2021). These issues we face on our grasslands today from ERC encroachment will only get worse if proper land management is not implemented. To combat these negative impacts of ERC encroachment, proper management should target ERC removal through prescribed fire, mechanical removal techniques, or a combination of both (Buehring et al. 1971). Due to the lack of aboveground vegetation and bud bank production directly underneath ERC canopies, post-removal restoration will likely require additional strategies (ie. direct seeding) from land managers to establish native perennial vegetation although some researchers suggest natural return to pre-encroachment vegetation is possible in only a couple of growing seasons (Alford et al. 2012, Limb et al. 2010, Pierce and Reich 2010). Once the targeted perennial vegetation reestablishes in these previously voided areas of ERC canopies, prescribed fire applications every 5-10 years should eliminate potential ERC encroachment and maintain a healthy grassland ecosystem. Since prescribed fire is a new concept for some landowners, the need for partnerships and volunteer prescribed burn associations is greater now more than ever and will be fundamental in halting the spread of woody vegetation onto our grassland ecosystems (Garmestani et al. 2021, Toledo et al. 2014).

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TABLES

Table 4.1 Characteristic summary of selected ERC trees for bud bank sampling at Site 1 (2020) and Site 2 (2021).

	Site 1 (2020)		Site 2 (2021)	
ERC Tree	Canopy Diameter (m)	Slope (%)	Canopy Diameter (m)	Slope (%)
1	5.25	16	6.60	18
2	5.65	24	7.65	19
3	6.55	28	6.00	16
4	6.00	28	8.00	17
5	7.10	31	6.00	22
6	8.35	17	6.90	14
7	5.25	25	6.20	13
8	5.60	23	5.80	23
9	5.35	24	6.30	15
10	5.30	19	8.00	14
Mean	***6.04***	***23.5***	***6.75***	***17.1***

Table 4.2 Total bud production per ERC by treatment for Site 1 in 2020 and Site 2 in 2021 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

	Site 1 (2020)					Site 2 (2021)				
Tree	**UC**	**CE**	**2M**	**GL**	**Total[1]**	**UC**	**CE**	**2M**	**GL**	**Total[1]**
1	194	628	1120	1024	**2966**	215	1354	1260	1581	**4410**
2	76	558	768	686	**2088**	46	1338	1273	2100	**4757**
3	24	853	1342	918	**3137**	166	1807	1177	949	**4099**
4	16	558	474	619	**1667**	169	1688	1616	1956	**5429**
5	13	246	543	545	**1347**	115	1421	1560	1535	**4631**
6	58	790	778	1268	**2894**	90	652	870	1303	**2915**
7	61	483	942	657	**2143**	5	901	1222	1162	**3290**
8	58	1106	1030	1415	**3609**	35	705	774	1323	**2837**
9	145	1007	1108	833	**3093**	111	948	741	946	**2746**
10	100	619	1044	1199	**2962**	17	830	1142	1006	**2995**
Total[2]	**745**	**6848**	**9149**	**9164**	**25906[3]**	**969**	**11644**	**11635**	**13861**	**38109[3]**

[1]Total bud production by tree (# live buds/0.013m^3)
[2]Total bud production by treatment (# live buds/0.031m^3)
[3]Total bud production by Site among all trees and treatments (# live buds/0.126m^3)

Table 4.3 Bud bank composition (%) by functional group at Site 1 in 2020 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Values indicate mean composition with standard error and letters indicate significance between treatments within the year found using Dunn's post hoc test. P-values were derived from Kruskal-Wallis tests on treatment medians.

Mean Bud Bank Composition by Functional Group at Site 1 (2020)												
Treatment	IGR	IGC	NGR	NGC	Unk. Graminoid	Graminoid	Forb	Shrub	Crown	Rhizome	Native	Introduced
UC	42.1 ± 9.16	30.2 ± 5.53	2.8 ± 2.02$_{a}$	5.0 ± 2.92$_{a}$	0	80.1 ± 4.19	15.8 ± 4.01	4.1 ± 1.96	50.1 ± 7.62	49.9 ± 7.62	7.8 ± 4.45$_{a}$	72.3 ± 6.55
CE	25.8 ± 1.71	40.4 ± 1.92	7.8 ± 0.73$_{ab}$	14.5 ± 0.57$_{ab}$	0	88.5 ± 2.04	10.4 ± 1.90	1.1 ± 0.58	63.3 ± 2.19	36.7 ± 2.19	22.3 ± 0.52$_{ab}$	66.2 ± 2.20
2M	25.1 ± 2.35	31.3 ± 1.63	13.9 ± 2.46$_{b}$	19.4 ± 1.86$_{b}$	0.33 ± 0.23	89.8 ± 1.85	9.4 ± 1.73	0.5 ± 0.23	56.9 ± 2.23	43.1 ± 2.23	33.3 ± 3.15$_{b}$	56.5 ± 3.39
GL	29.0 ± 2.22	31.7 ± 1.76	12.8 ± 1.42$_{b}$	15.7 ± 1.13$_{ab}$	0.33 ± 0.33	89.3 ± 1.73	9.8 ± 1.91	0.6 ± 0.28	53.9 ± 2.06	46.1 ± 2.06	28.6 ± 2.20$_{b}$	60.7 ± 2.85
P-value	***0.48***	***0.05***	***<0.01***	***<0.01***	***0.29***	***0.22***	***0.58***	***0.84***	***0.12***	***0.12***	***<0.01***	***0.04***
Notes: Origin; I = introduced, N = native, Life Form; G = graminoid, Propagule; C = crown, R = rhizome, Other; Unk = unknown.												

Table 4.4 Bud bank composition (%) by functional group at Site 2 in 2021 for treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). Values indicate mean composition with standard error and letters indicate significance between treatments within the year found using Dunn's post hoc test. P-values were derived from Kruskal-Wallis tests on treatment medians.

Mean Bud Bank Composition by Functional Group at Site 2 (2021)												
Treatment	IGR	IGC	NGR	NGC	Unk. Graminoid	Graminoid	Forb	Shrub	Crown	Rhizome	Native	Introduced
UC	31.3 ± 6.39	29.2 ± 9.05	4.9 ± 2.86$_{a}$	8.5 ± 4.72$_{a}$	2.0 ± 2.00	75.8 ± 9.48$_{a}$	21.2 ± 9.45	3.0 ± 1.42	56.4 ± 7.78	43.6 ± 7.78	13.4 ± 7.49$_{a}$	60.4 ± 10.27
CE	32.3 ± 3.66	26.5 ± 3.00	11.9 ± 1.31$_{ab}$	25.3 ± 5.67$_{ab}$	0.1 ± 0.10	96.1 ± 0.90$_{b}$	3.7 ± 0.92	0.1 ± 0.07	54.1 ± 2.94	45.9 ± 2.94	37.2 ± 6.62$_{ab}$	58.9 ± 6.57
2M	22.6 ± 2.71	21.7 ± 2.63	16.8 ± 1.23$_{b}$	36.4 ± 4.60$_{b}$	0.1 ± 0.07	97.5 ± 0.63$_{b}$	2.5 ± 0.63	0	59.7 ± 2.71	40.3 ± 2.71	53.2 ± 5.03$_{b}$	44.2 ± 5.14
GL	24.4 ± 2.03	21.7 ± 2.15	17.1 ± 1.13$_{b}$	32.7 ± 3.54$_{b}$	0.5 ± 0.27	96.3 ± 1.14$_{b}$	3.7 ± 1.14	0.01 ± 0.01	56.7 ± 2.43	43.3 ± 2.43	49.8 ± 3.78$_{b}$	46.0 ± 3.53
P-value	***0.06***	***0.41***	***<0.01***	***<0.01***	***0.34***	***<0.01***	***0.06***	***0.05***	***0.21***	***0.21***	***<0.01***	***0.07***
Notes: Origin; I = introduced, N = native, Life Form; G = graminoid, Propagule; C = crown, R = rhizome, Other; Unk = unknown.												

Table 4.5 Correlations between the main matrix NMS axes and bud bank functional groups for Site 1 in 2020 among treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

Functional Group Correlations with NMS Axes (Site 1)			
	Axis 1		
Functional Group	r	r-squared	tau
SHR	-0.172	0.029	-0.095
UGC	-0.334	0.112	-0.309
FR	-0.405	0.164	-0.483
FC	-0.712	0.507	-0.537
NGR	-0.761	0.580	-0.723
NGC	-0.865	0.748	-0.758
IGR	-0.942	0.887	-0.767
IGC	-0.971	0.943	-0.851
	Axis 2		
SHR	0.178	0.032	0.114
IGC	0.158	0.025	0.165
IGR	0.142	0.020	0.184
UGC	0.018	0.000	0.067
FR	-0.052	0.003	-0.010
FC	-0.223	0.050	0.005
NGC	-0.360	0.130	-0.108
NGR	-0.524	0.275	-0.149

Notes: Origin; I = introduced, N = native, Life Form; F = forb, G = graminoid, SHR = shrub, Propagule; C = crown, R = rhizome, Other; U = unknown.

Table 4.6 Correlations between the main matrix NMS axes and bud bank functional groups for Site 2 in 2021 among treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

Functional Group Correlations with NMS Axes (Site 2)			
	Axis 1		
Functional Group	r	r-squared	tau
SHR	0.303	0.092	0.243
FC	-0.086	0.007	-0.079
UGC	-0.229	0.053	-0.191
FR	-0.369	0.136	-0.312
UGR	-0.401	0.161	-0.335
NGC	-0.663	0.439	-0.484
IGC	-0.902	0.814	-0.742
NGR	-0.904	0.818	-0.727
IGR	-0.924	0.854	-0.772
	Axis 2		
NGC	0.741	0.549	0.420
NGR	0.262	0.069	0.160
FC	0.189	0.036	0.198

UGC	-0.044	0.002	-0.094
UGR	-0.055	0.003	-0.114
SHR	-0.079	0.006	-0.097
FR	-0.140	0.020	-0.183
IGR	-0.362	0.131	-0.289
IGC	-0.380	0.145	-0.311

Notes: Origin; I = introduced, N = native, Life Form; F = forb, G = graminoid, SHR = shrub, Propagule; C = crown, R = rhizome, Other; U = unknown.

Table 4.7 P-values found from perMANOVA comparisons for bud production and composition for Site 1 in 2020 and Site 2 in 2021 where under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

	Treatment Comparisons						
	Among	UC v. CE	UC v. 2M	UC v. GL	CE v. 2M	CE v. GL	2M v. GL
Site 1	<0.01	<0.01	<0.01	<0.01	0.06	0.10	0.69
Site 2	<0.01	<0.01	<0.01	<0.01	0.12	0.13	0.52

FIGURES

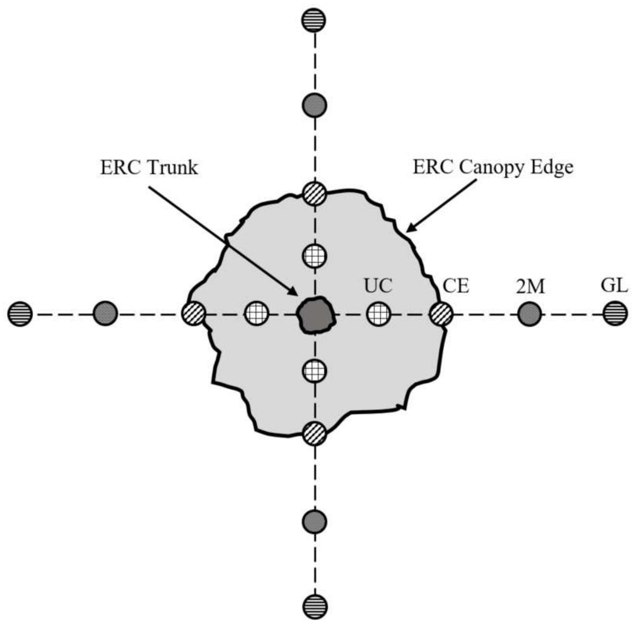

Figure 4.1 Aerial illustration of soil bud bank core sampling design with four transects extending from an ERC trunk where treatments contain under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

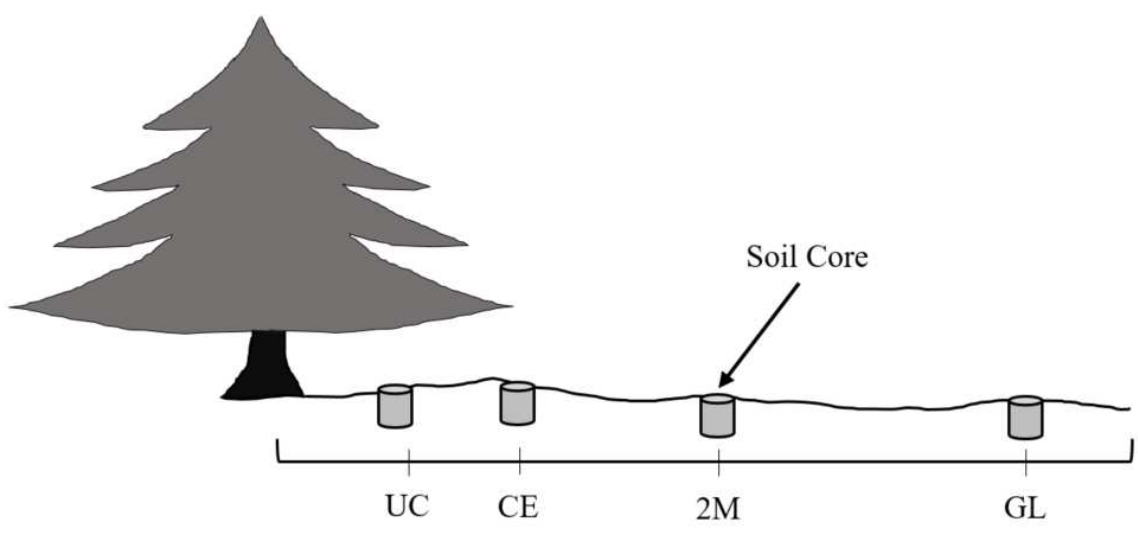

Figure 4.2 Lateral illustration of soil bud bank core sampling design of one transect out of four total per ERC tree, with treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

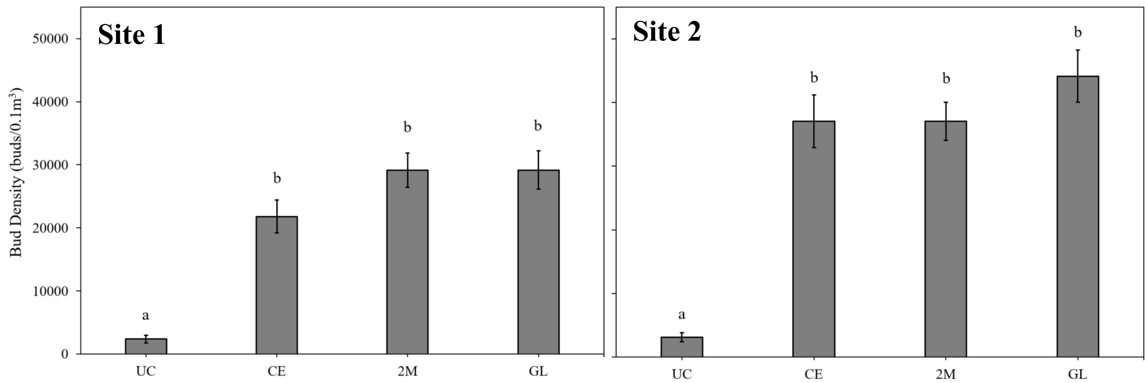

Figure 4.3 Average bud production (live buds/0.1 m^3) by treatment for Site 1 in 2020 and Site 2 in 2021. Mean, standard error, and letters of significance found from Tukey's HSD are represented for each treatment where under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL).

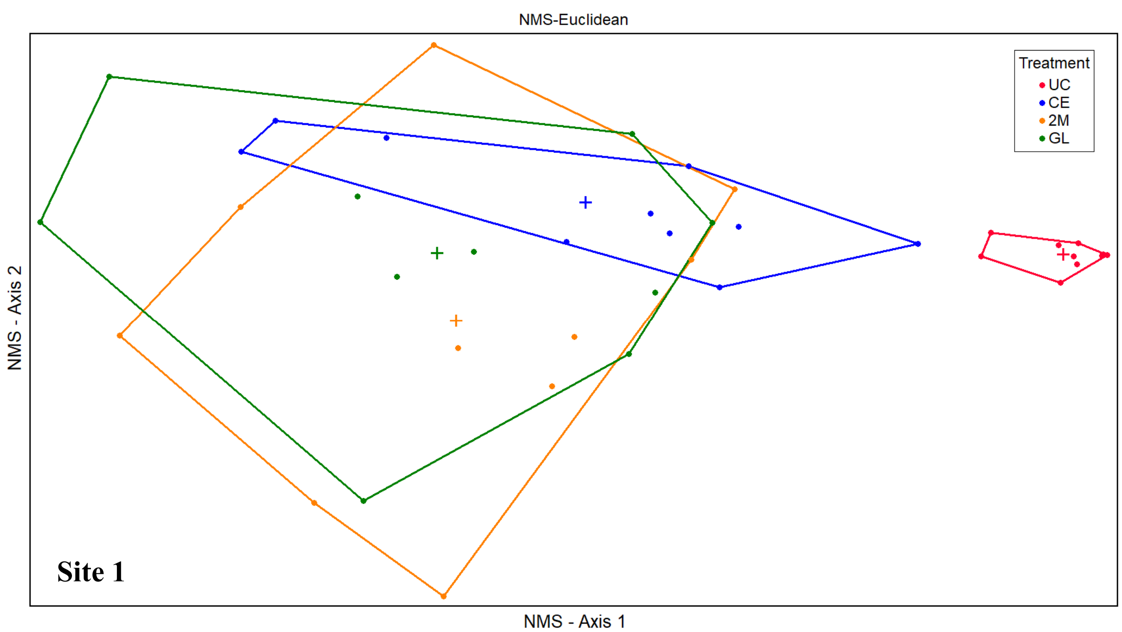

Figure 4.4 NMS ordination plots of belowground production based on crown and rhizome buds by functional group for Site 1 in 2020 with convex hulls and centroids among treatments, where under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). The centroid (cross symbol) represents the multivariate average for each treatment.

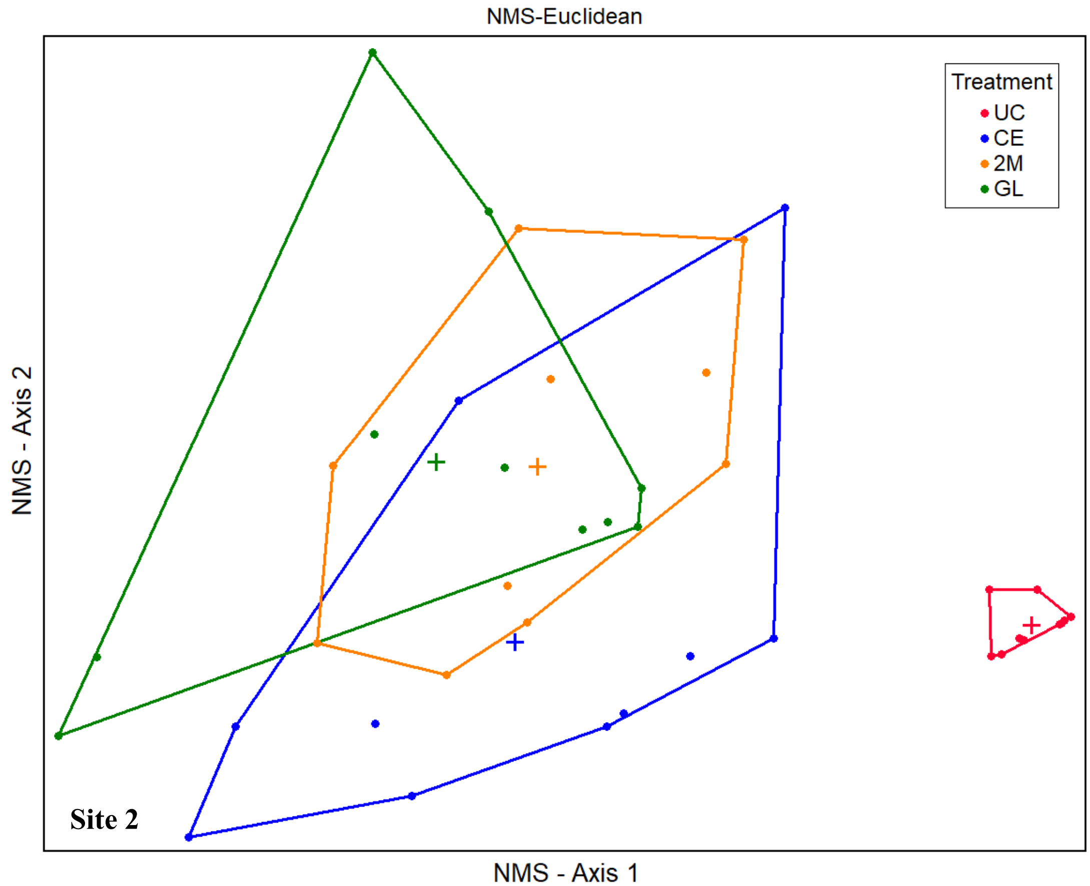

Figure 4.5 NMS ordination plots of belowground production based on crown and rhizome buds by functional group for Site 2 in 2021 with convex hulls and centroids among treatments, where under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL). The centroid (cross symbol) represents the multivariate average for each treatment.

APPENDIX A

Table A.1 Average monthly temperature (˚C) and 30-year (1990-2019) average monthly temperature (˚C) for Site 1 in 2020 and Site 2 in 2021. Deviations from the 30-year average are displayed as well as total 12-month average and a growing season (May-August) average (HPRCC 2022, Mesonet 2022).

	Average Temperature (˚C)													
	January	February	March	April	May	June	July	August	September	October	November	December	Total Average	Average (May-August)
2020														
Actual	-7.2	-4.4	2.8	6.7	13.3	22.2	23.3	22.2	16.1	6.1	3.3	-2.8	8.5	20.3
30-year	-7.5	-5.2	0.9	7.3	14.0	20.0	23.4	22.2	17.3	9.2	1.2	-5.2	8.1	19.9
Deviation	**0.3**	**0.8**	**1.9**	**-0.6**	**-0.7**	**2.2**	**-0.1**	**0.0**	**-1.2**	**-3.1**	**2.1**	**2.4**	**0.4**	**0.4**
2021														
Actual	-3.3	-10.0	3.9	7.2	14.4	23.3	23.3	22.8	18.3	10.6	3.3	-4.4	9.1	21.0
30-year	-6.4	-4.2	1.8	8.1	14.6	20.6	23.8	22.6	17.9	9.8	2.1	-4.2	8.9	20.4
Deviation	**3.1**	**-5.8**	**2.1**	**-0.9**	**-0.2**	**2.7**	**-0.5**	**0.2**	**0.4**	**0.8**	**1.2**	**-0.2**	**0.2**	**0.6**

Table A.2 Monthly precipitation (mm) and 30-year (1990-2019) average monthly precipitation (mm) for Site 1 in 2020 and Site 2 in 2021. Deviations from the 30-year average are displayed as well as total annual and growing season (May-August) precipitation (HPRCC 2022, Mesonet 2022).

	Precipitation (mm)													
	January	February	March	April	May	June	July	August	September	October	November	December	Total	Total (May-August)
020														
Actual	0.51	0.25	19.05	5.08	56.39	135.64	89.92	100.84	10.67	12.19	12.19	2.03	444.75	382.78
30-year	12.45	15.49	26.92	66.29	88.65	101.60	70.36	72.14	60.45	46.74	19.56	13.97	594.61	332.74
Deviation	**-11.94**	**-15.24**	**-7.87**	**-61.21**	**-32.26**	**34.04**	**19.56**	**28.70**	**-49.78**	**-34.54**	**-7.37**	**-11.94**	**-149.86**	**50.04**
2021														

Actual	4.32	1.02	27.94	36.07	45.72	14.73	88.65	65.53	45.47	66.55	2.79	1.02	399.80	214.63
30-year	12.70	17.78	30.99	74.93	93.98	94.74	73.41	75.69	65.53	51.82	22.61	16.76	630.94	337.82
Deviation	**-8.38**	**-16.76**	**-3.05**	**-38.86**	**-48.26**	**-80.01**	**15.24**	**-10.16**	**-20.07**	**14.73**	**-19.81**	**-15.75**	**-231.14**	**-123.19**

Table A.3 Summary of cover and frequency of plant species present at grassland control locations and underneath eastern redcedar canopies ranging from two to greater than seven meters in diameter at Site 1 in 2020 near Academy, South Dakota.

Species		ERC Canopy Diameter Classes (m)													
Common Name	Scientific Name	Grassland Control (n=120)		C (2-3) (n=103)		D (3-4) (n=77)		E (4-5) (n=76)		F (5-6) (n=64)		G (6-7) (n=36)		H (7+) (n=48)	
		Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)
Smooth brome	*Bromus inermis* Leyss.	84	25.15	73	4.90	78	3.59	84	2.18	73	2.33	56	0.99	67	1.10
Annual ragweed	*Ambrosia artemisifolia* L.	57	4.19	36	1.00	23	0.83	24	0.93	20	0.92	44	0.71	13	0.72
Kentucky bluegrass	*Poa pratensis* L.	49	3.35	40	2.34	36	0.88	30	1.35	34	1.06	28	0.38	15	0.56
Green needlegrass	*Nassella viridula* (Trin.) Barkworth	48	8.63	52	2.34	47	2.98	50	2.33	44	1.53	42	2.34	33	2.06
Scribner's rosette grass	*Dichanthelium oligosanthes* (Schult.) Gould var. *scribnerianum* (Nash) Gould	33	2.29	32	1.13	29	0.95	24	0.88	28	1.34	17	0.62	15	0.56
Big bluestem	*Andropogon gerardii* Vitman	28	16.71	28	4.14	12	3.68	11	1.39	8	2.02	19	0.96	10	2.06
Sweetclover	*Melilotus officinalis* (L.) Lam.	23	2.24	9	0.49	5	0.53	5	0.10	14	0.11	11	0.35	6	0.10
Aster	*Symphyotrichum spp.*	20	2.54	7	0.76	4	1.50	1	0.10			6	0.55	4	1.00
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	18	6.02	17	2.53	16	2.01	5	2.15						
Western poison ivy	*Toxicodendron rydbergii* (Small ex Rydb.) Greene	15	5.58	17	7.15	18	7.25	14	2.27	20	3.69	11	1.63	15	1.79
Flodman's thistle	*Cirsium flodmanii* (Rydb.) Arthur	14	3.00	17	2.17	14	1.92	4	1.83	5	3.00	6	1.00	2	1.00
Wild bergamot	*Monarda fistulosa* L.	14	5.94	10	1.69	10	1.39	1	1.00	5	7.17			2	0.10
Western snowberry	*Symphoricarpos occidentalis* Hook.	12	3.86	6	3.17	8	1.67	4	1.33	8	2.60	6	2.50	15	1.50
Composite dropseed	*Sporobolus compositus (*Poir.) Merr.	9	1.65	3	0.83										
Western wheat	*Agropyron smithii* (Rydb.) Á. Löve	9	4.36	14	1.19	8	0.27	1	0.10			8	1.40		
Prairie rose	*Rosa arkansana* Porter	8	3.11	4	3.50	9	3.43	5	2.50			3	7.00	2	1.00

Leafy spurge	*Euphorbia esula* L.	6	3.14							3	1.10	11	1.63	4	0.55
Anemone	*Anemone spp.*	6	2.70	5	1.60	4	0.83	3	2.00	2	2.00			2	0.50
Prickly lettuce	*Lactuca serriola* L.	5	3.67	2	3.50	1	3.00	1	0.10	3	0.30	3	0.10	2	0.30
Needleleaf sedge	*Carex duriuscula* C. A. Mey.	4	1.64	10	0.48	17	0.85	14	0.73	27	0.76	19	0.56	27	0.38
White sagebrush	*Artemisia ludoviciana* Nutt.	4	1.20	6	1.00	1	1.00	3	0.50						
Prairie sagewort	*Artemisia frigida* Willd.	3	1.25									3	2.00		
Lead plant	*Amorpha canescens* Pursh	3	3.38					3	2.75	3	3.00	3	0.50		
Canada thistle	*Cirsium arvense* (L.) Scop.	3	2.67	1	5.00										
Stiff goldenrod	*Oligoneuron rigidum* (L.) Small var. *humile* (Porter) G.L. Nesom	3	1.33	1	2.00									2	1.00
Common dandelion	*Taraxacum officinale* F.H. Wigg.	2	2.00	1	2.00	4	0.43	5	0.88	3	0.75			10	0.82
Canada bluegrass	*Poa compressa* L.	2	22.50	1	2.00			3	6.00					2	2.00
Blacksamson echinacea	*Echinacea angustifolia* DC.	2	1.00	1	0.50	3	2.50	1	1.00			3	1.00		
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	2	22.50					3	1.50						
Prairie junegrass	*Koeleria macrantha* (Ledeb.) Schult.	2	0.55												
Prairie sandreed	*Calamovilfa longifolia* (Hook.) Scribn.	2	3.50												
Eastern redcedar	*Juniperus virginiana* L.	2	5.00	9	0.90	16	0.59	34	0.68	34	0.77	44	0.88	46	1.45
Yellow salsify	*Tragopogon dubius* Scop.	1	1.00												
Alfalfa	*Medicago sativa* L.	1	1.00	1	5.00	1	2.00					3	2.00		
Field bindweed	*Convolvulus arvensis* L.	1	2.00												
Cheatgrass	*Bromus tectorum* L.	1	1.00												
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.	1	0.10							2	1.00				
Canada goldenrod	*Solidago altissmia* L.	1	2.00							3	2.50				
Cinquefoil	*Potentilla spp.*	1	1.00	4	1.25					2	2.00	3	1.00		
Common oxeye	*Heliopsis helianthoides* (L.) Sweet	1	8.00	1	3.00										
False boneset	*Brickellia eupatorioides* (L.) Shinners	1	1.00												
Prairie groundcherry	*Physalis hispida* (Waterf.) Cronquist	1	5.00							2	0.10			2	0.50
Missouri goldenrod	*Solidago missouriensis* Nutt.	1	0.50	1	1.00	1	1.00								
Small-leaf pussytoes	*Antennaria parvifolia* Nutt.	1	1.00	7	5.64	6	1.70	7	3.00	5	3.00	6	4.50	2	2.00

Rush skeletonplant	*Lygodesmia juncea* (Pursh) D. Don ex Hook.	1	1.00												
Hoary verbena	*Verbena stricta* Vent.	1	3.00			1	0.50			2	1.00	3	0.10		
Fall rosette grass	*Dichanthelium wilcoxianum* (Vasey) Freckmann	1	2.00	1	2.00										
Common mullein	*Verbascum thapsus* L.							3	0.50	2	0.50				
Nodding plumeless thistle	*Carduus nutans* L.					1	1.00	3	6.00	3	3.50				
Catnip	*Nepeta cataria* L.			1	0.10	1	0.10			2	0.10	3	1.00	2	4.00
White clover	*Trifolium repens* L.													2	1.00
Soft-hair marbleseed	*Onosmodium bejariense* DC. ex A. DC.					1	0.50			3	0.30				
Common milkweed	*Asclepias syriaca* L.											3	1.00		
Upright prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.			1	1.00										
Silverleaf Indian breadroot	*Pediomelum argophyllum* (Pursh) J. Grimes			1	0.20										
Prairie violet	*Viola pedatifida* G. Don									2	0.20	8	1.00	2	1.00
Virginia strawberry	*Fragaria virginiana*											3	1.00		
Common yellow oxalis	*Oxalis stricta* L.			1	0.10							3	0.10		
Marsh muhly	*Muhlenbergia racemosa* (Michx.) Britton, Sterns & Poggenb.					1	4.00								
Cactus	*Opuntia spp.*			1	2.00										
Gooseberry	*Ribes spp.*									2	0.10			2	2.00
Unknown forb	---					1	0.20	7	0.48	9	0.75	6	0.50	21	1.46

Table A.4 Summary of cover and frequency of plant species present at grassland control locations and underneath eastern redcedar canopies ranging from two to greater than seven meters in diameter at Site 2 in 2021 near Platte, South Dakota.

Species		ERC Canopy Diameter Classes (m)													
		Grassland Control (n=120)		C (n=99)		D (n=78)		E (n=91)		F (n=47)		G (n=27)		H (n=14)	
Common Name	Scientific Name	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)	Freq. (%)	Cover (%)
Sideoats grama	*Bouteloua curtipendula* (Michx.) Torr.	74	3.95	33	1.60	27	1.29	16	1.23	4	0.88	4	0.75	7	2.00
Big bluestem	*Andropogon gerardii* Vitman	60	9.97	64	4.83	49	5.34	63	3.29	38	1.31	22	1.73	14	1.35
Little bluestem	*Schizachyrium scoparium* (Michx.) Nash	56	11.36	43	5.48	27	2.10	13	1.53	26	2.13			21	2.17
Kentucky bluegrass	*Poa pratensis* L.	49	6.12	72	6.08	90	3.36	78	2.46	89	2.22	85	1.06	79	1.50
Needle and thread	*Hesperostipa comata* (Trin. & Rupr.) Barkworth	47	2.87	28	2.04	47	2.56	35	1.14	19	0.79	19	0.85	14	0.10
Blue grama	*Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths	28	3.42	14	2.39	10	1.16	7	0.75	2	0.40	4	0.20		
Hoary verbena	*Verbena stricta* Vent.	20	0.41	2	0.10										
Buffalograss	*Bouteloua dactyloides* (Nutt.) J. T. Columbus	19	2.13	9	1.35	6	0.40	7	0.23	11	0.42	15	0.25		
Blacksamson echinacea	*Echinacea angustifolia* DC.	8	0.90	11	1.20	6	0.42	4	0.99	2	0.75				
Upright prairie coneflower	*Ratibida columnifera* (Nutt.) Wooton & Standl.	8	0.93	1	0.40			1	0.10	2	0.40				
Eastern redcedar	*Juniperus virginiana* L.	8	0.28	21	0.75	33	0.76	43	1.23	40	0.97	44	0.69	21	0.17
Small-leaf pussytoes	*Antennaria parvifolia* Nutt.	8	1.23	12	0.63	5	0.93	4	0.74	13	0.73	7	0.38		
Needleleaf sedge	*Carex duriuscula* C. A. Mey.	7	1.17	5	1.04	4	0.75	1	0.50			4	0.10	7	0.75
Western snowberry	*Symphoricarpos occidentalis* Hook.	7	2.25	8	1.32	1	1.00	5	0.26	15	0.74	4	0.20	36	0.60
White heath aster	*Symphyotrichum ericoides* (L.) G.L. Nesom	6	1.21	2	1.50			2	1.60						
Composite dropseed	*Sporobolus compositus* (Poir.) Merr.	6	4.03	1	1.00							4	4.50		
Green needlegrass	*Nassella viridula* (Trin.) Barkworth	6	2.46	3	1.67	4	0.50	1	0.50			4	0.70		
Scribner's rosette grass	*Dichanthelium oligosanthes* (Schult.) Gould var *scribnerianum* (Nash) Gould	6	0.66	4	0.88	1	0.50	2	0.18	4	0.35				
Dotted blazing star	*Liatris punctata* Hook.	4	1.95	3	2.17										

Plains muhly	*Muhlenbergia cuspidata* (Torr. ex Hook.) Rydb.	4	1.68	1	1.00			1	1.00	2	0.10	4	1.00
Missouri milkvetch	*Astragalus missouriensis* Nutt.	3	0.71	3	1.10	1	0.50	3	1.02				
Prairie dropseed	*Sporobolus heterolepis* (A. Gray) A. Gray	3	2.81										
Smooth brome	*Bromus inermis* Leyss.	3	1.08	11	1.30	10	1.43	12	1.22	2	0.10		
Snow on the mountain	*Euphorbia marginata* Pursh	3	1.07										
Flodman's thistle	*Cirsium flodmanii* (Rydb.) Arthur	3	2.37	1	5.00								
Purple prairie clover	*Dalea purpurea* Vent.	3	2.25	1	1.50					2	0.20		
Western silver aster	*Symphyotrichum sericeum* (Vent.) G. L. Nesom	3	0.30										
White sagebrush	*Artemisia ludoviciana* Nutt.	3	3.50										
Purple threeawn	*Aristida purpurea* Nutt.	3	3.50	3	2.42	3	1.55	2	3.25				
Marsh muhly	*Muhlenbergia racemosa* (Michx.) Britton, Sterns & Poggenb.	3	1.67										
Prairie sagewort	*Artemisia frigida* Willd.	3	0.65	2	1.25	1	0.15						
Prairie fleabane	*Erigeron strigosus* Muhl. Ex Willd.	2	0.40	1	0.50	3	2.50	1	0.80			4	0.10
Fringed willowherb	*Epilobium ciliatum* Raf.	2	0.35	2	0.55	4	0.63	1	0.50	2	0.25	4	0.20
Missouri goldenrod	*Solidago missouriensis* Nutt.	2	3.75	2	3.00								
Stiff goldenrod	*Oligoneuron rigidum* (L.) Small var. *humile* (Porter) G.L. Nesom	2	2.38	4	0.51								
Shortbreak sedge	*Carex brevior* (Dewey) Mack.	2	0.35										
Prairie sandreed	*Calamovilfa longifolia* (Hook.) Scribn.	2	3.50										
Leafy spurge	*Euphorbia esula* L.	1	0.50			3	0.75			2	0.20		
Hairy rockcress	*Arabis hirsuta* (L.) Scop.	1	0.20										
Lesser fringed gentian	*Gentianopsis virgata* (Raf.) Holub	1	0.20	1	0.20								
Drummond's false pennyroyal	*Hedeoma drummondii* Benth.	1	0.30										
Candle anemone	*Anemone cylindrica* A. Gray	1	0.50					1	0.75				
Curlycup gumweed	*Grindelia squarrosa* (Pursh) Dunal	1	2.00	1	0.50								
False gromwell	*Onosmodium bejariense* DC. ex A. DC.	1	0.50	1	0.10								
Purple meadow-rue	*Thalictrum dasycarpum* Fisch. & Avé-Lall	1	0.75										
Silverleaf Indian breadroot	*Pediomelum argophyllum* (Pursh) J. Grimes	1	0.10	1	0.10								
Indiangrass	*Sorghastrum nutans* (L.) Nash	1	1.00										

Porcupinegrass	*Hesperostipa spartea* (Trin.) Barkworth	1	2.00												
Sand dropseed	*Sporobolus cryptandrus* (Torr.) A. Gray	1	0.20												
Prairie rose	*Rosa arkansana* Porter	1	18.00												
Black medick	*Medicago lupulina* L.			1	0.75			1	0.75						
Field pennycress	*Thlaspi arvense* L.									2	0.40	4	1.00		
Common mullein	*Verbascum thapsus* L.					1	0.75								
Tall tumblemustard	*Sisymbrium altissimum* L.									2	0.50				
Common dandelion	*Taraxacum officinale* F.H. Wigg.			1	0.50					4	0.15				
Curly dock	*Rumex crispus* L.							1	1.50						
Field bindweed	*Convolvulus arvensis* L.			1	1.00	1	0.50								
Yellow foxtail	*Setaria pumila* (Poir.) Roem. & Schult.													7	0.10
Canada bluegrass	*Poa compressa* L.							1	2.00					7	0.10
Quackgrass	*Elymus repens* (L.) Gould			3	0.58										
Canadian horseweed	*Conyza canadensis* (L.) Cronquist			1	0.10										
Ribseed sandmat	*Chamaesyce glyptosperma* (Engelm.) Small													7	0.10
Common yellow oxalis	*Oxalis stricta* L.											4	0.20		
Hairy false goldenaster	*Heterotheca villosa* (Pursh) Shinners			1	0.20	1	0.75								
White panicled aster	*Symphyotrichum lanceolatum* (Willd.) G. L. Nesom													7	2.00
Inland rush	*Juncus interior* Wiegand														
Leadplant	*Amorpha canescens* Pursh			1	3.00										
Missouri foxtail cactus	*Escobaria missouriensis* (Sweet) D. R. Hunt							1	1.00						
American elm	*Ulmus americana* L.					3	0.75			2	0.30				
Unknown Forb 1	---			1	0.10										
Unknown Forb 2	---			3	0.40			1	0.50						
Unknown Shrub	---					1	0.50	3	0.14	2	0.10	4	0.10		

Table A.5 Foliar cover functional group analyses dependent variables response to ERC canopy diameter treatments for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

					ERC Canopy Diameter Classes (m)												
Dependent Variables	Analysis Test	*P-Value*	Grassland Control		C(2-3)		D(3-4)		E(4-5)		F(5-6)		G(6-7)		H(7+)		
2020																	
Forb	KW	*0.02*	10.00	ab	4.76	ab	4.55	ab	0.45	a	6.46	ab	26.83	b	12.92	ab	
Unknown Forb	KW	*<0.01*	0	a	0	a	0	a	0	a	0	ab	0	b	0	ab	
FIA/B	KW	*<0.01*	0	a	0	b	0	b	0	ab	0	ab	0	ab	0	ab	
FIP	KW	*0.02*	0	ab	0	a	0	ab	0	ab	0	ab	0	ab	0	b	
FNA/B	KW	*<0.01*	2.35	a	0	abc	0	bc	0	bc	0	bc	0	ab	0	c	
FNP	KW	*<0.01*	0.26	a	0	ab	0	abc	0	c	0	abc	0	abc	0	bc	
Graminoid	KW	*<0.01*	86.36	a	83.33	ab	76.60	ab	73.92	ab	66.94	bc	40.37	c	44.74	c	
GIA	KW	*0.47*	0		0		0		0		0		0		0		
GIP	KW	*<0.01*	60.03	a	33.33	abc	36.84	abc	33.33	ab	28.17	bc	10.10	bc	10.10	c	
GNP	KW	*0.18*	16.17		25.00		16.67		15.15		18.19		9.02		7.28		
ShNP	KW	*0.83*	0		0		0		0		0		0		0		
TNP	KW	*<0.01*	0	a	0	a	0	ab	0	bc	0	bc	0	c	0	c	
2021																	
Forb	KW	*<0.01*	0.75	a	0	ab	0	b	0	b	0	ab	0	ab	0	ab	
Unknown Forb	KW	*0.34*	0		0		0		0		0		0		0		
FIA/B	KW	*0.37*	0		0		0		0		0		0		0		
FIP	KW	*0.28*	0		0		0		0		0		0		0		
FNA/B	KW	*0.39*	0		0		0		0		0		0		0		
FNP	KW	*<0.01*	0	a	0	ab	0	b	0	b	0	b	0	b	0	ab	
Graminoid	KW	*0.04*	98.27	a	98.69	a	100	a	95.24	a	93.55	a	91.84	a	89.73	a	
GIA	KW	*<0.01*	0	a	0	a	0	a	0	a	0	a	0	a	0	b	
GIP	KW	*<0.01*	0.326	a	26.32	b	31.41	b	38.46	b	46.51	b	62.50	b	66.94	b	
GNA	KW	*0.87*	0		0		0		0		0		0		0		

GNP	KW	*<0.01*	89.32	a	58.54	b	55.78	bc	38.46	bc	23.81	bc	6.25	c	0	c
ShNP	KW	*<0.01*	0	a	0	a	0	a	0	a	0	ab	0	a	0	b
TNP	KW	*<0.01*	0	a	0	ab	0	bc	0	c	0	bc	0	c	0	abc

Notes: Origin; I = introduced, N = native, Life Form; F = forb, G = graminoid, Sh = shrub, T = tree, Life Span; A = annual, B = biennial, P = perennial, Other; U = unknown.

Table A.6 Foliar cover community analyses dependent variables response to ERC canopy diameter treatments for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

			ERC Canopy Diameter Classes (m)													
Dependent Variables	Analysis Test	*P-Value*	Grassland Control		C(2-3)		D(3-4)		E(4-5)		F(5-6)		G(6-7)		H(7+)	
2020																
FQI	KW	*<0.01*	10.98	a	9.53	ab	9.33	bc	8.66	bc	8.8	bc	8.8	bc	7.79	c
Species Richness	KW	*<0.01*	5	a	4	b	4	b	3	b	3.5	b	4	b	3	b
Native Richness	KW	*<0.01*	3	a	3	ab	3	abc	2	c	2	bc	2.5	abc	2	c
Diversity (H')	KW	*0.21*	1		1.04		0.98		0.92		0.98		1.05		0.78	
Evenness	KW	*<0.01*	0.64	a	0.76	b	0.72	ab	0.8	b	0.77	b	0.81	b	0.76	ab
2021																
FQI	KW	*<0.01*	11	a	9.53	b	8.66	bc	7.79	cd	6.71	d	4.33	d	4.72	d
Species Richness	KW	*<0.01*	5	a	4	b	3	bc	3	cd	3	cd	2	d	2.5	cd
Native Richness	KW	*<0.01*	4	a	3	b	2	bc	2	c	2	c	1	c	1	c
Diversity (H')	KW	*<0.01*	1.12	a	1.01	ab	0.91	bc	0.82	bcd	0.69	cde	0.64	e	0.65	de
Evenness	KW	*0.02*	0.76	a	0.76	a	0.77	a	0.75	a	0.66	a	0.7	a	0.58	a

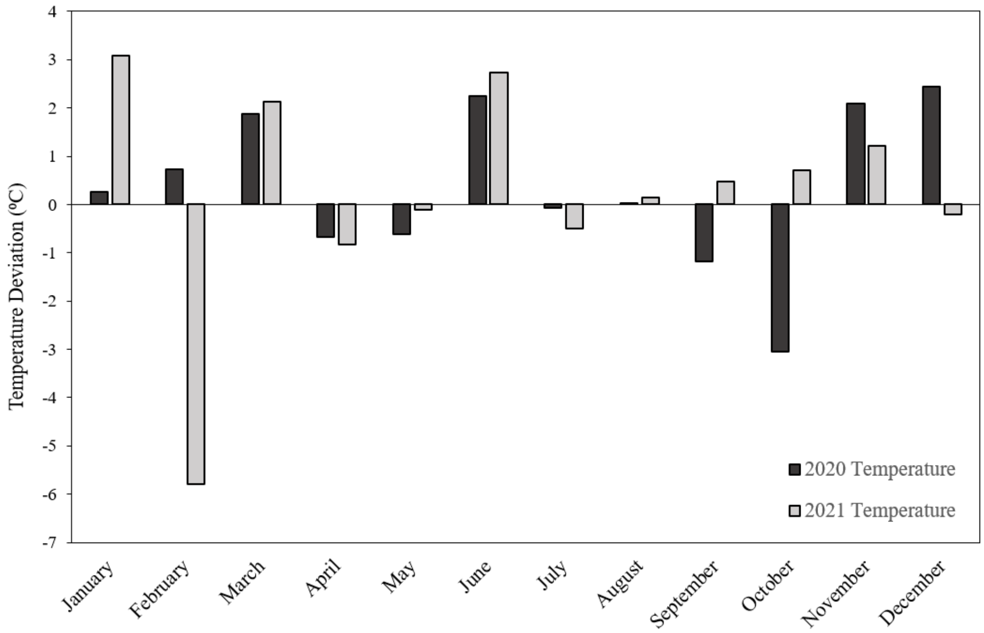

Figure A.1 Deviations of monthly temperature (˚C) from the 30-year (1990-2019) average for Site 1 (2020) using Brule County and Site 2 (2021) using Charles Mix County in South Dakota (HPRCC 2022, Mesonet 2022).

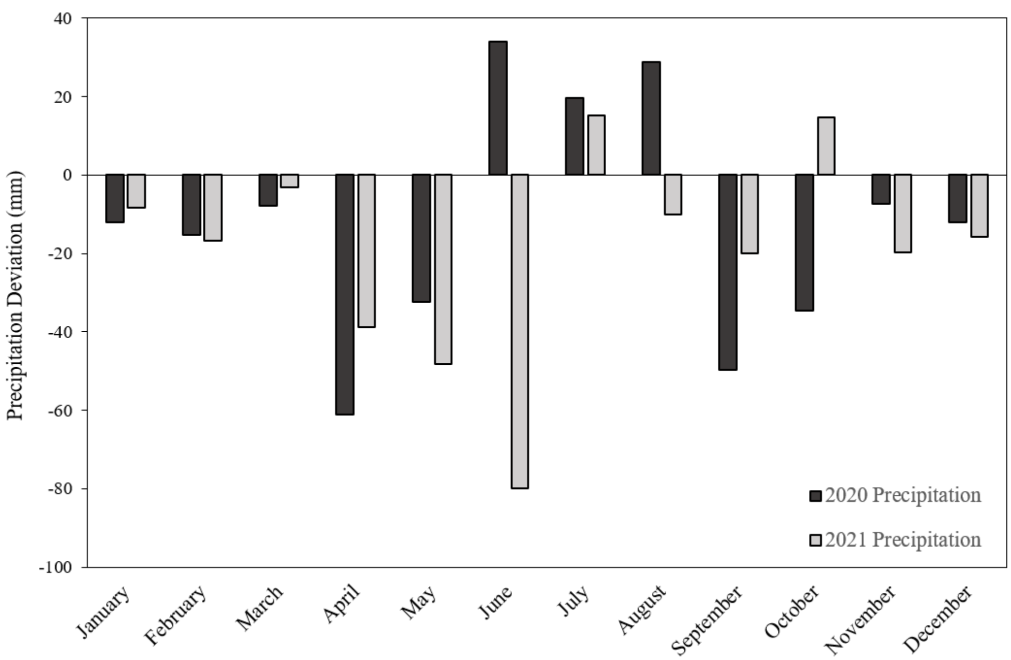

Figure A.2 Deviations of monthly precipitation (mm) from the 30-year (1990-2019) average for Site 1 (2020) using Brule County and Site 2 (2021) using Charles Mix County in South Dakota (HPRCC 2022, Mesonet 2022).

APPENDIX B

Table B.1 Seed bank composition functional group analyses dependent variables response to treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

			Treatments							
Dependent Variables	Analysis Test	*P-Value*	UC		CE		2M		GL	
2020										
Forb	KW	*0.48*	38.50		38.03		32.23		35.64	
FIA/B	KW	*0.30*	3.45		2.44		4.47		5.06	
FIP	KW	*<0.01*	1.32	a	0.00	ab	0.00	c	0.00	bc
FNA/B	KW	*0.05*	10.12	a	5.77	ab	6.06	ab	3.36	b
FNP	KW	*0.26*	16.97		12.00		17.61		22.38	
Unknown Forb	KW	*0.40*	0.00		0.00		0.00		0.00	
Graminoid	KW	*0.45*	60.67		61.97		67.77		64.36	
GIA	KW	*0.01*	0.00	a	0.00	ab	0.00	ab	0.00	b
GIP	KW	*0.71*	54.97		54.55		57.38		56.05	
GNP	KW	*0.01*	2.23	a	2.56	ab	5.00	b	2.70	ab
ShNP	KW	*0.58*	0.00		0.00		0.00		0.00	
TIP	KW	*<0.01*	0.00	a	0.00	b	0.00	b	0.00	b
Intro	KW	*0.74*	63.78		67.65		67.01		67.20	
Native	KW	*0.74*	36.22		32.35		32.99		32.80	
A/B	KW	*0.80*	13.64		10.71		14.74		14.34	
Perennial	KW	*0.80*	86.36		89.29		85.26		85.66	
2021										
Forb	KW	0.29	60.23		67.71		60.50		55.54	
FIA/B	KW	<0.01	0.00	a	0.00	ab	0.00	ab	0.00	b
FIP	KW	0.14	0.00		0.00		0.00		0.00	
FNA/B	KW	0.05	34.79	a	40.59	a	28.57	a	29.10	a
FNP	KW	0.06	18.59		20.76		28.57		22.40	
Unknown Forb	KW	0.64	0.00		0.00		0.00		0.00	
Graminoid	KW	0.29	39.77		32.29		39.50		44.46	
GIA	KW	<0.01	5.48	a	0.00	b	0.00	b	0.00	b
GIP	KW	0.34	24.40		22.84		19.44		24.90	
GNA	KW	0.57	0.00		0.00		0.00		0.00	
GNP	KW	<0.01	0.00	a	5.72	ab	13.14	c	11.56	bc
Unknown Graminoid	KW	0.23	0.00		0.00		0.00		0.00	
ShNP	KW	0.39	0.00		0.00		0.00		0.00	
Intro	KW	0.01	37.17	a	25.79	ab	23.26	b	26.97	ab
Native	KW	0.01	62.83	a	74.21	ab	76.52	b	71.71	ab
A/B	KW	<0.01	44.50	a	42.16	a	32.58	b	30.53	ab
Perennial	KW	<0.01	55.50	a	56.78	ab	66.67	b	66.82	ab

Notes: Origin; I = introduced, N = native, Life Form; F = forb, G = graminoid, Sh = shrub, T = tree, Life Span; A = annual, B = biennial, P = perennial, Other; U = unknown.

Table B.2 Seed densities (seeds/0.1m^3) analyses dependent variables response to treatments under canopy (UC), canopy edge (CE), two meters for canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

			Treatments							
Dependent Variables	Analysis Test	*P-Value*	UC		CE		2M		GL	
2020										
Seed Bank Density	KW	*0.27*	10313		8021		9358		8722	
ERC Seed (Intact)	KW	*<0.01*	6812	a	1082	b	127	c	0	c
ERC Seed (Broken)	KW	*<0.01*	16034	a	3639	b	23	c	23	c
ERC Seed (Hole)	KW	*<0.01*	5093	a	828	b	0	c	0	c
ERC Seed (Total)	KW	*<0.01*	26905	a	6090	b	150	c	23	c
2021										
Seed Bank Density	KW	*0.74*	4520		4011		3692		4202	
ERC Seed (Intact)	KW	*<0.01*	23491	a	4329	b	0	c	0	c
ERC Seed (Broken)	KW	*<0.01*	17329	a	2476	b	0	c	0	c
ERC Seed (Hole)	KW	*<0.01*	8658	a	446	b	0	c	0	c
ERC Seed (Total)	KW	*<0.01*	50945	a	7681	b	127	c	0	c

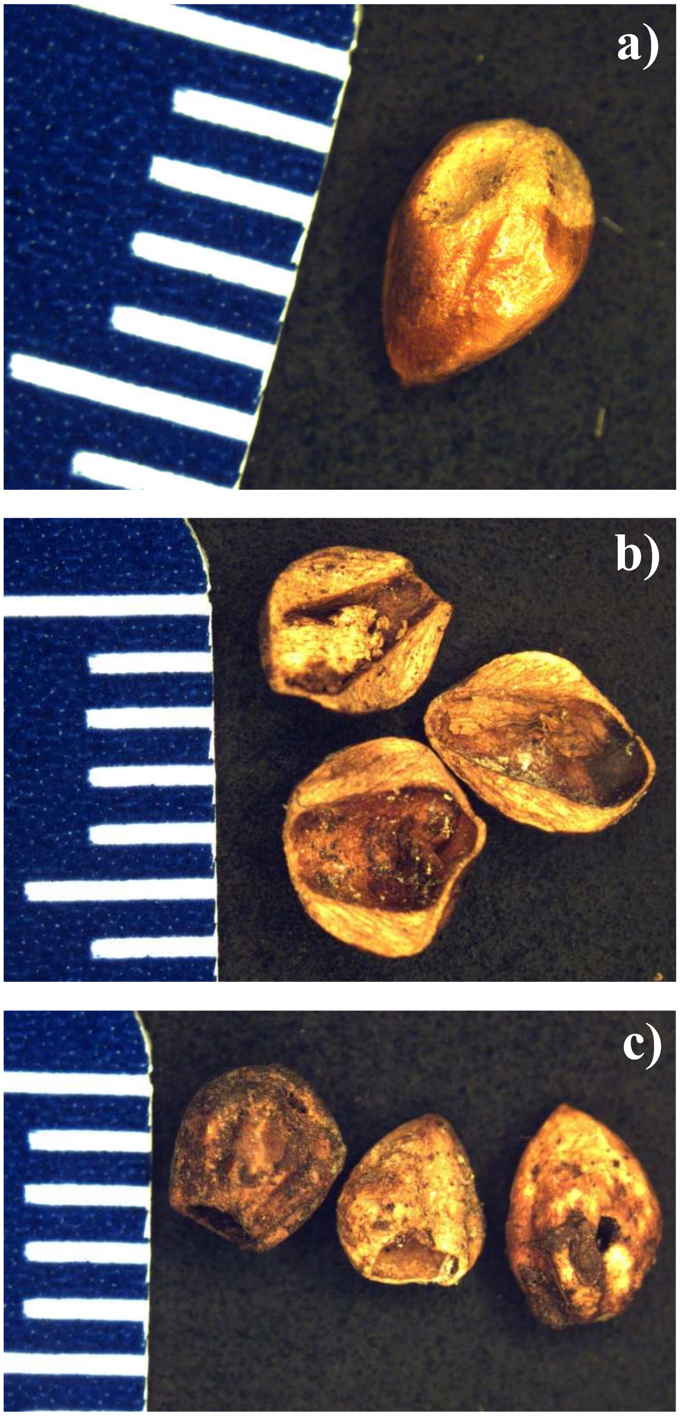

Figure B.1 ERC seeds underneath the dissecting microscope at 13.5x magnification with a ruler (mm) on the left side where a) intact, b) broken, and c) hole.

APPENDIX C

Table C.1 Live bud densities (buds/0.1m^3) response to treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

			Bud Production (#/0.1m^3)							
	Analysis Test	*P-Value*	UC		CE		2M		GL	
Site 1 (2020)	KW	*<0.01*	1894	a	19847	b	31385	b	27868	b
Site 2 (2021)	KW	*<0.01*	3199	a	36383	b	38181	b	41794	b

Table C.2 Bud bank functional group composition analyses with dependent variables response to treatments under canopy (UC), canopy edge (CE), two meters from canopy edge (2M), and grassland control (GL) for Site 1 in 2020 and Site 2 in 2021. Treatment medians with their respective letters of significance from Kruskal-Wallis (KW) tests are displayed.

			Treatments							
Dependent Variables	Analysis Test	*P-Value*	UC		CE		2M		GL	
Site 1 (2020)										
IGR	KW	*0.48*	33.85		26.27		25.25		30.01	
IGC	KW	*0.05*	29.83		39.80		29.98		33.14	
NGR	KW	*<0.01*	0.00	a	7.99	ab	12.33	b	12.29	ab
NGC	KW	*<0.01*	0.00	a	13.76	ab	19.92	b	16.39	ab
Unk. Graminoid	KW	*0.29*	0.00		0.00		0.00		0.00	
Graminoid	KW	*0.22*	75.89		90.36		90.19		89.66	
Forb	KW	*0.58*	15.65		8.74		8.52		9.09	
Shrub	KW	*0.84*	0.00		0.00		0.00		0.21	
Crown	KW	*0.12*	58.48		61.74		59.59		53.58	
Rhizome	KW	*0.12*	41.52		38.26		40.41		46.42	
Native	KW	*<0.01*	0.00	a	22.56	ab	34.50	b	30.37	b
Introduced	KW	*0.04*	73.05		68.32		55.10		61.68	
Site 2 (2021)										
IGR	KW	0.06	36.30		36.06		23.18		23.40	
IGC	KW	0.41	26.67		29.92		21.36		22.52	
NGR	KW	<0.01	0.00	a	11.50	ab	17.13	b	17.27	b
NGC	KW	<0.01	0.90	a	19.65	ab	33.30	b	31.71	b
Unk. Graminoid	KW	0.34	0.00		0.00		0.00		0.00	
Graminoid	KW	<0.01	85.83	a	96.87	b	97.55	b	97.21	b
Forb	KW	0.06	10.07		2.81		2.45		2.79	
Shrub	KW	0.05	0.00		0.00		0.00		0.00	
Crown	KW	0.21	49.63		50.96		58.78		58.36	
Rhizome	KW	0.21	50.37		49.04		41.22		41.64	
Native	KW	<0.01	2.97	a	30.00	ab	54.05	b	48.21	b

Introduced	KW	0.07	77.73	66.47	45.58	47.31

Notes: Origin; I = introduced, N = native, Life Form; F = forb, G = graminoid, Propagule; C = crown, R = rhizome, Other; Unk = unknown.

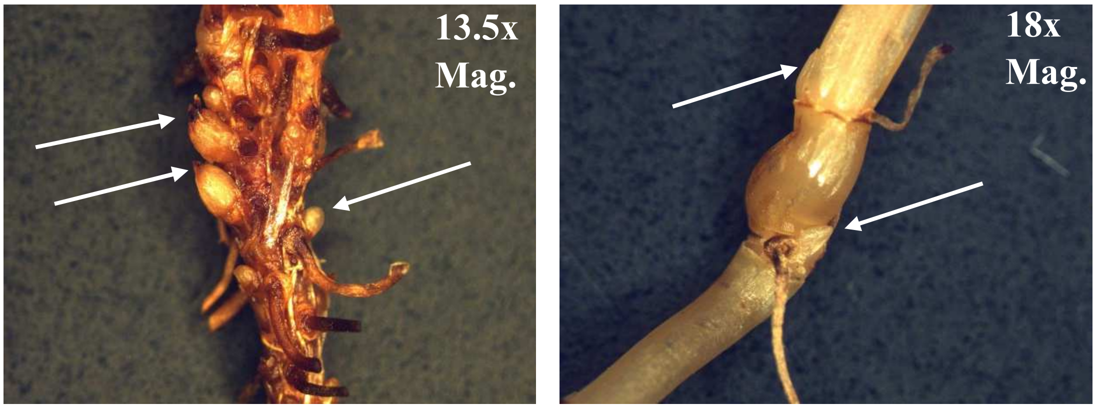

Figure C.1 Live buds of *Poa pratensis* L. (Kentucky bluegrass) underneath a dissecting microscope with arrows pointed at live buds where a) crown and b) rhizome.