

THE INVESTIGATION OF RESTORATION TECHNIQUES FOR TWO DEGRADED  
RANGELAND SITES IN NORTH DAKOTA

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**Title**

The Investigation of Restoration Techniques for Two Degraded Rangeland  
Sites in North Dakota

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota  
State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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## **ABSTRACT**

Vast areas of rangeland have been degraded or lost to row crop agriculture and urban development in the United States since European colonization. Two projects were undertaken to investigate strategies to improve diversity and forage production, while reducing invasive species on degraded prairie and also the revegetation of prairie susceptible to erosion. The projects sought to determine the effects of several pre-seeding burn and herbicide treatments paired with the interseeding of native species and to develop a seed mixture that is effective for restoring highly erodible areas devoid of vegetation. Results indicate that treatments pre-treated with herbicide have significantly higher biomass production and diversity along with a lower abundance of introduced species than the control. A seed mix selected for sites subject to high erosion paired with a topsoil layer addition resulted in higher native species richness, native species percent cover, and lower introduced species percent cover than the control.

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# **CHAPTER 1. GRAZING, BURNING, HERBICIDE, AND SEEDING AS RANGELAND RESTORATION TOOLS IN SOUTHEASTERN NORTH DAKOTA**

## **Abstract**

Many prairie areas in North Dakota are experiencing degradation from biodiversity loss, invasion by introduced species, and reduced forage quality. To address these issues and to develop a strategy to restore ecosystem functions on these prairies, the effectiveness of five treatments: 1) control (no treatment used), 2) native seeds drilled into the existing conditions, 3) spring burn followed by drill seeding, 4) glyphosate treatment followed by drill seeding, and 5) spring burn with glyphosate application followed by drill seeding were applied in 2010 on an invaded pasture. The pasture was divided into two sides, one side with grazing by cattle and the other was excluded from grazing. The five treatments were randomly selected for three blocks per side with each treatment being 40 x 100 m. Cattle were reintroduced to the entire pasture two years post-treatment. Aboveground biomass data were gathered in 2019 and 2020 by clipping eight 0.25 m<sup>2</sup> quadrats per treatment per block. In the spring of 2020, a prescribed burn was done on the site to evaluate the effectiveness of fire as a post-management strategy. Results from the 2019 and 2020 clipping data indicate that seed and herbicide treatments had significantly higher native warm-season grass biomass, average total biomass, and lower smooth brome biomass than control treatments. The findings of this study will potentially have implications for future restoration and management of degraded prairie areas. The findings of this study will potentially have implications for how highly erodible sites are revegetated and how to manage a noxious weed early in the restoration timeline.

## Introduction

Ecological restoration is a process of actively assisting the recovery of a degraded, damaged, or destroyed ecosystem such that it can function as a self-supporting ecosystem resilient to disturbances (Urbanska et al. 1997; Society for Ecological Restoration International Science and Policy Working Group 2004). Many of the restoration efforts in grassland ecosystems have been concerned with reducing introduced species and boosting the diversity and abundance of native species as goals to determine restoration success (Corbin et al. 2004; Rook et al. 2011). The basis for this is higher biodiversity is required for the maintenance of ecosystem services, ecosystem resiliency, and ecosystem function (Isbell et al. 2011; Tilman et al. 2014). Unfortunately, many restoration projects are done with little or no long term monitoring to evaluate if treatment effects remain (Ruiz-Jaen and Aide 2005).

An issue with restoring degraded prairie systems is there is typically a lack of a native species seed bank present which can limit restoration success (Clark et al. 2007). To address this problem, interseeding native species into the existing vegetation is an option. When seeding an area, there are two main methods: broadcast seeding and drill seeding. Broadcast seeding essentially tosses the seeds out randomly on the surface while a seed drill places seeds close together in rows under the soil surface (Howe and Brown 1999; Ambrose and Wilson 2003). Both methods have their advantages and disadvantages. Seeds that are drilled have greater soil-to-seed contact, less risk of predation, and the areas seeded are more resistant to invasion, but it is more labor intensive (Ambrose and Wilson 2003; Larson et al. 2011). Broadcast seeding has the advantage that it is much easier to do as it not impaired by rocks and other debris that may damage a seed drill. The random scattering of seeds from the broadcaster spreads the seeds out on the soil surface in a way that the risk of competition from neighbors is reduced, but because

the seeds are on the soil surface there is a higher risk of predation (Dixon et al. 2017). With either method, it is important to incorporate species in the seed mix that will establish and spread from their initial colonization point (Neubert and Caswell 2000; Fuchs 2001).

### **Literature Review**

Across the United States, grasslands have become an imperiled land type as between 82 to 99% have been lost since European arrival. Losses and degradation to these lands are the result of mass conversions of prairie to land tilled for row crop agriculture, overexploitation by grazing, urban development, and recreation (Samson and Knopf 1994). As the effects of global climate change become more pronounced and apparent, grassland health will be an area of major concern for limiting the impacts of climate change due to grasslands' ability to act as carbon sinks (Seastedt and Knapp 1993).

Much of the degradation of the remaining grasslands in North Dakota can be attributed to invasions by non-native species, such as Kentucky bluegrass (*Poa pratensis*) and smooth brome (*Bromus inermis*). Due to the highly competitive nature of these two species, they have invaded both native cool-season and warm-season grasslands to the point that they are two of the most recognizable and widespread plants across North American grasslands (Bahm et al. 2011; DeKeyser et al. 2015). The prevalence of Kentucky bluegrass is so high that in some areas on the northern Great Plains, especially North and South Dakota, surface coverage percentages are greater than 50% (Toledo et al. 2014). The successful invasion of smooth brome and Kentucky bluegrass can be attributed to their competitively advantageous trait of being strongly rhizomatous, which results in the establishment of a thick, mat-like structure that can choke out many native species (Bahm et al. 2011; DeKeyser et al. 2015).

Invasion by these two problem species has the potential to alter ecosystem functions, lower plant diversity, and alter seasonal forage distributions (Hendrickson and Lund 2010). A major impact of invasion by Kentucky bluegrass, smooth brome, and invasive species as a whole is that they reduce biodiversity by out competing native species (Toledo et al. 2014; DeKeyser et al 2015). The shift in species composition from native dominated to invasive cool-season species dominated plant communities can shift the energy flow of the carbon cycle from being a full season cycle to that of a system that only flows during spring and fall. This potentially could impact how much carbon is sequestered by the plant community in a given growing season (Printz and Hendrickson 2015).

Natural prairies developed under grazing by wild herbivores are now typically grazed by domestic livestock. Grazing impacts prairie systems' plant community composition, diversity, soil properties, nutrient cycling, and microbial activities (Wagle and Gowda 2018). Many impacts grazing has on grassland function and can be traced to grazing intensity and frequency (Launchbaugh 2003). Overgrazing a pasture can result in increased invasion rates by non-native species, but not grazing at all can have the same effect. The key to using grazing as a tool for maintaining biodiversity lies somewhere in between the two extremes (Launchbaugh 2003). Grazing impacts community composition and diversity in several ways. As observed in the tallgrass prairie region of the southern Great Plains, many large herbivores prefer to eat dominant C4 grasses over subdominant C3 grasses and forbs on areas with short fire intervals (Veen et al. 2008; Collins and Calabrese 2012). This will lead to a reduction in shading and competition allowing more C3 grasses and forbs to survive, therefore increasing diversity. Also, grazing by livestock can be used to target invasive species. Targeting introduced cool-season grasses could reduce their competitiveness and improve the plant diversity of northern tallgrass prairies (Smart

et al. 2013). With targeted grazing, land managers can place grazers on a plot when only weedy species are growing or are the most palatable plants. Depending on the target species, the right type of herbivore must be used to be effective. Cattle are best for consuming fibrous plants like graminoids, goats are well adapted to stripping leaves from branches or even consuming branches themselves, so they are good for control of species such as juniper, and sheep are excellent for controlling herbaceous weeds (Launchbaugh 2003).

Above ground impacts are not the only aspect of prairies that grazing influences, as soil properties, nutrient cycling, surface hydrology, and microbial activity are all also impacted. Some of these changes include soil compaction, higher bulk densities, and soil structure deterioration when subjected to overgrazing (Greenwood and McKenzie 2001). The changes to soil structure, bulk density, and reduced vegetative cover associated with heavily grazed systems has been shown to decrease microbial biomass by changing soil temperature and water characteristics (Holt and McIvor 1994). Furthermore, grazing can shift plant root-to-shoot ratios towards having more root biomass than shoot biomass to have stored resources for regrowth (Anderson 2011). Surface hydrology can also be improved by properly stocked grazing systems relative to both overgrazed and under stocked systems. If grazers can effectively graze thatch-forming species such as Kentucky bluegrass, water will infiltrate into the soil instead of running off due to the lack of a thatch layer (Toledo et al. 2014)

Like grazing, prescribed burning is a tool that can have many impacts, such as increasing growth and productivity, altering species composition, changing nutrient cycles and water availability, and modifying microbial activities when compared to systems where fire has been suppressed (Knapp et al. 1984). Grasslands that have been subjected to burning have shown a community shift from a community dominated by fungal microbiota pre-fire to a bacteria

dominated microbiota community post-fire possibly due to decreased soil moisture (Fultz et al. 2016). When comparing burned and unburned grasslands, burned grasslands show increases in plant growth and production due to litter removal, added nutrients from burnt biomass, and increased light exposure (Sharrow and Wright 1997). Burning also helps to maintain grassland by preventing woody encroachment (Wagle and Gowda 2018).

The timing and type of burn play a large role in what impact burning will have on a plant community (Hendrickson and Lund 2010). Studies have shown that winter and spring burns are best for increasing species richness, while spring burns also reduce forb diversity (Towne and Owensby 1984; Towne and Kemp 2003). Another option instead of relying on seasonal burn schedules is to determine the ideal growth stage to burn a target species and calculate the accumulated growing degree days (AGDD) it takes for the plant to reach that stage. For example, smooth brome is most susceptible during the tiller elongation phase (Wilson and Stubbendieck 1997). In northern tallgrass prairies, 95% of smooth brome populations can be expected to reach 50% elongation between 946 AGDD and 1566 AGDD (Priester et al. 2019). As for burn type, heterogeneous patch burning has resulted in higher diversity for both forbs and grasses when compared to homogenous burning over the course of four years (Fuhlendorf and Engle 2004). These findings were supported in a study done in South Dakota's Grand River Grasslands where the effectiveness of two fire treatments (patch-burn and homogenous burn) for returning the site to reference site conditions was investigated (Delaney et al. 2016). The results of the study indicated that patch-burn was more effective as it turned the landscape into a heterogeneous mosaic, which promotes a greater level of diversity at the landscape level relative to a landscape burned homogeneously.

Burning can impact both aboveground and below-ground nutrient cycling. In an experiment examining the effects of fire and large herbivores on canopy nitrogen in tallgrass prairies, Ling et al. (2019) determined that canopy nitrogen content was highest in areas burned in the spring with short fire intervals, relative to treatments with longer fire return intervals in other seasons, suggesting that plant productivity and forage quality are promoted by early and frequent fires. If fires occur too frequently though, they may hinder carbon and nitrogen from being decomposed and incorporated into the soil due to it being burned off before it can be broken down (Wagle and Gowda 2018).

Herbicide application is another tool commonly used in grassland restoration, but by itself, it rarely provides sustainable control options for invasive species management because a desirable seedbank is often lacking (Endress et. al 2012). Herbicide is often effective when paired with other management techniques such as seeding, burning, and grazing (Link et al. 2017). Much of the success of herbicide treatments also depends on the level of invasion a land manager is trying to combat. In a study conducted on the Sheyenne National Grasslands, three sites were chosen with varying Kentucky bluegrass invasion levels (Ereth et al. 2017). Results showed that plots burned in the fall accompanied by spring glyphosate application had nearly 40% more relative basal cover of native grass and almost 60% less bluegrass cover than the plots that were not subjected to burning or herbicide with high invasion levels (> 91% Kentucky bluegrass species composition). The results were less pronounced at lower invasion levels. In another experiment, seeding was paired with glyphosate on a heavily invaded grassland and the site was then left alone for six years (Endress et al. 2012). The researchers found the native grass species percent foliar cover was increased by 52%, but much of the introduced forbs that were negatively impacted by the herbicide were replaced by other introduced species (Endress et al.

2012). Further, Slopek and Lamb (2017) found smooth brome abundance decreases, but Kentucky bluegrass abundance increases after herbicide application when studying the long-term effects of glyphosate for smooth brome control. This indicates Kentucky bluegrass most likely replaced the smooth brome that was eradicated by the herbicide treatment.

While the previously mentioned restoration methods are all effective management tools when used in situations in which a viable native seed bank is present, the lack of native seed availability in invaded prairies will limit native species establishment thus limiting the likelihood of reaching restoration goals (Foster and Tilman 2003). Several studies have found that seed additions to restoration projects have resulted in significantly higher ground cover and native species cover (Brudvig et al. 2011; Stanley et al. 2011).

Initial predictions stated at the start of the study in 2010 were treatments with the greatest amount of competition reduction, i.e. blocks that were grazed and treated with herbicide and fire, would result in the highest biomass production and lowest levels of smooth brome and Kentucky bluegrass invasion. These predictions were based upon the results from other studies whose results have indicated the coordination of multiple restoration strategies along with interseeding is key for successful results because it will likely cause the greatest reduction of competition (Bakker et al. 2003; Corbin et al. 2004; Thorpe and Stanley 2011). 10 years post-treatment application, we predicted the seed and herbicide combination would be the most effective for controlling invasive species, increasing biomass production, and the highest species diversity. This prediction is based on previous studies conducted at the site that found the seed and herbicide treatment to be the most effective (Huffington 2011; Link et al. 2017). The purpose of this study was to investigate the legacy effects of multiple pre-seeding treatments and their impacts on biomass production and invasive species control.

## Methods

The project site is located at 46°32'19.5"N and 97°08'35.0"W (legal description: T135N, R51W, NE ¼ Section 6) on 12.1 ha (30 ac) of pastureland within the Ekre Grassland Preserve in Richland County, North Dakota. This area is located within the Sheyenne River Delta, which was formed by the prehistoric meanderings of the Sheyenne River (Bryce et al. 1998). The site was subject to rotational grazing by cattle for longer than ten years before treatment application. To better understand the impacts of grazing, 6.1 ha of the study site was fenced off to grazing by livestock, while the scheduled rotational grazing was continued on the remaining 6 ha.

The soil of the study site is predominately composed of an Aylmer-Bantry complex. The Aylmer series consists of very deep, moderately well-drained, rapidly permeable soils formed by wind-worked sands on outwash plains and delta plains on slopes of 0 to 6 percent and is classified as a mixed, frigid Aquic Udipsamment (USDA-NRCS 2020a). The Bantry series consists of very deep, somewhat poorly drained, rapidly permeable soils that formed in windblown glaciofluvial deposits. Both series are described as not prime farmland and are primarily used for pasture and hay (USDA-NRCS 2020a).

The climate of the area is described as continental, with cold winters and hot summers. The annual mean temperature for the site is 5.4 °C and receives 55.7 cm of rainfall on average, 79% of which falls during the growing months of April through September (Figures 1.1 and 1.2). The average frost-free period is 130 days, with soil thaw typically complete by May 1<sup>st</sup> (Manske and Barker 1988; NDAWN 2020).

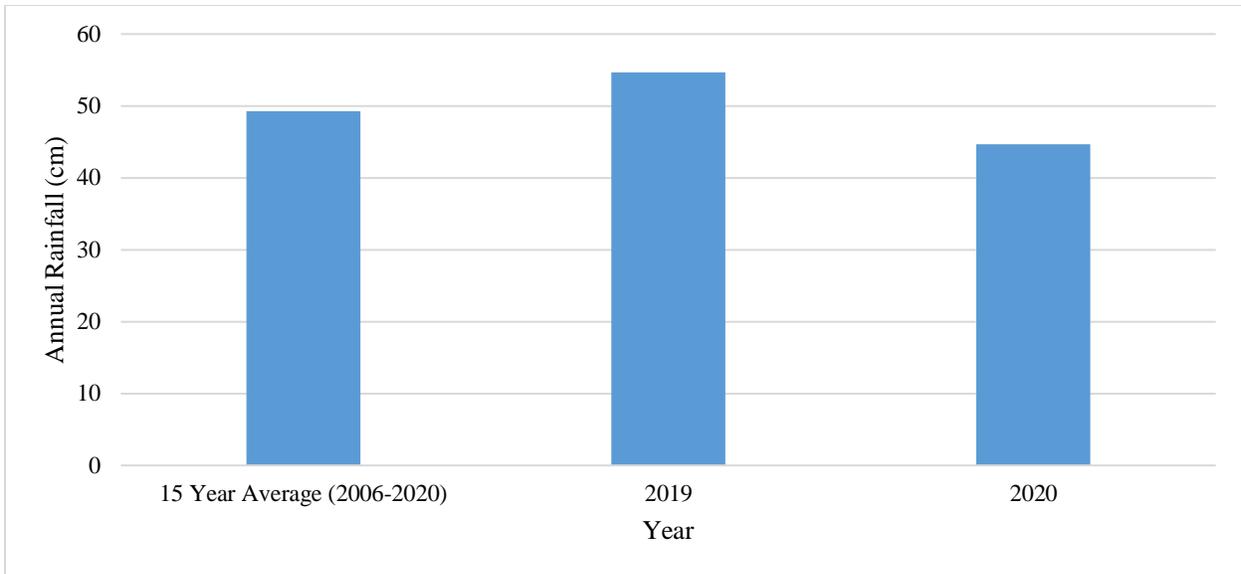


Figure 1.1. Yearly rainfall totals for the Ekre NDAWN Station located at the study site.

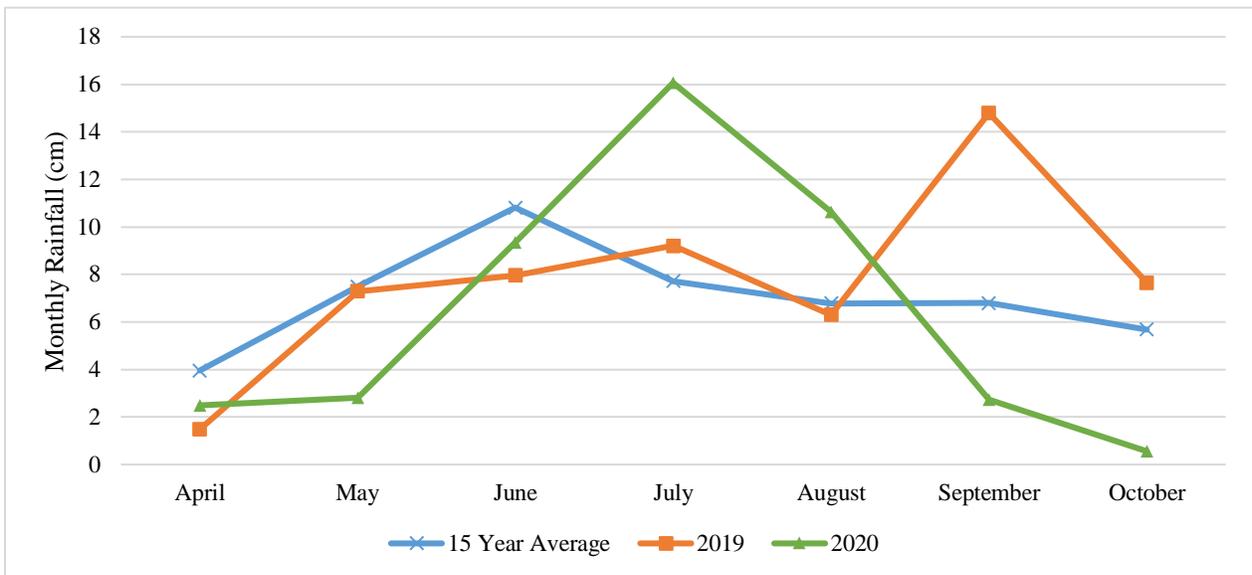


Figure 1.2. Monthly rainfall totals by year at the Ekre NDAWN Station located at the study site.

The pasture where the study site is located is currently considered a degraded tallgrass prairie pasture. The site was at one point cultivated but was likely reseeded in the 1970s (DeKeyser personal communication). Since then, there has been minimal recolonization by traditional, warm-season tallgrass species such as big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), Indian grass (*Sorghastrum nutans* L.),

and switchgrass (*Panicum virgatum* L.). Within the site, there are a variety of cool-season grasses present, including smooth brome (*Bromus inermis* Leyss.) and Kentucky bluegrass (*Poa pratensis* L.). Kentucky bluegrass is a non-native invasive perennial grass with European origins that is widespread throughout the region and has been observed invading grasslands and displacing native species (Murphy and Grant 2005; DeKeyser et al. 2009). Prior to the start of this study, a forage production analysis was conducted on every pasture in the Ekre Grassland Preserve and found that the study site pasture was underperforming by roughly 560 kg/ha compared to the rest of the preserve and was also the lowest scoring pasture of the entire preserve (Huffington 2011).

The study design consisted of three blocks of five treatments per plot resulting in 15 treatments per plot and a total of 30 total treatments (Figure 1.3). Treatments measured 40 m × 100 m in size, and each treatment in the block was randomly assigned one of the following; control, seed only, burn seed combination, seed herbicide combination, and burn seed herbicide combination.



Figure 1.3. Treatment layout of the Ekre study site.

To avoid potentially negatively impacting early germination and seedling success, burning and herbicide treatments were applied three weeks before interseeding. The initial spring burns were administered as strip burns, which ultimately resulted in head fires for the treatments selected. The herbicide utilized in this study was RoundUp® Concentrate Plus (The Scotts Company LLC, Worldwide Rights Reserved) mixed with water at a ratio of 60:1. The mix was applied using a boom sprayer at a rate of 23 L/ha to the appropriate treatments before the burn applications. Seeding of the trial blocks, with exception of the control treatments, was conducted on July 16<sup>th</sup> and 17<sup>th</sup>, 2010, using a Truax FLEX II drill model FLXII-818 (Truax Company Inc., New Hope, MN). Interseeding was done at a 20 cm spacing interval at a seeding depth of 0.25 to

1.25 cm. At the time of interseeding, the soil was moist to wet with a few small areas of inundation. The seed mixture used was sourced from the Natural Resource Conservation Service – Ecological Site Description for subirrigated sands for Major Land Resource Area 56 (USDA-NRCS 2020b). The species composition of the mix as well as the seeding density and ratios were selected to restore the pasture to the tallgrass prairie plant community that was historically present. The seed used for the study was sourced from Millborn Seeds in Brookings, SD, and a complete list of the species planted as well as the seeding densities is shown in Table 1.1 below.

Table 1.1. Seed mix species composition and seeding densities.

<b>Species</b>	<b>Scientific Name</b>	<b>Kg/ha</b>
Big Bluestem – Bison	<i>Andropogon gerardii</i>	2.69
Prairie Sandreed – Goshen	<i>Calmolvilfa longifolia</i>	1.12
Canada Wildrye – Mandan	<i>Elymus canadensis</i>	0.56
Little Bluestem	<i>Schizachyrium scoparium</i>	0.56
Switchgrass – Dakota	<i>Panicum virgatum</i>	0.56
Western Wheatgrass – Rodan	<i>Pascopyrum smithii</i>	0.56
Sand Bluestem	<i>Andropogon hallii</i>	0.34
Green Needlegrass – Lodorn	<i>Nassella viridula</i>	0.28
Indiangrass – Tomahawk	<i>Sorghastrum nutans</i>	0.28
Purple Prairie Clover	<i>Dalea purpurea</i>	0.28
White Prairie Clover	<i>Dalea candida</i>	0.28
Blue Grama - Bad River	<i>Bouteloua gracilis</i>	0.17
Prairie Cordgrass - Red River Germplasm	<i>Spartina pectinata</i>	0.17
Porcupine Grass - South Dakota native collection	<i>Hesperostipa spartea</i>	0.11
Prairie Junegrass	<i>Koeleria macrantha</i>	0.01

On July 15, 2010, cattle were rotated off the pasture and did not reenter until 2011 to prevent impacts to newly established seedlings. Starting in mid-May of 2011, the seeded pasture was the first to be grazed in order to limit competition from cool-season grasses. For the next two years of the study, the grazed portion of the pasture was subjected to twice-over rotational grazing. Once it was determined that there were no significant differences in average native warm-season grass biomass and average total forage biomass between the grazed and ungrazed

pastures, the entire site was opened up to grazing, so it could be used as a management tool (Link et al. 2017). On April 30<sup>th</sup>, 2020, a second prescribed burn was applied to the entire study area. Conditions at the time of the burn were 17.78 °C (64 °F) air temperature, wind speed of 9.66 kph (6 mph) with wind gusts up to 20.92 kph (13 mph), and 38% relative humidity (NDAWN 2020).

Starting in late July and ending in early August of 2019 and 2020, biomass data were collected by clipping eight 0.25 m<sup>2</sup> quadrats per treatment. Grasses were clipped and sorted by species while forbs, shrubs, and sedges were treated as groups. Biomass clippings were dried at a temperature of 37.78 °C for a minimum of 72 h before weight measurements were recorded.

The study design for this project was a split plot, randomized complete block experimental design. Treatment and year were treated as whole plot factors. Treatment type had five levels (control, burn seed, seed, seed herbicide, and burn seed herbicide) and year had two levels (2019, 2020). The split plot factor was grazing, with two levels (rotational grazing by cattle and excluded from grazing by cattle). Total warm-season grass biomass, total biomass, grass species richness, Kentucky bluegrass biomass, and smooth brome biomass were evaluated using the analysis of variance (ANOVA) procedure in SAS Enterprise Guide 7.1 (Copyright © 2017 by SAS Institute Inc. Cary, NC, USA) with Tukey's (HSD) tests to evaluate pair-wise comparisons of treatment populations. A log transformation was applied to satisfy distributional assumptions prior to analysis to smooth brome biomass, Kentucky bluegrass biomass, and total biomass. No transformations were applied to native warm-season grass biomass or grass species richness because these variables met distributional assumptions without a transformation

## Results

ANOVA indicated ( $F = 5.6$ ,  $P < 0.0001$ ,  $DF_{\text{effect}} = 39$ ,  $DF_{\text{error}} = 20$ ) that average native warm-season grass biomass responded to treatment type ( $P < 0.0001$ ) and that there was a significant interaction term (Treatment x Year) ( $P = 0.0081$ ). When aggregated across years, the seed herbicide, burn seed herbicide, seed, and burn seed treatments increased average native warm-season grass biomass over the control (Figure 1.4). However, the treatments responded differently in 2019 and 2022 (Figure 1.5). Grazing had no effect on average native warm-season grass biomass ( $P = 0.1597$ ).

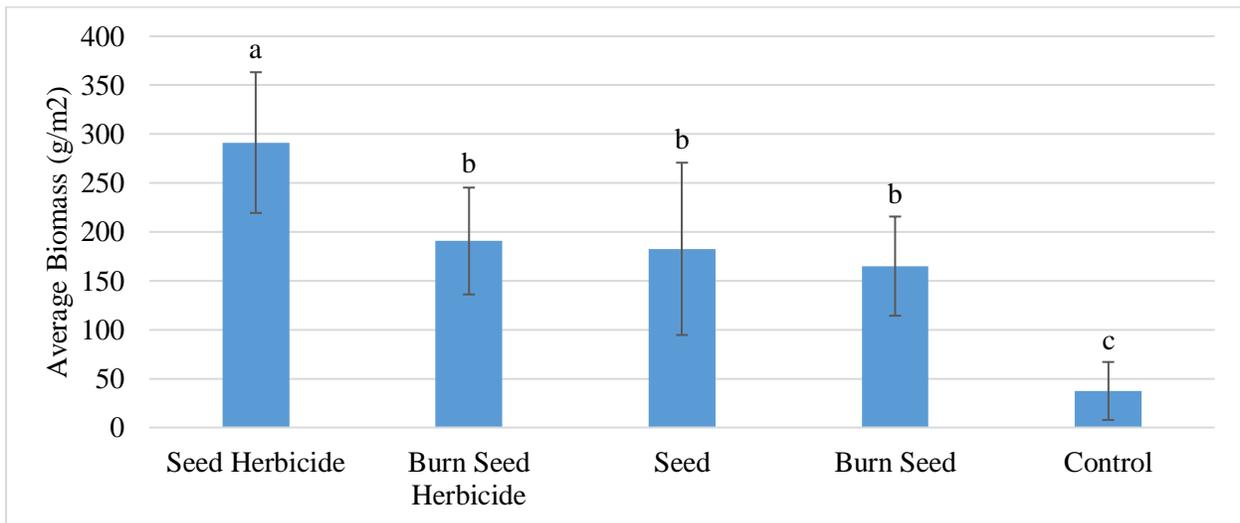


Figure 1.4. Average native warm-season grass biomass ( $\pm$  standard deviation) by treatment for 2019 and 2020. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

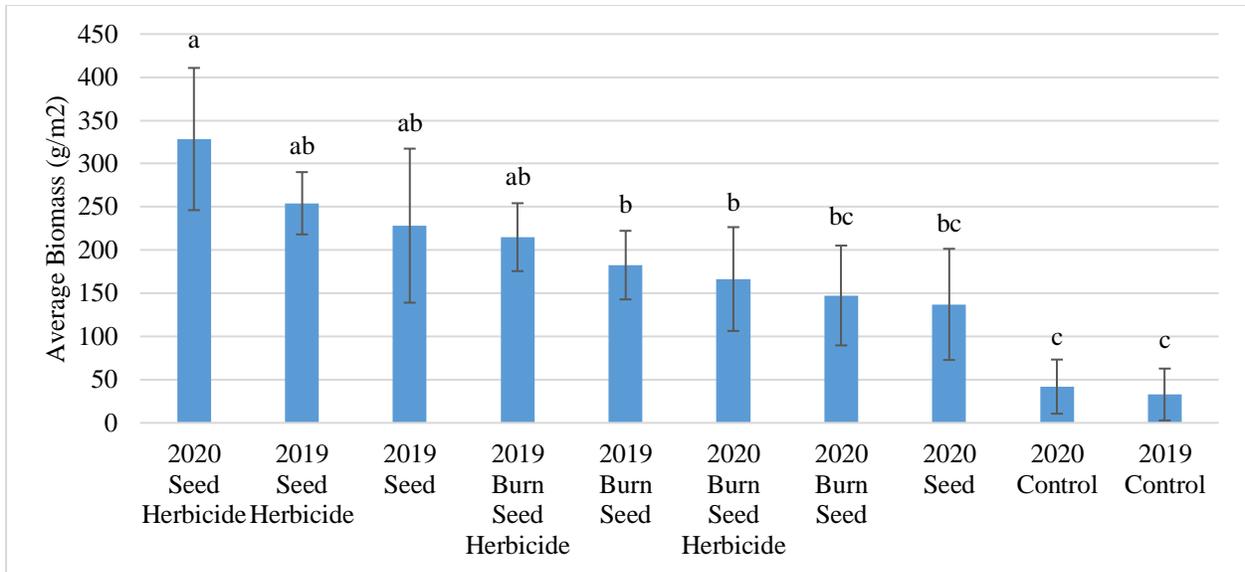


Figure 1.5. Average native warm-season grass biomass ( $\pm$  standard deviation) by year and treatment. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

Average total biomass responded ( $F = 2.56$ ,  $P = 0.0137$ ,  $DF_{\text{effect}} = 39$ ,  $DF_{\text{error}} = 20$ ) to treatment ( $P < 0.0001$ ) and year ( $P = 0.0009$ ). The seed herbicide treatment increased the average total biomass more than the seed, burn seed herbicide, burn seed, and control treatments (Figure 1.6). In addition, average total biomass was also higher ( $P = 0.0009$ ) in 2020 than 2019 (Figure 1.7) while no differences were attributable to grazing ( $P = 0.4259$ ).

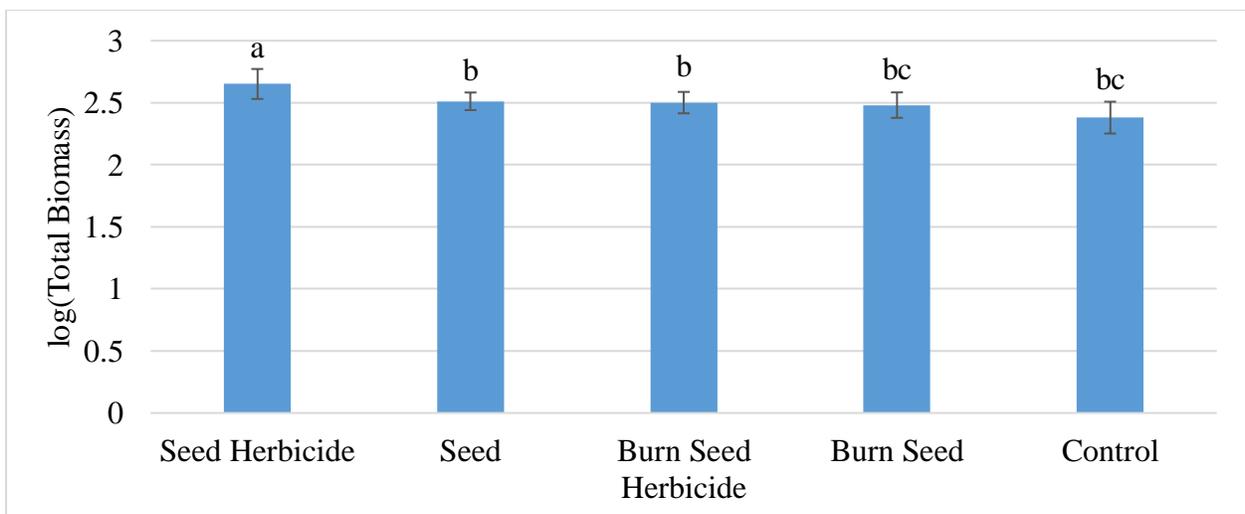


Figure 1.6. Average total biomass ( $\pm$  standard deviation) by treatment for 2019 and 2020. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

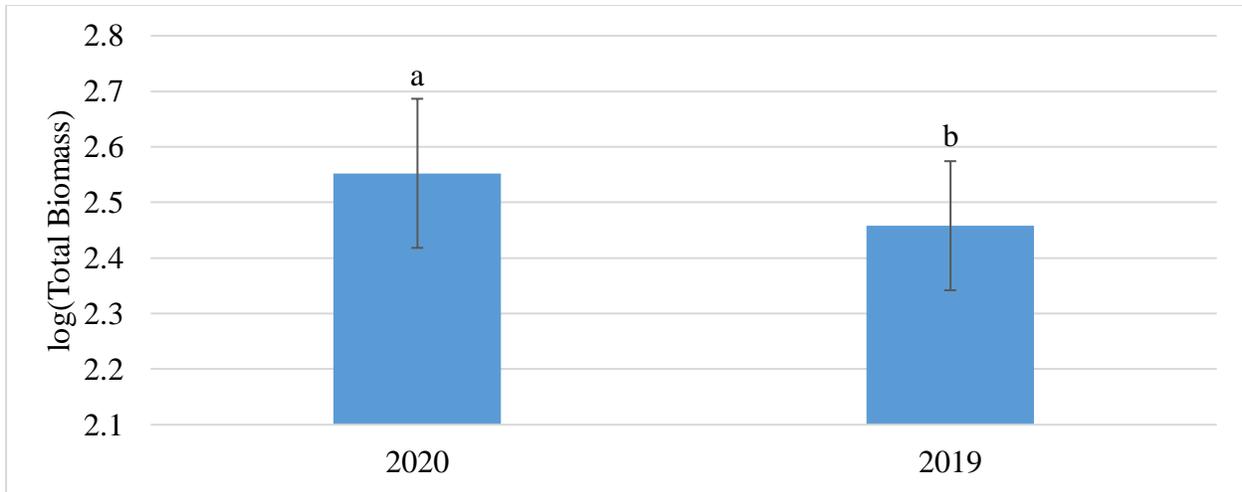


Figure 1.7. Average total biomass ( $\pm$  standard deviation) by year. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

Average grass species richness ( $F = 3.35$ ,  $P = 0.0027$ ,  $DF_{\text{effect}} = 39$ ,  $DF_{\text{error}} = 20$ ) responded to treatment ( $P < 0.0001$ ) and year ( $P = 0.0074$ ). Across both years, the burn seed herbicide, seed herbicide, burn seed, and seed treatments increased average species richness over the control treatment (Figure 1.8). Average grass species richness was higher in 2020 than 2019 (Figure 1.9), while grazing ( $P = 0.8812$ ) did not have an effect on average grass species richness.

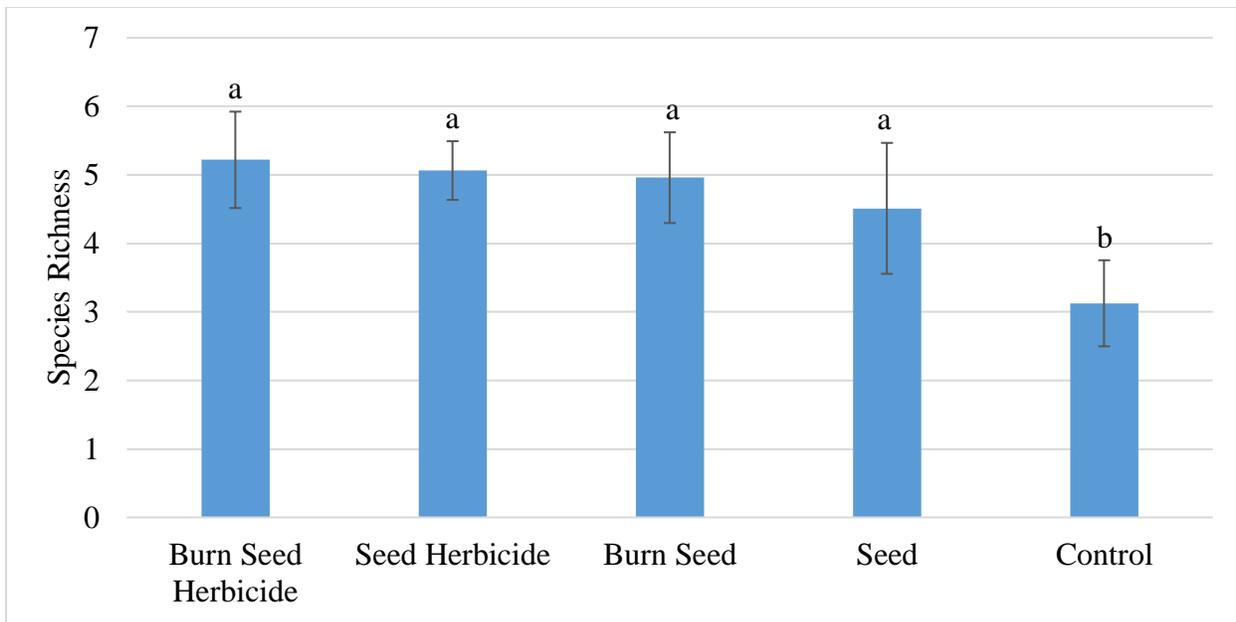


Figure 1.8. Average grass species richness ( $\pm$  standard deviation) by treatment for 2019 and 2020. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

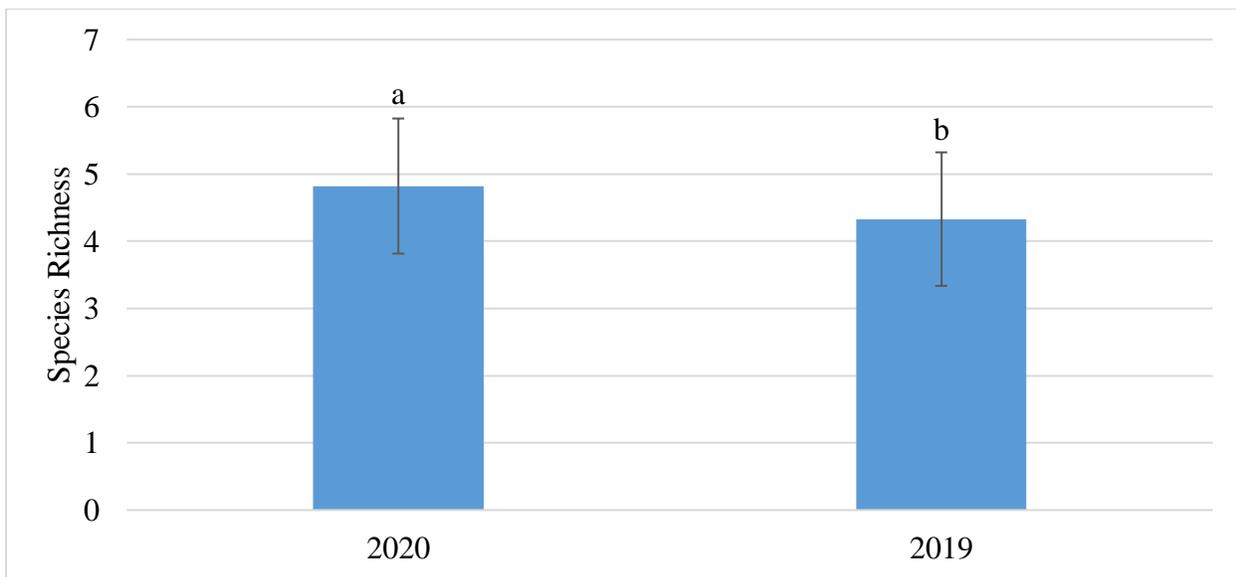


Figure 1.9. Average grass species richness ( $\pm$  standard deviation) across by year. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

ANOVA did not yield significant model for Kentucky bluegrass biomass ( $F = 0.89$ ,  $P = 0.6379$ ,  $DF_{\text{effect}} = 39$ ,  $DF_{\text{error}} = 20$ ) or smooth brome biomass ( $F = 1.06$ ,  $P = 0.4619$ ,  $DF_{\text{effect}} = 39$ ,  $DF_{\text{error}} = 20$ ).

## Discussion

In this study, we tested the effectiveness of several different pre-seeding treatments to reduce competition from exotic invasive species, increase biomass production, and increase species richness nine and ten years post-treatment application. The findings of the study indicate the use of herbicide before interseeding 10 years ago has continued to result in higher native warm-season grass biomass and total biomass relative to the control treatment. The use of any of the treatment methods (burn seed herbicide, seed herbicide, burn seed, and seed) all resulted in higher average grass species richness over the control.

The results are partially consistent with the prediction that a seed and herbicide combination would result in the greatest biomass production, highest species diversity, and the most effective invasive species control because it was this treatment that increased those measures the most nearly a decade ago. The results are similar to studies conducted previously at the site by Huffington (2011) and Link et al. (2017), which found the seed herbicide combination to be the most effective for seedling establishment as well as increasing warm-season plant biomass and total plant biomass. Additionally, the findings are consistent with studies that have observed the combination of seeding and some form of disturbance to be an effective method for reducing introduced perennial grasses, increasing native diversity, and native species cover (Bakker et al. 2003; Thorpe and Stanley 2011; Stanley et al. 2011).

Between 2019 and 2020 two notable differences were observed. The average total biomass and grass species richness of all the treatments were higher in 2020 than what was recorded in 2019. In 2020, the study site received nearly double the monthly rainfall for July than it did in July of 2019. This increase in rainfall right before biomass clipping started could have caused heightened biomass production contributing to the increased biomass totals observed. It is

also important to note that between the data collection events, a spring prescribed burn was applied to the entire site. However, due to low fuel load and improper timing, the fire burned unevenly resulting in an unintentional patch burn. The impacts on increased biomass are consistent with Knapp et al.'s findings (1984) that spring burns increase biomass production, due to the removal of litter and greater infiltration of solar radiation to the subcanopy post-burn. While the patch burn result may have been unintentional, it created a heterogeneous landscape that favors higher species diversity. This is due to the increased spectrum of niches that can be occupied as supported by findings from Fuhlendorf and Engle (2004) and Delaney et al. (2006) and as a result could explain the increased species diversity observed in 2020.

While the burn may have positively influenced biomass production and diversity, it did not appear to impact smooth brome biomass. As found by Kobiela et al. (2017), smooth brome requires two or more burns within four years of each other to reduce smooth brome cover. It may be possible that another burn applied by 2024 may result in a reduction of smooth brome that was not apparent after one burn. The lack of impacts to smooth brome biomass could also be attributable to the timing of the burn. As noted by Wilson and Stubbendieck (1997), smooth brome is most susceptible to burning during the tiller elongation phase, as it is during this time plant growth is exposed above from the soil surface and is devoting the majority of its resources to growth rather than belowground storage. In the case of our burn, the fire was applied at 448.6 AGDD. Priester et al. (2019) showed elongation occurs 946 AGDD and 1566 AGDD. When our burn took place it is likely the smooth brome population was nowhere near elongation, thus had the majority of its resources stored belowground and sheltered from burning. Average grass species richness was also different by block. The average grass species richness was higher in

block three than in block one. A possible explanation for the difference could be it is a result of the natural variability in the plant community and study site.

In this study, the treatments applied 10 years ago no longer showed any effects for controlling smooth brome and Kentucky bluegrass. As noted by Trowbridge et al. (2017), the effects of disturbance-based treatments in their study had become almost undetectable in terms of exotic grass species control within 10 years of treatment application, consistent with what was seen with Kentucky bluegrass and smooth brome in this study. This finding highlights the importance of monitoring and adaptive management in restoration projects to ensure goals continue to be met.

The main driver of restoration success in this study appears to be linked to increasing biodiversity. For each variable tested, whether it was significant or not, the control treatment scored the poorest whether it was total biomass production, warm-season grass biomass, or species richness. The role biodiversity plays in maintaining an ecosystem cannot be overstated. In a study by Tilman et al. (1996) the results indicated that ecosystem productivity is greatest in systems with high diversity due to greater utilization of soil nutrients. Further, there have been observed relationships between aboveground biomass and diversity where grasslands with high diversity show significantly higher biomass production (Biondini 2007). The benefits of biodiversity relate to more than just biomass production though. There has been evidence showing that restoring high plant diversity to degraded lands has the potential to increase the carbon sequestration capability of the land, something that will only become more important with the uncertainty of climate change (Yang et al. 2019). Lastly, increasing biodiversity has been shown to make a grassland ecosystem more resistant to invasion by problem species as it

limits the number of unoccupied niches that an invasive species could occupy (Tilman et al. 2014).

This study provided insight on the persistence of multiple pre-seeding treatment combination effects, but future research is still needed to develop management plans to maintain the observed effects of the treatments as well as to restore effects that have been reduced or lost over time. As noted by other studies, these plans should utilize the combination of multiple strategies to ensure the highest level of success. Further, future research should also be done to determine lasting treatment effects at timescales greater than what was observed in this study. This study will provide useful information for managers in the tallgrass region of southeastern North Dakota on how using a mixture of herbicide and interseeding can improve the quality of their rangelands by increasing biomass production and species richness.

### **Conclusion**

The initial goal set forth at the start of this project 10 years ago by Huffington et al. (2011) was to determine how the study site plant community changed over time, and how much of the change could be attributed to interseeding. This study addressed that goal with the finding that the combination of herbicide and interseeding has continued to promote higher native warm-season biomass, total biomass production, and grass species richness. However, residual treatment effects for controlling Kentucky bluegrass and smooth brome no longer remain as they did in 2012. Still, this study points to herbicide combined with interseeding as an effective tool for improving degraded grasslands by increasing native-warm season grass biomass, total biomass, and grass species richness. Further research should seek to investigate adaptive management strategies such as the reapplication of fire and herbicide to ensure initial restoration goals continue to be met.

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## **CHAPTER 2. REVEGETATING A HIGHLY ERODIBLE MILITARY SITE USING ECOLOGICAL SITE DESCRIPTION DERIVED SEED MIXES**

### **Abstract**

Land use changes and overuse have caused increased rates of erosion and degradation in vast areas of rangeland across the globe. The degradation of these rangelands can be expected to only worsen as plant communities transition to alternative states. To avoid further damages, land managers should revegetate areas where erosion is of great concern by planting species adapted to local conditions. This study looked to test three different seed mixtures derived from ecological site descriptions, as well as the effects of a topsoil layer addition, to determine the most effective treatment for seedling establishment, native species diversity, and surface cover percentage. The treatments (topsoil, special, standard, and guard) consisted of three different seed mixes and fourth treatment consisting of a topsoil addition before seeding. The treatments were applied in the summer of 2019. Four blocks were established for each treatment. Initial grass and forb seedling counts were conducted roughly one month after seeding in August 2019, followed by a percent cover by species survey in August 2020. An herbicide was applied to half of each plot in the summer of 2020 to test its usefulness for introduced species control. Analysis of the data showed the combination of a native seed mix formulated for the ecoregion and a topsoil addition resulted in the highest native species richness, cover, and lower introduced species cover relative to the other treatments. The herbicide application showed higher native species ground cover and lower introduced species richness than subplots that were left unsprayed.

## Introduction

Rangelands make up a significant portion of Earth's land surface with an estimated global coverage between 18-80% (Lund 2007). Changes in land use and intensification have caused increased soil erosion rates and land degradation resulting in 20-73% of the world's rangelands being classified as degraded (Lal 1990; Lund 2007). When these lands are subjected to land-use change and degradation, the plant communities may be lost or transition to an alternative state with different ecological processes and feedbacks. This can often increase soil erosion even further and make restoration more difficult or impossible (Ash et al. 1994; Bestelmeyer et al. 2009). The seeding of herbaceous vegetation on lands susceptible to erosion is a common practice for remediating erosion risk, but is most effective when the species selected are adapted for the present conditions (Van Epps and McKell 1983; Kimiti et al. 2017). The goal of this study was to configure an effective seed mix for revegetating highly erodible lands using comparable ecological sites as the basis for the mix and to determine how the mixes would impact seedling establishment, native and introduced species richness, and native and introduced species ground cover.

This study consisted of broadcast seeding three different seed mixtures; a North Dakota National Guard mix, a standard mix, and a specialty mix. The National Guard mix represented the mixture that had been historically used for all revegetation projects at the site. The standard mix was created using Natural Resource Conservation Service ecological site descriptions for the area, while the specialty mix used site descriptions from the Badlands Region of North Dakota. In addition to seeding the plots with just the mixes, a fourth treatment was created by spreading a layer of topsoil before seeding with the standard mix. Seeding occurred in July of 2019 and seedling counts were conducted the following month. In July of 2020, an herbicide application

was randomly applied to half of each treatment to investigate the impacts of the herbicide on introduced and native species cover. In August of 2020 surface coverage data by species was collected and analyzed. Predictions for the study were that the standard seed mix paired with a topsoil addition would result in the highest seedling establishment, native species richness, and native species cover. This prediction is based on studies that have found using reference sites that closely match the present conditions will result in higher seedling establishment success and also the addition of topsoil will provide a functioning substrate that contains nutrients and soil microorganisms required for seedling establishment (White and Walker 1997; Rokich et al. 2000; Holmes 2001).

### **Literature Review**

Across the United States the Department of Defense manages roughly 4.6 million ha of land that it uses for its military bases, training ranges, testing grounds, etc. (Vincent et al. 2014). As required by the National Environmental Policy Act (NEPA), the military is responsible for ensuring that significant short-term and long-term impacts on the environmental conditions and natural resources are minimized. They are required to maintain ecological diversity, the productivity of the land, as well as conserve species that are listed by the Threatened and Endangered Species Act along with their required habitat conditions (16 U.S.C. §§1531-1544; Goran et al. 1983). Further, Executive Order 13112 recommends that “Federal agencies prevent the introduction of invasive species, control existing invasive populations in a cost-effective and environmentally sound manner, and, whenever possible, restore native species and habitat conditions in ecosystems that have been invaded” (Exec. Order No. 13112, 1999). The Department of Defense has a responsibility to maintain the environmental conditions of their current lands to ensure usable training grounds are sustained for future utilization.

On military installations, the usage of large tracked vehicles is widespread, as they are a means for troop movement as well as construction. The use of these large machines has the potential to directly cause injury or death to plants, alter soil water and nutrient dynamics, and alter root penetration abilities along with seedling establishment (Voorhees et al. 1986; Alakukku and Elonen 1995). In a study done at a military base in the Mojave Desert, soil compaction and altered plant species compositions effects from a single tank pass could still be seen 40 years after operations were halted at the area (Prose 1985). The intensive use of heavy military equipment can cause areas to become entirely devoid of vegetation which can lead to increased erosion. A study at a military installation in Idaho that examined the impacts of M1A2 Abrams tank maneuvers on plant communities and soil loss found that a single turn with the tank removed the upper 15 to 38 centimeters of soil along with the vegetation (Grantham et al. 2001).

In systems where the topsoil has been removed or altered, it could take decades or centuries for soil genesis to occur to a point where there is a soil capable of supporting the previous community (Allen 1995). These soils will be challenging to revegetate due to low soil organic matter, poor soil moisture retention, and decreased nutrient cycling (Elseroad et al. 2003). Additionally, soils left exposed without vegetation or topsoil are susceptible to habitat degradation, soil compaction, extreme erosion, and sediment transport (Forman and Alexander 1998; Spellerberg and Morrison 1998; Coffin 2007). An option to amend this issue is to spread topsoil over the affected areas prior to revegetation (Tormo et al. 2007). The basis for this practice is the spread topsoil will contain a seed bank, plant propagules, nutrients, and soil microorganisms lacking from sites without topsoil, which will increase the likelihood of returning the soil to a functioning substrate (Rokich et al. 2000; Holmes 2001).

To address nutrient limitations in systems where topsoil has been spread or is lacking, soil amendments can be added, however, amendments can have negative effects on revegetation and nutrient cycling if used improperly (Ramlow et al. 2018). Organic fertilizers, wood strand mulch, and biochar are three commonly used amendments. Organic fertilizers are a relatively short term option that will increase nitrogen availability in the soil for initial revegetation, but should not be used excessively as they can promote invasive species over native species if usage rates are too high (Gendron and Wilson 2007). Wood strand mulch immobilizes excess nitrogen in the soil due to a high carbon to nitrogen ratio and gradually releases mineralized nitrogen over time, thus limiting invasive species while still allowing rapid recolonization by native species, along with the added benefit of also boosting soil water content (Blumenthal et al. 2003; Rhoades et al. 2017). Biochar is a material that is created from pyrolyzation of woody materials that can then be added to the soil (Kerré et al. 2016). Biochar has the benefits of allowing additional storage of carbon in the soil, increased soil water retention and plant-available water, increased nutrient holding capacity, and increased soil microbial abundance and diversity relative to untreated soil (Lehmann et al. 2011; Blanco-Canqui 2017; Jackson et al. 2017). The drawback of using biochar is it can limit the plant available nutrients derived from decaying organic matter, which studies have shown to be more negatively impactful on growth and establishment of grasses than forbs (Eschen et al. 2006; Lehmann et al. 2011; Adams et al. 2013).

In addition to soil nutrients, soil texture and structure play a large role in whether a revegetation project will be successful or not (Fehmi and Kong 2012). Variations in soil structure and texture will provide micro-environments, or safe sites, within the seedbed. Additionally, these safe-sites can be provided by surface cracks, depressions, and plant litter (Harper et al.

1965). Safe-sites provide better water availability and soil contact for seeds, protection from predation, and limit seed/seedling desiccation than if sown just on the soil surface (Nelson et al. 1970; Evans and Young 1972; Campbell and Swain 1973). While soil safe-sites are primarily applicable to broadcast seeding, oftentimes reclamation sites are too rocky or have too steep of slopes to effectively drill seed (Vallentine 1989). To increase seedling establishment on bare ground, straw mulch and wood chips can be added after seeding. These two applications have been found to reduce surface runoff, increase infiltration, lower extreme surface temperatures, and decrease evapotranspiration (Fehmi and Kong 2012; Fehmi et al. 2020).

When selecting which species to plant in a restoration project, it is important to make sure the seed mix is comprised of the correct species for the conditions present. Plants are more likely to withstand competition from invasive weeds if they are better adapted to a site (Van Epps and McKell 1983). Finding reference sites with similar conditions to the restoration site can be used to determine which species will likely establish during the revegetation process (White and Walker 1997). Historically, across semiarid regions in the United States, revegetation projects had a primary goal of providing maximum forage production while also remaining low cost, rather than restoring ecosystem functions. Seed mixes as a result were often comprised of non-native invasive species such as crested wheatgrass (*Agropyron cristatum*), smooth brome (*Bromus inermis*), and yellow sweet clover (*Melilotus officinalis*) (Simmers and Galatowitsch 2010; Redmond et al. 2013; Wood et al. 2015). These plantings often resulted in communities low in diversity with a major change in ecosystem function from native grasslands (Kirmer et al. 2012). Currently, restoration goals have shifted toward conservation, and as a result, mainly native species are planted (West 1993). Regardless of the goal, planting a diverse mix should yield better results than planting a monoculture, as studies have found higher

diversity leads to higher productivity, richness, limiting resource utilization, and cover (Naeem et al. 1995; Tilman et al. 1996; Hooper et al. 2005; Porkorny et al. 2005).

### **Methods**

The study site is located on the Tank Ditch Area of Camp Grafton South (CGS) in Eddy County, North Dakota (47°43'26.4" N, 98°39'40.4" W). The legal description is T149N, R63W, NE ¼ Section 13. This study site is 1.44 ha (3.56 acres) of what was rangeland prior to 1990 that has since been used by the North Dakota National Guard for heavy equipment operation training. The training consisted of using large machinery, such as bulldozers and scrapers, to construct anti-tank ditches and for other military maneuvers for 30 years. These practices resulted in the complete removal of the A soil horizon and the mixing of the lower soil horizons when the ditches were refilled. The removed top soil was not stockpiled or replaced at the site, instead it was spread on other sites at CGS or simply mixed with the other soil horizons. The site was also used for grazing by cattle between training operations.

The study site lies in the Transitional Grasslands prairie region on the boundary between the End Moraine Complex, which was formed by blocks of surface material being scraped off and thrust up by glaciers at the southern edge of the Devils Lake Basin, and the Drift Plain, formed from lacustrine deposits left behind by the retreating Wisconsinan glaciers (Barker and Whitman 1988; Bryce et al. 1996). The soil of the study site is currently classified as predominately composed of an Udorthents loamy, borrow area, with 0 to 15 percent slopes. This soil is classified as a loamy, mixed, superactive, calcareous, frigid Udorthents. The soil of the site is considered to be deep, well-drained, and is in the medium runoff class. The water table is considered to be very deep at more than 203 cm (80 in) below the soil surface. The site is

categorized as not prime farmland and not suited for forage in terms of forage suitability (USDA-NRCS 2020).

The climate of the study area is considered humid continental, characterized by cold winters and hot summers (Peel et al. 2007). The hundred-year (1919-2019) average air temperature at McHenry, ND, the closest available weather data station located 24 km (15 miles) southeast of CGS, was 4.78 °C (40.6 °F). The hundred-year average annual precipitation totaled 53.82 cm (21.19 in) per year with 75-77% falling as rain during the growing season (April through September) (Lym et al. 1997; USDA-NRCS 2006; NOAA 2019a).

The vegetation of this region is comprised of a mixed-grass and tallgrass prairie species matrix (Whitman and Wali 1975). The CGS graminoid communities are dominated by Kentucky bluegrass (*Poa pratensis* L.), smooth brome (*Bromus inermis* Leyss.), little bluestem (*Schizachyrium scoparium* Michx.), western wheatgrass (*Pascopyrum smithii* Rydb.), blue grama (*Bouteloua gracilis* Willd. Ex Kunth), and needle-and-thread (*Hesperostipa comata* Trin. & Rupr.). Common forbs found at CGS include narrowleaf purple coneflower (*Echinacea angustifolia* DC.), Flodman's thistle (*Cirsium flodmanii* Rydb.), stiff sunflower (*Helianthus pauciflorus* Nutt.), common yarrow (*Achillea millefolium* L.), groundplum milkvetch (*Astragalus crassicaulus* Nutt.), white prairie aster (*Symphotrichum ericoides* (L.) G.L. Nesom), curlycup gumweed (*Grindelia squarrosa* Pursh.), purple prairie clover (*Dalea purpurea* Vent.), Missouri goldenrod (*Solidago missouriensis* Nutt.), dotted blazing star (*Liatris punctata* Hook.), Canada goldenrod (*Solidago Canadensis* L.), Canada thistle (*Cirsium arvense* L.), and leafy spurge (*Euphorbia esula* L.) (Lym et al. 1997; Prosser 1998; Prosser 2003). Leafy spurge prevalence is high at CGS with some areas experiencing 80% cover (Lym et al. 1997).

The study site consists of 1.44 ha in size, divided into four blocks. The site is located on a slight, gradual slope with block one at the highest position blocks two, three, and four at progressively lower positions on the slope. Along this slope there are noticeable signs of rill surface erosion. Each block consisted of four 30m × 30m plots randomly selected to be treated with one of the four treatments. The four treatments consisted of a 5.08 cm (2 in) deep topsoil addition to the soil surface with a standard seed mix, the standard seed mix applied alone, a specialty seed mix, and the National Guard mix. The entire site was fenced off before treatment application to exclude cattle from grazing.

In 2020, the four plots in each block were split into subplots with one subplot randomly selected for an herbicide treatment to determine the effects of a leafy spurge control measure and the other subplot left unsprayed (Figure 2.1). Herbicides used in this study were glyphosate paired with methylated seed oil (MSO) (Southern Agricultural Insecticides, Inc. Worldwide Rights Reserved) at a rate of 2.33 L/ha (32 fl oz/ac) and 1.75 L/ha (23.95 fl oz/acre) respectively. Facet® (BASF Corporation, Worldwide Rights Reserved) paired with Overdrive® (BASF Corporation, Worldwide Rights Reserved), and MSO sprayed at a rate of 4.68 L/ha (64 fl oz/acre), 0.42 kg/ha (6 oz/acre), 1.75 L/ha (23.95 fl oz/acre) was applied in year two of the study. All herbicide was applied using a boom sprayer.

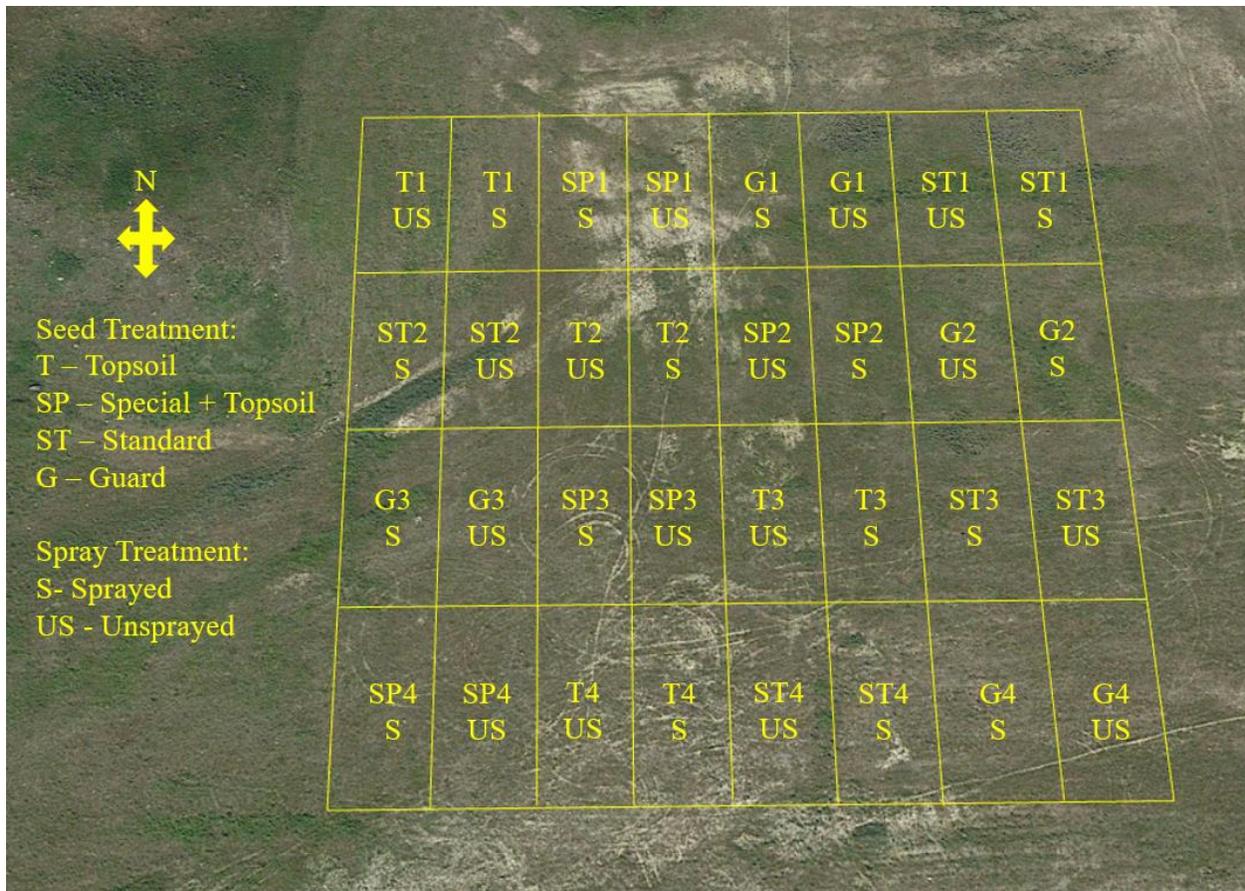


Figure 2.1. Camp Grafton treatment and spray application layout.

The topsoil addition was done using topsoil that had been stored in a pile for over a year from an area of CGS within 3.22 km of the study site and can be seen in Figure 2.2. Two rounds of glyphosate were applied on June 19<sup>th</sup> and June 26<sup>th</sup>, 2019, to eradicate vegetation growing at the site prior to seeding. After the herbicide was applied, a soil conditioner was used on the study site to knock down and remove the remaining standing vegetation as well as break up the soil surface to create an appropriate seedbed.



Figure 2.2. Picture of the Camp Grafton study site. The darker squares depict the topsoil addition treatments.

Seeding was done using a broadcast seeder with the seeding rate dependent on the seed mix (Tables 2.1, 2.2, and 2.3). Once seeding was complete, the entire study site was raked with a tractor-mounted harrow. All seeding was done on July 7<sup>th</sup>, 2019. At the time of planting the study site was classified as abnormally dry which can result in the slowing of planting and growth of crops and pastures (NOAA 2019b). The specialty seed mixture was derived from the Natural Resource Conservation Service – Ecological Site Descriptions for MLRA 58C, due to that region having highly erodible soils similar to the study site. The special mix consisted of ten different native species of grasses, five native forbs, and one native sedge. The standard seed mix was derived using the Natural Resource Conservation Service – Ecological Site Descriptions for MLRA 55b and consisted of seven different native grasses and five different native forbs (USDA-NRCS 2019). The National Guard mix was the standard mix the North Dakota National Guard had been using at CGS and consisted of seven grass species, not all of which are native,

and one non-native clover. The purpose of this mix was to prioritize quick establishment and forage production at CGS. All of the seed mixes were sourced from Millborn Seeds in Brookings, SD.

Table 2.1. Species composition and seeding rate for the specialty mix.

<b>Common Name</b>	<b>Scientific Name</b>	<b>Seeding Rate (kg/ha)</b>
Little Bluestem - MN native Badlands	<i>Schizachyrium scoparium</i>	5.949
Thickspike Wheatgrass - certified Critana	<i>Elymus lanceolatus</i>	2.852
Sideoats Grama - Pierre	<i>Bouteloua curtipendula</i>	2.050
Bluebunch Wheatgrass - certified Goldar	<i>Pseudoroegneria spicata</i>	1.348
Canada Wildrye - Mandan	<i>Elymus canadensis</i>	1.158
Indian Ricegrass - certified Rimrock	<i>Achnatherum hymenoides</i>	0.917
Western Yarrow	<i>Achillea millefolium</i>	0.657
Bluebunch Wheatgrass - Anatone	<i>Pseudoroegneria spicata</i>	0.652
Sand Dropseed - ND native sourced	<i>Sporobolus cryptandrus</i>	0.531
Prairie Junegrass	<i>Koeleria macrantha</i>	0.366
Blue Grama - Bad River	<i>Bouteloua gracilis</i>	0.326
Prairie Dropseed - MN native sourced	<i>Sporobolus heterolepis</i>	0.246
Blanketflower	<i>Gaillardia aristata</i>	0.210
Brown Fox Sedge - IA native sourced	<i>Carex vulpinoidea</i>	0.100
Prairie Onion - IA native sourced	<i>Allium stellatum</i>	0.090
Dotted Blazing Star - ND native sourced	<i>Liatris punctata</i>	0.070
Prairie Sage - IA sourced	<i>Artemisia frigida</i>	0.010

Table 2.2. Species composition and seeding rate for the standard mix.

<b>Common Name</b>	<b>Scientific Name</b>	<b>Seeding Rate (kg/ha)</b>
Little Bluestem - Badlands	<i>Schizachyrium scoparium</i>	6.926
Western Wheatgrass - certified Rosane	<i>Pascopyrum smithii</i>	3.498
Slender Wheatgrass - certified Revenue	<i>Elymus trachycaulus</i>	3.368
Sideoats Grama - Pierre	<i>Bouteloua curtipendula</i>	2.386
Switchgrass - Sunburst	<i>Panicum virgatum</i>	1.133
Prairie Junegrass	<i>Koeleria macrantha</i>	0.431
White Prairie Clover - MN native sourced	<i>Dalea candida</i>	0.281
Blue Gramma - Bad River	<i>Bouteloua gracilis</i>	0.251
Prairie Coneflower - IA native sourced	<i>Ratibida columnifera</i>	0.170
Canada Milkvetch - ND native sourced	<i>Astragalus canadensis</i>	0.110
Western Yarrow	<i>Achillea millefolium</i>	0.050
Prairie Sage - IA native sourced	<i>Artemisia frigida</i>	0.010

Table 2.3. Species composition and seeding rate for the National Guard mix.

Common Name	Scientific Name	Seeding Rate (kg/ha)
Jerry Oats	<i>Avena sativa</i>	12.178
Western Wheatgrass - certified Rosana	<i>Pascopyrum smithii</i>	2.977
Big Bluestem - Bison	<i>Andropogon gerardii</i>	2.345
Sideoats Grama - Pierre	<i>Bouteloua curtipendula</i>	2.285
Green Needlegrass - Lodorm	<i>Nassella viridula</i>	2.215
Yellow Blossom Sweet Clover	<i>Melilotus officinalis</i>	2.205
Crested Wheatgrass - certified Hycrest	<i>Agropyron cristatum</i>	1.694
Switchgrass - Sunburst	<i>Panicum virgatum</i>	1.443

Initial seedling counts were conducted on August 15<sup>th</sup>, 2019 using a 0.25 m<sup>2</sup> quadrat randomly placed four times per plot for a total of 64 samples. The number of grass and forb seedlings were counted within each quadrat. One-way analysis of variance (ANOVA) was employed in SAS Enterprise Guide 7.1 (Copyright © 2017 by SAS Institute Inc. Cary, NC, USA) to determine whether forb and/or grass seedling establishment was affected by seed mix treatments (guard mix, special mix, standard mix, and topsoil) in 2019. In addition, Tukey's HSD tests were used to make pair-wise comparisons of seedling counts between seed mix treatments (SAS Enterprise Guide 7.1 (Copyright © 2017 by SAS Institute Inc. Cary, NC, USA)).

The year two herbicide application was completed on June 15<sup>th</sup>, 2020, roughly one month before the surface coverage data collection. On August 4<sup>th</sup> and 5<sup>th</sup>, 2020, a surface coverage survey was conducted utilizing six 1 m<sup>2</sup> quadrats per plot (three per spray/unsprayed subplot) for a total of 96 samples. Foliar ground cover percentages were determined by each species present

within the quadrat. Sampling was distributed randomly throughout the plot/subplot. The cover data collected in the field was used to determine native species richness, introduced species richness, leafy spurge relative ground cover, introduced species relative ground cover, and native species relative ground cover.

ANOVA was used to determine whether native species richness, introduced species richness, leafy spurge relative ground cover, introduced species relative ground cover, and native species relative ground cover were affected by seed mix treatments (guard mix, special mix, standard mix, and topsoil) and/or herbicide applications (sprayed or unsprayed) (SAS Enterprise Guide 7.1 (Copyright © 2017 by SAS Institute Inc. Cary, NC, USA)). Tukey's HSD tests were again used to make pair-wise comparisons (SAS Enterprise Guide 7.1 (Copyright © 2017 by SAS Institute Inc. Cary, NC, USA)).

### **Results**

ANOVA indicated ( $F = 17.73$ ,  $P = 0.0002$ ,  $DF_{\text{effect}} = 6$ ,  $DF_{\text{error}} = 9$ ) that 2019 forb seedling counts responded to the seed treatment type ( $P < 0.0001$ ). The guard treatment increased the average number of forb seedlings over the special, standard, and topsoil treatments (Figure 2.4).

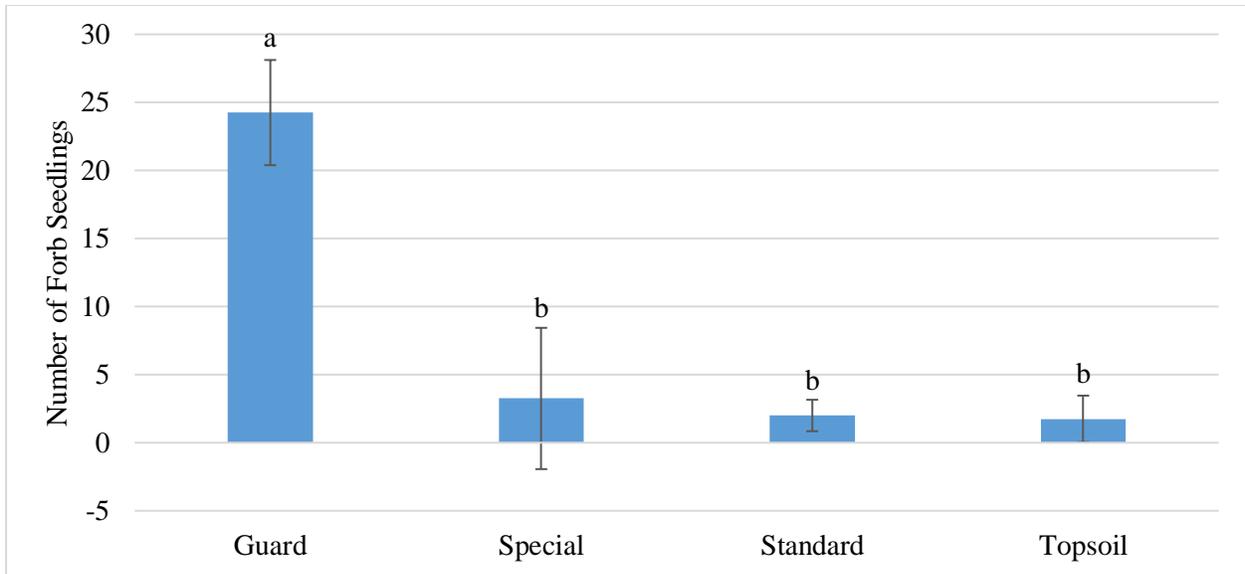


Figure 2.3. Average number of forb seedlings ( $\pm$  standard deviation) by treatment in 2019. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

While ANOVA indicated ( $F = 3.62$ ,  $P = 0.0413$ ,  $DF_{\text{effect}} = 6$ ,  $DF_{\text{error}} = 9$ ) that 2019 average grass seedling counts responded to the seed treatment type, the Tukey's HSD tests found no significant differences ( $P = 0.052$ ) in the pair-wise comparisons of seed treatment types (Figure 2.4).

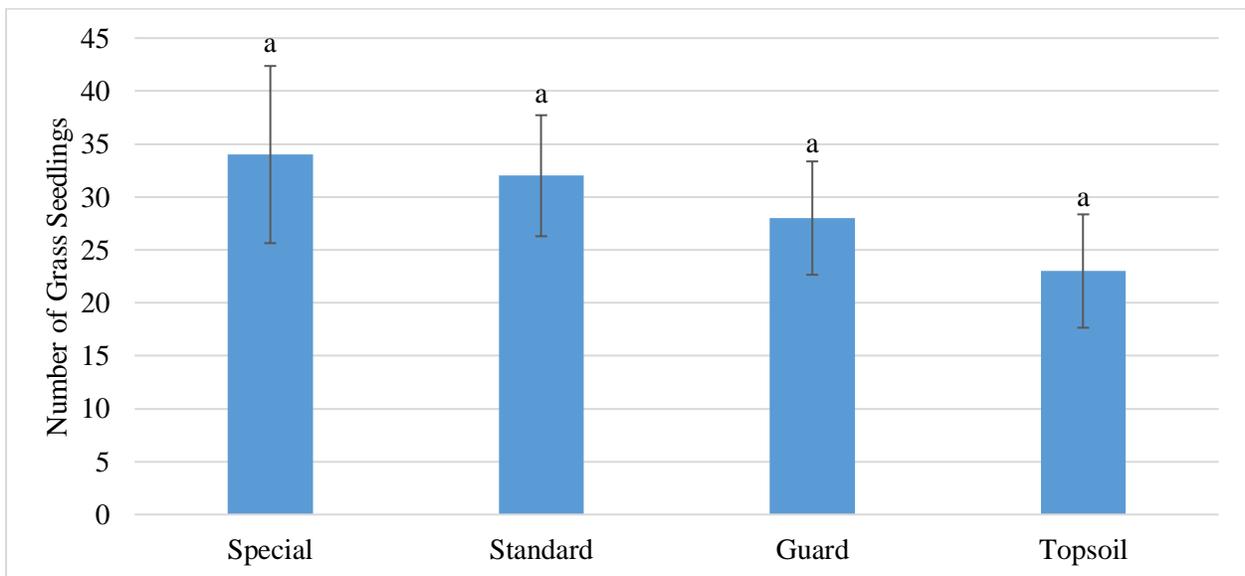


Figure 2.4. Average number of grass seedlings ( $\pm$  standard deviation) by treatment in 2019. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

In 2020, ANOVA indicated ( $F = 2.77$ ,  $P = 0.0376$ ,  $DF_{\text{effect}} = 19$ ,  $DF_{\text{error}} = 12$ ) average native species richness was influenced by seed treatment type ( $P = 0.0163$ ) and block ( $P = 0.014$ ). The topsoil treatment increased average native species richness over the guard treatment (Figure 2.5) and block two possessed higher average native species richness than blocks three and one (Figure 2.6). Herbicide application did not affect average native species richness ( $P = 0.9543$ ).

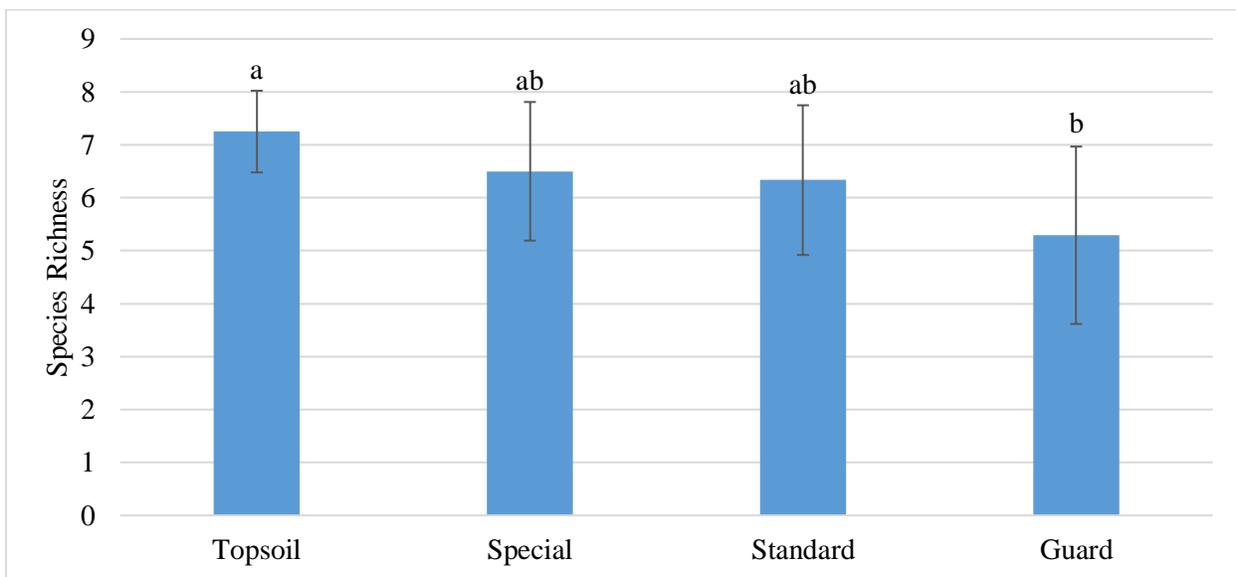


Figure 2.5. Average native species richness ( $\pm$  standard deviation) by treatment per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

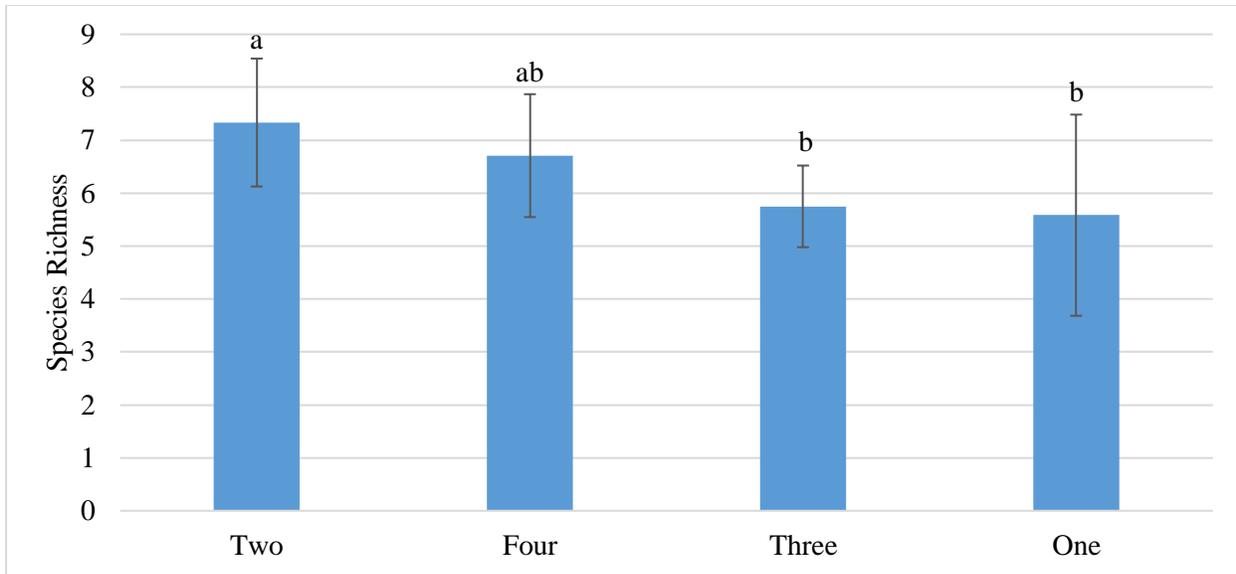


Figure 2.6. Average native species richness ( $\pm$  standard deviation) by block per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

ANOVA indicated ( $F = 5.58$ ,  $P = 0.002$ ,  $DF_{\text{effect}} = 19$ ,  $DF_{\text{error}} = 12$ ) that average native species relative ground cover was affected by seed treatment type ( $P < 0.0001$ ) and herbicide application ( $P = 0.0233$ ) in 2020. The topsoil, standard, and special seed mix treatments increased average native species relative ground cover over the guard mix treatment (Figure 2.7). Average native species relative ground cover was higher in sprayed subplots of the treatments over subplots left unsprayed (Figure 2.8).

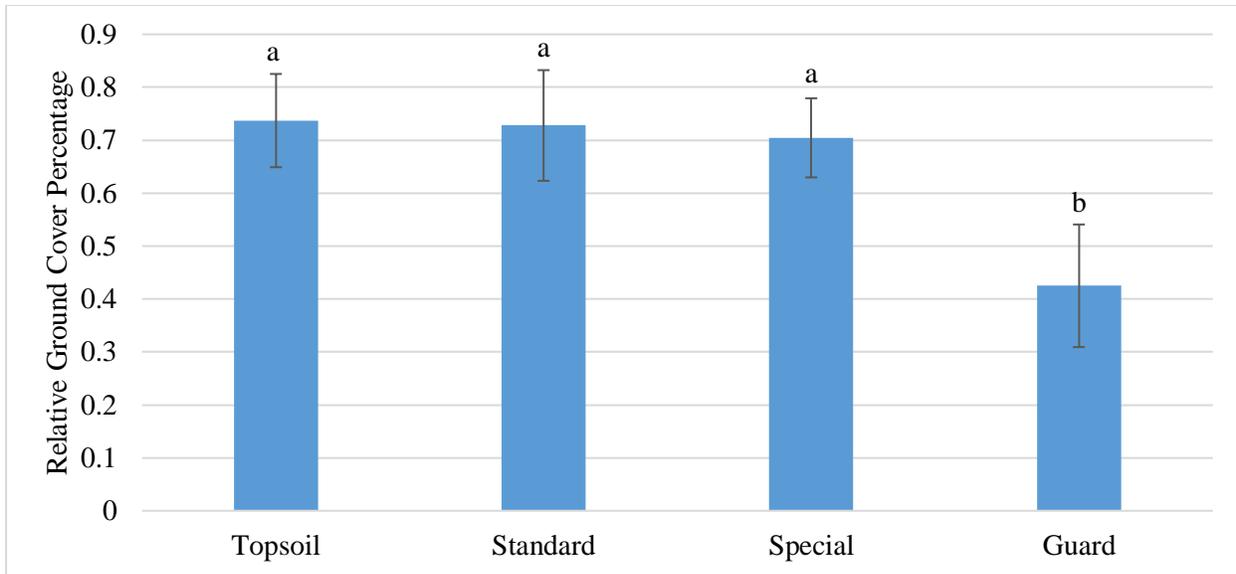


Figure 2.7. Average native species relative ground cover ( $\pm$  standard deviation) by treatment per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

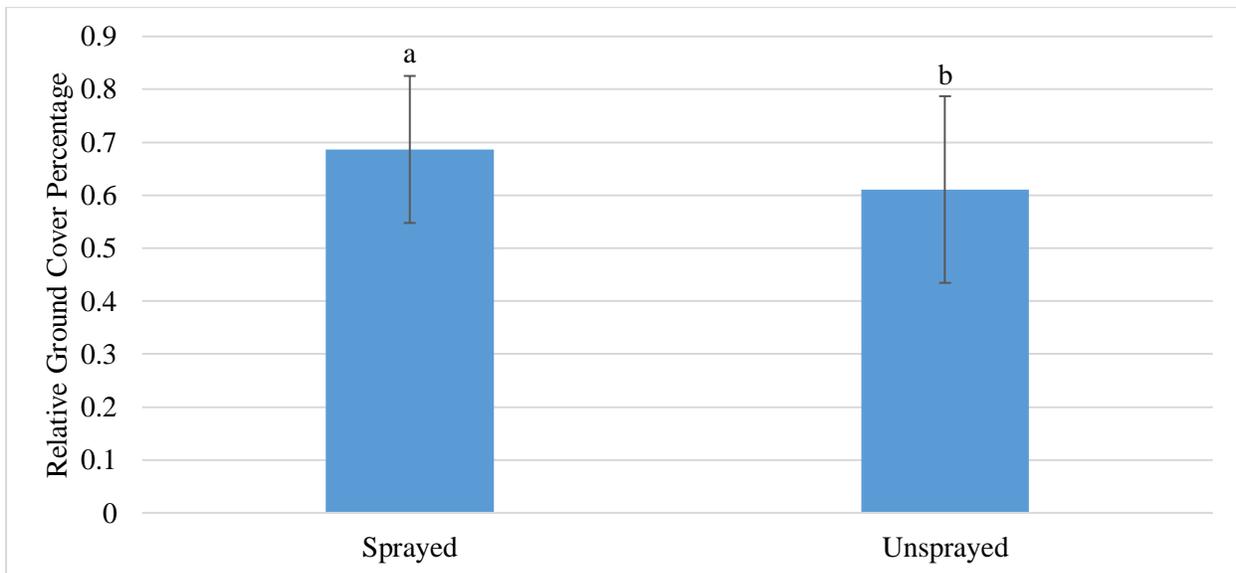


Figure 2.8. Average native species relative ground cover ( $\pm$  standard deviation) by herbicide application per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

ANOVA indicated ( $F = 2.73$ ,  $P = 0.0395$ ,  $DF_{\text{effect}} = 19$ ,  $DF_{\text{error}} = 12$ ) that 2020 average introduced species richness was influenced by herbicide application ( $P = 0.0035$ ) and block ( $P = 0.0013$ ). Average introduced species richness was higher in the unsprayed subplots of each plot than in subplots that were sprayed (Figure 2.9). Block four had higher average introduced species

richness than blocks 3 and 1 (Figure 2.10). Block two resulted in higher average introduced species richness over block one (Figure 2.10).

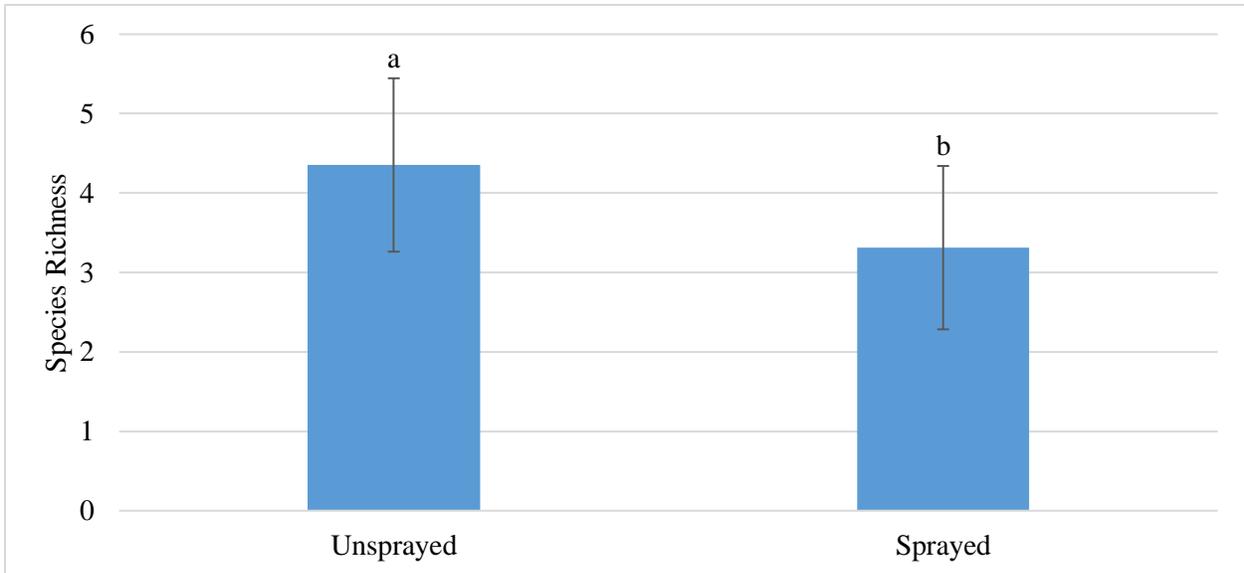


Figure 2.9. Average introduced species richness ( $\pm$  standard deviation) by herbicide application per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

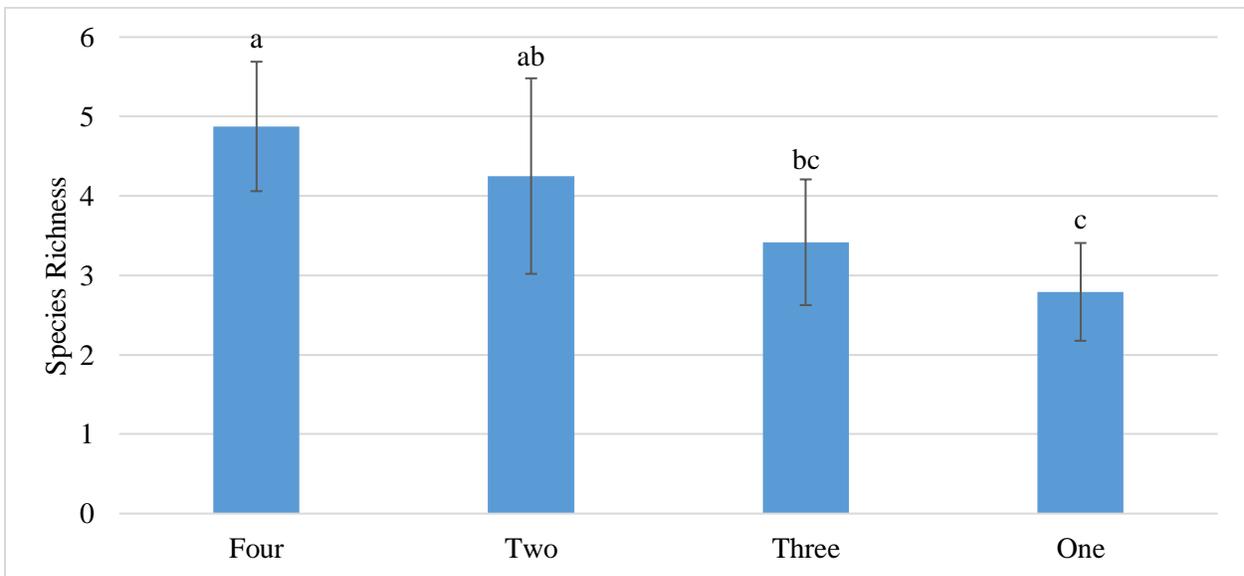


Figure 2.10. Average introduced species richness ( $\pm$  standard deviation) by block per  $m^2$ . A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

ANOVA indicated ( $F = 5.58$ ,  $P = 0.002$ ,  $DF_{\text{effect}} = 19$ ,  $DF_{\text{error}} = 12$ ) that the 2020 average introduced species relative cover responded to treatment type ( $P < 0.0001$ ) and the herbicide

application ( $P = 0.0233$ ). Average introduced species cover was lower in the special, standard, and topsoil treatments than the guard seed mix treatment (Figure 2.11). Average introduced species relative ground cover was higher in unsprayed subplots than sprayed subplots (Figure 2.12).

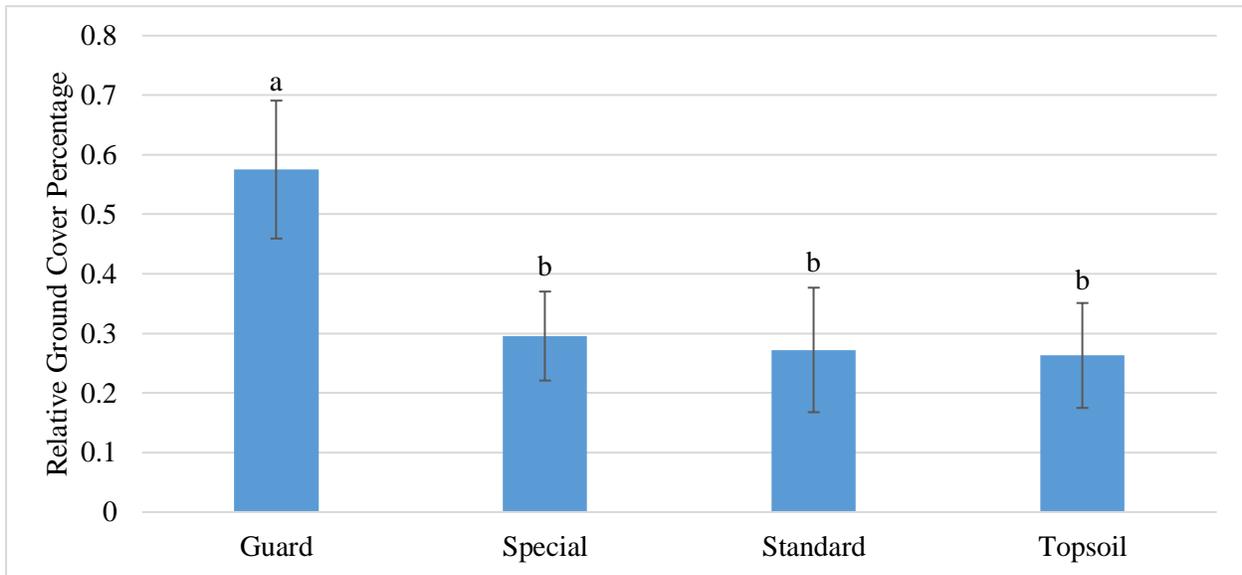


Figure 2.11. Average introduced species relative cover ( $\pm$  standard deviation) per  $m^2$  by treatment. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

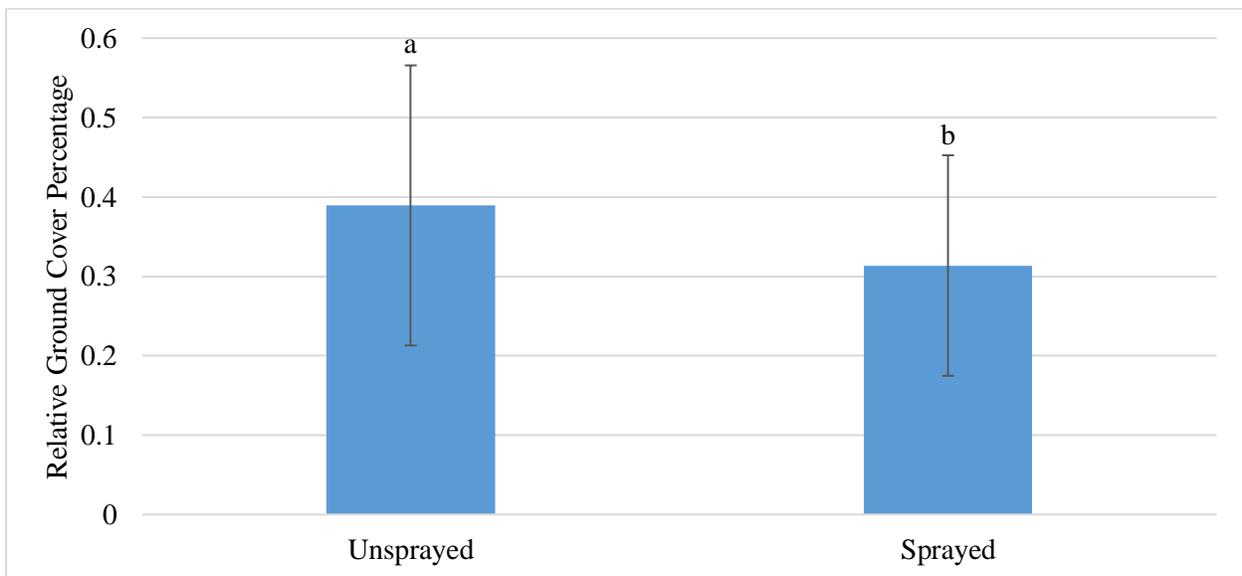


Figure 2.12. Average introduced species cover ( $\pm$  standard deviation) per  $m^2$  by herbicide application. A statistical difference at  $\alpha = 0.05$  is indicated by different letters.

ANOVA did not yield a significant model for 2020 leafy spurge ground cover ( $F = 1.19$ ,  $P = 0.3896$ ,  $DF_{\text{effect}} = 19$ ,  $DF_{\text{error}} = 12$ ).

### **Discussion**

The goal of this study was to determine a seed mix for revegetating rangelands subject to high rates of erosion that had high seedling establishment, high native species diversity and cover, and low introduced species cover and diversity. Our initial prediction was that treatment blocks subjected to a topsoil addition and a standard mix would best meet the study goals. Even though the guard seed mix resulted in the highest forb seedling counts in the first year of the study, this was the result of the successful establishment of non-native yellow sweet clover, which was the only forb in the mix. There were no differences between seed mix treatments for grass seedling counts. The initial prediction that the topsoil and standard seed mix combination would have the greatest seedling establishment because it provided a viable substrate for the seeds to grow from, was incorrect as the topsoil scored the lowest total for both the forb and grass seedling counts, albeit a non-significant difference from the standard only and special mixes.

While our initial prediction was incorrect for seedling establishment, our prediction that the topsoil and standard seed mix combination would result in the highest species richness and native species cover was partially borne out. The topsoil and standard mix resulted in higher ( $p \leq 0.05$ ) species richness than the National Guard mix but did not differ in richness from the special and standard only mixes. This is not surprising, as the topsoil and standard mix combination had the highest species diversity while also providing a viable substrate for plant growth. This finding is mirrored by Hooper et al. (2005) who found seed mixes with higher richness led to increased diversity due to a broader range of limiting resource utilization. Like average native

species richness, the average native species relative ground cover was higher ( $p \leq 0.05$ ) in the blocks treated with the topsoil and standard mix than the Guard mix. The topsoil treatment was also higher ( $p > 0.05$ ) than the standard only and special mixes. The addition of topsoil prior to seeding can provide nutrients, organic matter, and soil microbiota that can otherwise limit successful seed propagation in sites that are lacking topsoil (Rokich et al. 2000; Holmes 2001), and could explain the higher richness and native species cover observed in topsoil treated blocks.

The seed mix treatments not only had impacts on average native species relative cover, but it also impacted average introduced species relative cover. Our study found the guard mix had much higher ( $p \leq 0.05$ ) average introduced species relative ground cover than the special, standard, and standard with topsoil treatments. This difference is likely the result of the inclusion of several introduced species in the guard mix. Another factor that could have contributed to the higher introduced species cover in the guard mix treated plots is the high prevalence of yellow sweet clover and its role as a nitrogen fixer. Studies by Lesica and DeLuca (2000) and Van Riper et al. (2010) have found yellow sweet clover readily establishes in the Northern Great Plains and fixes nitrogen at much higher rates than native legumes. If native plant species have low nitrogen requirements then they can be outcompeted by plant species that have high nitrogen requirements when yellow sweet clover is present because of increased nitrogen availability. This was shown by Dornbusch (2018) who found that this excess nitrogen can facilitate a change in the competitive balance to a system that favors plants suited for high nitrogen levels and invasion by introduced species.

Between the two sampling events, we observed leafy spurge invading the test plots and responded with a Facet (quinclorac) herbicide application. Results showed the average native species relative ground cover was higher ( $p \leq 0.05$ ) in the subplots that received the herbicide

versus those that were not sprayed. Additionally, sprayed subplots showed lower ( $p \leq 0.05$ ) average introduced species richness and average introduced species ground cover. The herbicide used in this study has a narrow spectrum of species it will control with the benefit of having minimal impacts on native grasses and forbs and has been found by Enache and Ilnicki (1991) and Erikson et al. (2006) to be an effective control measure for leafy spurge in rangeland settings. These studies mirror this study in a way that quinclorac effectively decreased invasive species growth and establishment while not having any apparent negative impacts on native species.

A final observation from this study was there were differences found in introduced and native species richness between blocks. Block four had higher ( $p \leq 0.05$ ) average introduced species richness than blocks one and three while block two had higher ( $p \leq 0.05$ ) average native species than blocks one and three. This could be attributable to natural variations within the site.

Studies have shown the use of diverse seed mixtures matched to the present site conditions can be effective for revegetating rangelands with degraded soils and the effectiveness of the seed mixture can be increased by pairing the mix with a topsoil application (Van Epps and McKell 1983; Holmes 2001; Kimiti et al. 2017). However, at this point of the study, there is no evidence that the special site adapted seed mix performed better than the standard mix in terms of grass and forb seedling establishment, native species relative ground cover, introduced species relative ground cover or native species richness. The findings in this study also point to a change being needed to the CGS management plan as the seed mix they had been using tallied the poorest results across every metric examined in this study except for forb seedling establishment. This research has also indicated that targeted herbicide application for problem species has benefits for desired native species establishment.

The scope of this study is limited at this point because there has only been one-year post-seeding that has passed and the effects of restoration may not yet be fully apparent at the timescale of this study. Further research is required to determine how the effects of a combination of a specially formulated seed mix, topsoil additions, and introduced species control develop as time progresses.

### **Conclusion**

This study sought to test the effectiveness of several different seeding mixtures as well as the addition of a topsoil layer for revegetating highly erodible rangelands using ecological site descriptions. Initial data shows the use of a ecoregionally adapted seed mixture paired with a pre-seeding topsoil addition can result in higher native species diversity and native species relative ground cover compared to the other treatments. We have also determined the use of a post-seeding herbicide application one-year after seeding to be effective for controlling introduced species with minimal impact to desirable native species. Additional investigations on how the plant communities develop over the course of the restoration will be crucial to determine the overall effectiveness of the treatments for revegetating this heavily degraded site.

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