INVASIONS IN THE PRAIRIE POTHOLE REGION: ADDRESSING THE EFFECTS OF EXOTIC PLANTS ON WETLAND AND GRASSLAND ECOSYSTEMS AND RESTORATION EFFORTS

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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

Three wetland restoration methods: seeding, seeding + hay mulch, and seeding + hay mulch + vegetation plugs were compared via the plant community within a formerly cropped wetland in southeastern North Dakota. Arrangement of plugs were also compared to assess the success of native species establishment. Mean relative cover for native species and introduced species were recorded and analyzed to compare the restoration methods and plug arrangement. Three herbicide treatments were studied on upland prairie sites with and without prescribed burning to test effects on leafy spurge (*Euphorbia esula*) control and seeded native establishment. There is no difference native species richness between the restoration methods six years post restoration, and no difference in plant cover in the different arrangement of plugs. Quinclorac significantly reduced leafy spurge cover; however, glyphosate treatments had higher cover of seeded native species.

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Introduction

Ecological Services Provided by Wetlands

Wetlands provide a wide array of services that conserve biodiversity and benefit organisms within and outside the habitat's boundaries (Bobbink et al. 2006; Mitsch & Gosselink 2007). Wetlands can function as the foundation for food webs that take place in the area. One example can be seen in marshes, where cordgrass (*Spartina*) and rush (*Juncus*) species provide food for herbivorous insects, which are then eaten by frogs, whose tadpole larvae feed the waterfowl, whose eggs then become food for the red fox (Mitsch et al. 2009). To add another branch to the complex network of energy transfers, decaying matter from plants and wildlife provide tissue for decomposers such as bacteria, fungi, and Oligochaete worms to break down and release nutrients back into the environment. The hydrology and fertile soils of wetlands allow diverse plant species to flourish, providing protective cover for wildlife. Faulkner et al. (2011) found seventy migratory bird species, two of those being the wood duck (*Aix sponsa*) and mallard duck (*Anas platyrhynchos*), rely on the wetlands' specific hydrologic regime and plant communities to provide favorable breeding habitats and reliable resources. Steen et al. (2012) & (2014) conducted a survey focusing on the effects climate change are having on the environment and bird populations in the Prairie Pothole Region (PPR). They found several bird species of

1 The material in this chapter was co-authored by Cheyenne Durant, Dr. Shawn DeKeyser, and Breanna Kobiela. Cheyenne had primary responsibility for conducting surveys in the field and analyzing the data collected from these surveys. Cheyenne was the primary developer of the conclusions that are advanced here. Cheyenne also drafted and revised all versions of this chapter. Dr. DeKeyser served as proofreader and Breanna checked the math in the statistical analysis conducted by Cheyenne.

significance like the black tern (*Chlidonias niger*) depend primarily on prairie potholes in North and South Dakota for breeding and foraging grounds. A more recent estimate shows a larger range of migrating waterfowl that depend heavily on the PPR's specific hydrologic regime and plant communities. One hundred fifteen species of North America's waterfowl and water bird, including the dabbling and diving duck, populations migrate through the PPR. They seek out these wetlands for their breeding grounds, as the vegetation provides adequate cover, forage, and habitat for the aquatic insects they feed on.

The productivity varies among wetlands and their components. Wetland plant communities with herbaceous species, like wooly sedge (*Carex pellita*) and cordgrass, rival tropical rainforests in having extremely high productivity (Cronk & Fennessy 2001; Marani et al. 2006). In the PPR, this high productivity can be attributed to its unique formation and connections with the grasslands (Dyke & Prest 1987; Dahl 2011; Van Meter & Basu 2015; Cohen et al. 2016). The retreat of glaciers from the Wisconsin Glacial Episode carved depressions throughout the area. These depressions, called potholes, gather water and materials from the surrounding grasslands, functioning as wetlands. These depressions extend over an area of 700,000 kilometers from Alberta, Canada, to the Dakotas, Minnesota, and northcentral Iowa. Historically these potholes covered about sixteen to eighteen percent of the PPR (Dyke & Prest 1987; Dahl 2011; Van Meter & Basu 2015; Cohen et al. 2016). There are more than 2.5 million wetlands in the region, making it the one of the largest wetland complexes in North America (Van der Valk 2005; Keddy et al. 2009).

Besides wildlife habitat, one of the more well-known wetland functions is water purification. Wetlands are given the name "Nature's kidneys" due to the soil's ability to break down chemical compounds found in fertilizers and pesticides carried with water flowing from the uplands (Mitsch & Gosselink 2015). The benefits of wetland plants and soils are extensive as their ability ranges from detoxifying polluted water systems to protecting shorelines from erosion.

Along with stabilizing the soil, native plants can lock away excessive carbon within their tissues, preventing the compounds from entering the atmosphere (Samson & Knopf 1994; Euliss et al. 2006; Stern et al. 2007). This makes wetlands some of the densest carbon sinks of all terrestrial ecosystems. While wetlands only cover about four percent of land surface area of the world, they are the largest store of carbon, locking about thirty-three percent of the globe's carbon within their organic soils. This makes them superior carbon sinks in comparison to forest trees in similar areas.

Wetland Losses and Consequences

Despite the vast benefits wetlands provide to the earth, humans were unaware of their value until immense damage had already been done. Dahl (1990) summarized how much of the colonial United States contained wetlands and how much was lost between the 1780's and 1980's. Before early settlement, the lower 48 states held 221 million acres of wetland. Within those two hundred years, the loss of intact wetland occurred at a rate of over sixty acres per hour. Samson & Knopf (1994) add further detail to the impact of negligence in protecting not only wetlands, but also the prairies surrounding them. Even more concerning is the amount of sequestered carbon that is released into the atmosphere when these wetlands are disturbed or destroyed (Dahl 2011; Gage et al. 2016).

These heavy losses place biodiversity at risk, including species not in proximity of the affected wetland(s). Craft et al. (2007) & Craft (2016) findings show that while on-site stressors involve change or removal of a wetland's hydrology, off-site stressors can include the timing and severity of flooding as well as increased agricultural activities such as fertilizer use.

Smaller wetlands, such as the depressions in the PPR, face the most drainage and run-off pollution. Van Meter & Basu (2015) showed that the loss of these smaller wetlands has a heavier impact on overall ecological services and biodiversity. Small wetlands process biochemicals and recharge ground water more efficiently than larger wetlands, as well as provide better habitat for wildlife and migratory birds. This is due to the smaller depressions' shorter hydroperiod which allows for potential prey (amphibian and insect larvae) yet limit competition from predatory fish (Van Meter & Basu 2015).

Research and models show that current trends for stressor influences for smaller wetlands could cause these wetlands to lose their ability to support reliant species (Oslund et al. 2010; Steen et al. 2014). Models based on these trends predict wetlands in North and South Dakota will be too dry to provide sufficient vegetation cover needed for breeding waterfowl, and total populations of these birds could be half than what they are currently. The mallard duck is one of the species that could be impacted the heaviest.

Mitsch & Day (2006) and Marton et al. (2014) found the alarming effects of wetland degradation and loss can extend far past the areas of drainage. With much of the United States' wetlands drained, their function of filtering excess nutrients is lost. The excess nitrogen found in fertilizers used on nearby cropland can flow freely into groundwater and streams, ultimately leading to oxygen depletion in rivers and open oceans. The Gulf of Mexico has a hypoxic zone of 20,000 square kilometers, which was created due to eutrophication of the waters in the Midwest flowing through the Mississippi River. This hypoxic zone has made much of the ocean inhospitable for marine life, which in turn impacts the fishing and tourism industry along the Gulf and neighboring coastal areas (Mitsch & Day 2006; Marton et al. 2014).

Invasive Species and Associated Complications in Wetland and Upland Restoration

Invasive plants have altered wetlands to the point where the whole ecosystem and its functions have changed permanently. Prairie Pothole Region wetlands are especially susceptible to invasion. Sheley et al. (2006) and Vinton & Goergen (2006) add that wetlands and grasslands in the Northern Great Plains have been altered to where usual processes favor invasive plant species. This is due to their service of harboring carbon enriched soils, large amounts of nutrients, sediments, salts, and heavy metals. The high concentrations of nutrients prove beneficial these invasive plants to propagate the area. The supply of nutrients allows more aggressive plants to form monotypes, out-competing the native species that once contributed to the wetland's original makeup (Galatowitsch & Van der Valk 1996a, 1996b; Seabloom & Van der Valk 2003; Mulhouse & Galatowitsch 2003; Aronson & Galatowitsch 2008; Zedler & Kercher 2010).

Even when restoring wetland areas, invasive species are a consistent concern and many have shown simply restoring a wetland's hydrology isn't enough (Galatowitsch & Van der Valk 1996a, 1996b; Seabloom & Van der Valk 2003; Mulhouse & Galatowitsch 2003; Aronson & Galatowitsch 2008; Zedler & Kercher 2010). Restored wetlands were shown to be distinct from intact wetlands and uplands in the fact they were dominated by invasive species. Zedler & Kercher (2010) not only support that while the reintroduction of a wetland's hydrology isn't enough to bring them back to their original functions, they found that invasive plants prevented restoration efforts from becoming truly successful.

While highly variable in appearance, invasive species share common traits which negatively impact the ecosystems and economies depending on these ecosystems. A definition of invasive species provided by Rejmánek & Richardson (1996) is a species that takes over a newly invaded area quickly and efficiently. The ability to do so can be attributed to traits invasive species share. These traits include high growth rate, short generation time, long photosynthetic periods, and rapid production of leaves (Rejmánek & Richardson 1996; Grotkopp & Rejmánek 2007).

Another part that contributes to the invasion of non-native plant species is the Enemy Release Hypothesis (Davis 2009). When these plants are introduced to new areas, they escape from their predators, parasites, and pathogens that usually keep populations in control in their native ranges. As these plants no longer need to allocate some of their resources to defense, they can distribute more resources towards reproduction. This gives the introduced species an advantage over native plants as native species must still contend with their specialist enemies (Davis 2009).

In order to address the difficulties of invasive plants cause in restoration efforts, one must understand and identify the main exotic species and how they take over wetlands. In North Dakota's PPR, there are two species that are causing the most degradation to wetland restoration efforts: reed canarygrass (*Phalaris arundinacea*) and hybrid cattail (*Typha x glauca*).

Hybrid Cattail

Larkin et al. (2011) describes hybrid cattails as having a similar history to that of reed canarygrass. This species of cattail is a hybrid of the native broad-leaved cattail (*T. latifolia*) and the European narrow-leaved cattail (*T. augustifolia*). Narrow-leaved cattail was first introduced in the Great Lakes region. Hybrid cattails share the same qualities of the other hybridized invaders such as the ability to form dense monocultures, rapid reproduction, and growth to displace native plants for resources. Hybrid cattails have an additional advantage over established species: leaving large amounts of biomass once the plants die. Farrer & Goldberg (2009) and Mitchell et al. (2011) provides detail on how the collected litter affects other plant species and the ecosystem. Spongy stems of hybrid cattails are slow to decompose and build up into layered mats, preventing establishment and access to sunlight and other resources to native species. The litter has shown to shade up to ninety-eight percent of available sunlight from the usually shorter, native species.

Hybrid cattails litter's extensive presence significantly impacts the composition of wetland communities, so much so that it effects the wetland's ability to function. Dense litter alters temperatures of the soil and the hydrological cycle of the wetland complex and how nutrients and other resources, such as sunlight, are allocated and exclude native plant species (Farrer & Goldberg 2009; Larkin et al. 2011; Mitchell et al. 2011). Hybrid cattails' negative impacts severely harm wetlands' ability to provide ecological services and can occur at a rapid rate. Mitchell et al. (2011) discovered that litter accumulation doubled in only ten years after hybrid cattail invasion, while species diversity fell more than fifty percent within 25 years of invasion.

Since hybrid cattails displace native species, the ecosystem's ability to provide for wildlife is severely compromised. While studies on specific wetland bird species do not reveal a consistent direct negative correlation with increasing cattail cover, they still endure effects these plants have on their habitat. Hybrid cattails take up much of a wetland's overall area, resulting in inhospitable conditions for species adapted to marshes and ponds (Kantrud 1986, 1992; Sojda & Solberg 1993; Solberg & Higgins 1993; Linz et al.1996a, b; Linz et al. 1997; Anderson et al.

2019). Native plants, such as sedges, provide ample amount of seeds to feed foraging water birds (Krapu & Reinecke 1992; Haukos & Smith 1993; Dugger et al. 2007; Greer et al. 2007; Hagy & Kaminski 2012) and these native plants are often outcompeted. Not only do aggressive hybrid cattails eliminate the variety of habitat options, but their small seeds make an inadequate replacement for food. Hybrid cattails also outcompete submerged vegetation, which provide energy-rich roots, tubers, and seeds, making them unavailable to dependent waterfowl (Krapu & Reinecke 1992).

Other significant prey animals, such as insects, tadpoles, and snails are also less accessible to the water birds and waterfowl, as they rely on the aquatic vegetation displaced by hybrid cattails (Krapu & Reinecke 1992; Anteau et al. 2011; Anteau 2012). Not only does hybrid cattails limit water availability for breeding amphibians, but hinders their larvae's growth. Studies show that wood frog (*Lithobates sylvaticus*) tadpoles do not develop into juveniles in areas dominated by hybrid cattails. Though the cause for this negative relationship isn't clearly understood, the slow decomposition of cattail stems, its resulting hypoxia and excessive carbon, phosphorus, and nitrogen is suggested to influence the halted development (Rose & Crumpton 1996; Christensen & Crumpton 2010; Stephens et al. 2013).

Reed Canarygrass

Nelson et al. (2013) identified reed canarygrass as a native species to North America; however, European varieties, repeatedly introduced accidentally and intentionally for agricultural and ornamental purposes, have crossbred with the North American ecotype. These hybrids have high genetic diversity, allowing them to adapt faster to the physical and chemical changes of the affected area. Along with high tolerance to varying environmental conditions, hybridized reed canarygrass has a long growing period, rapid growth rates, high productivity, and the ability to asexually reproduce.

Turner et al. (2013) attributed these advantages to hybrid vigor, which frequently leads to increased rates of growth and reproduction. Not only does the hybrid reed canarygrass have an advantage in its biology but also in the historical disturbance of the PPR. Restoring the hydrology in severely degraded areas provide open areas and these areas are then vulnerable to aggressive species such as the reed canarygrass (Lindig-Cisneros & Zedler 2002). Chen et al. (2017) notes reed canarygrass' aggressive invasion is exasperated by sediment and nutrient-rich soils caused by fertilizer run-offs from adjacent agricultural areas (Chen et al. 2017). Reports in 2012 show that the invasion of this aggressive variety of reed canarygrass has successively spread over forty-three states in the U.S., including those of the Northern Great Plains (USDA-NRCS 2019 PLANTS Database).

Restoration Methods

Passive restoration was once thought to be the answer in wetland restoration. It was a common practice to restore a wetland's hydrology (whether by removing drain tiles or filling drainage areas) and allow it to sit idle. This approach toward the restoration of PPR wetlands relied on the efficient community hypothesis, which states that native species once found in the area would eventually recolonize restored areas (Galatowitsch & Van der Valk 1996b). Galatowitsch and Van der Valk (1996b) compared the floristic composition of 10 restored wetlands with nearby wetlands in Iowa and found that species richness was lower in the seedbanks of restored areas. Wet meadow and submerged natives were also completely absent in restored wetlands. In a similar study, 37 restored wetlands in Iowa, Minnesota, and South Dakota were observed over a span of nineteen years with their floristic composition recorded during

1989, 1990, 1991, 2000, and 2007 (Aronson & Galatowitsh 2008). Aronson and Galatowitsch (2008) determined native wetland species' composition in restored areas are less than that of natural wetlands.

With multiple invasive species being of concern, finding the most effective method in controlling them is vital in curbing their harmful effects. Though there are a variety of methods in invasive species control such as burning, grazing, and herbicides, a combination of these methods could have the most impact with these resilient species.

Successful wetland and grassland restoration depend not only on finding the most cost-effective method in limiting the prevalence of invasive plants, but also finding the most effective method in reestablishing native plants. High costs are often an obstacle for restoration efforts, as overall costs can potentially outweigh the benefits. Direct costs in prairie and wetland restoration range from purchasing land, purchase of native seed and seedlings, labor involved with seeding, and pre- and post-restoration management. Indirect costs must also be considered; these costs can include the risk of the restoration effort failing and the loss of grazing potential as the newly planted seeds need time to establish themselves. Native seeds tend to be expensive due to their limited availability and are not selected for agricultural purposes (Rayburn & Laca 2013). Persuading private landowners to comply with restoration efforts is also difficult as the benefits require a long time to be visible and are marketable to only a few, ecologically-focused businesses.

One of the most common methods in restoration is applying native seed. Seedbanks of restored sites have lost traces of native species and need supplementation to make the return of these species possible (Van der Valk 2013). While applying additional seed can help native plants reestablish, active management is needed to prevent the invasive species, such as reed canarygrass, from taking over the seedbanks. If reed canarygrass is allowed to spread, it will once again displace the native seeds (Adams & Galatowitsch 2006). If managed properly, reestablished native plants are able to limit the space for competitive invasive plants (Lindig-Cisneros & Zedler 2002). Not only must one consider the management needed to keep invasive species from displacing natives in the seedbank but must also consider the quality of native seeds used. Applying a seed mix high in species diversity is more beneficial than applying a mix low in diversity (Leps et al. 2007).

Though the use of native hay to assist in planting seeds is a new practice, studies show that its use not only helped planted native species grow, but also inhibit the growth of invasive plants (Török et al. 2012). The hay's ability to protect the soil from drying facilitates an improved environment for native seeds, which often have a longer germination period than invasive weeds (Fowler 1988). The hay's coverage also prevents fluctuations of soil temperature, which invasive species use as a signal to germinate (Foster & Gross 1998). Transferring hay forms a barrier against not just changing temperatures, but against wind-dispersed invasive seeds and light for weeds as well (Wedin & Tilman 1993; Foster and Gross 1998). Applying native hay has another beneficial function, in which it can aid in applying more native seed into the area. Galatowitsch & Van der Valk (1996a) found that native species are limited in the ability to disperse. Native hay can overcome this limitation by keeping the seeds in place. For this to be successful, however, the hay must be cut at specific times. The best time to cut hay is when there is a high diversity and density of desired native species, and these species must be ready to seed (Kiehl & Wagner 2006; Rasran et al. 2006).

Native hay transfer has shown to facilitate the growth of native seeds, but the use of vegetation plugs also yield promise for both prairies and wetlands. Transplanting whole cores containing native plants, along with inter-seeding, have shown to increase species diversity, species richness, and success of restoration compared to seeding alone (Middleton et al. 2010). Though it is a highly preferred method, the high labor and monetary costs restrict the ability to apply vegetation plugs. Not only that, but these plugs must be removed from a healthy donor site. The removal leaves empty spaces that could be infiltrated by invasive weeds (Davis & Short 1997). Removing smaller plugs, rather than larger ones, could prevent continuous exposure. The prevention of habitat edge increase could also allow native species to recolonize the empty areas. Removing plugs from wetlands that will be removed during mitigation projects is another option as it prevents a total loss of wetlands (Strehlow 2015).

An experiment by Silliman et al. (2015) that yielded promise for native species occurred on the mud flats along the Florida and Netherlands coasts. The experiment tested how differing layout of plugs containing native grasses would survive and take root in these restored areas. The two layouts tested were having the plugs evenly dispersed and arranging them into clumps. Results showed that having the plugs clumped together increased survivorship and above-ground biomass of these native grasses. This success in both Florida and the Netherlands could be attributed to the positive interactions leading to increased healthy competition (Silliman et al. 2015). Since this experiment yielded positive results in the coastal wetlands, the question is could the same occur in wetlands in the PPR?

Methods

Study Area

Experiments regarding the effectiveness of different restoration techniques occurred on the Albert Ekre Grassland Preserve, which is in Richland County, North Dakota. This preserve is two kilometers east of the Sheyenne National Grassland and is at a Latitude of 46.526224 and

Longitude of -97.132370 (Strehlow 2015). The climate of the study site is continental humid, marked by having warm summers and cold winters, with varying temperatures and precipitation throughout the year. Average rainfall from 1980-2010 was 47.2 cm during the growing season of April through October (USDOC NOAA 2015). Average total rainfall for the growing seasons of the following years was: 2015 (43.87 cm), 2016 (48.44 cm), 2017 (39.01 cm), and 2018 (41.91 cm), and 2019 (54.69 cm) (NDAWN 2019). The soils on the study site can be described as sandy, formed by an ancient river that ran through the area and into the glacial Lake Agassiz (Strehlow 2015).

Experimental Design

The study site contains approximately 11 hectares and was divided into nine plots (three treatments by three replications). Replications were randomly assigned to the following treatments: 1) native seed only; 2) a combination of native seed and hay mulch from nearby native wet meadows; and 3) native seed, hay mulch, and vegetation plugs collected from nearby native wet meadows (Figure 1.1). Before installing the plot treatments, Glyphosate – Roundup Ready ® soybeans and glyphosate were used throughout an entire growing season to diminish the existing weed seedbank and provide a fresh start for the native seed and plugs. The vegetative plugs and seeds were applied during the summer and fall, 2014. A complete description of the experimental design including herbicide rates can be found in Strehlow (2015).

In the summers of 2015, 2016, 2017, 2018, and 2019, plant species surveys were conducted on these plots. The surveys were conducted along four permanent line transects located in the center of each replication (Figure 1.2). The initial transect lines were located randomly by generating a random number and multiplying by 360. The initial transect (i.e. created in 2015) was arranged utilizing a compass direction from the center of each replication, and the other transects would be located 90 degrees from the initial transect line. The identification of each plant species, Daubenmire Cover Class of each species, and percent cover of bare ground and litter were all ocularly estimated using a total of 13 - 1 m^2 quadrats (Daubenmire 1959). All species level data, bare ground, and litter were compared in blocking analysis test using SAS Enterprise Guide 6.1.

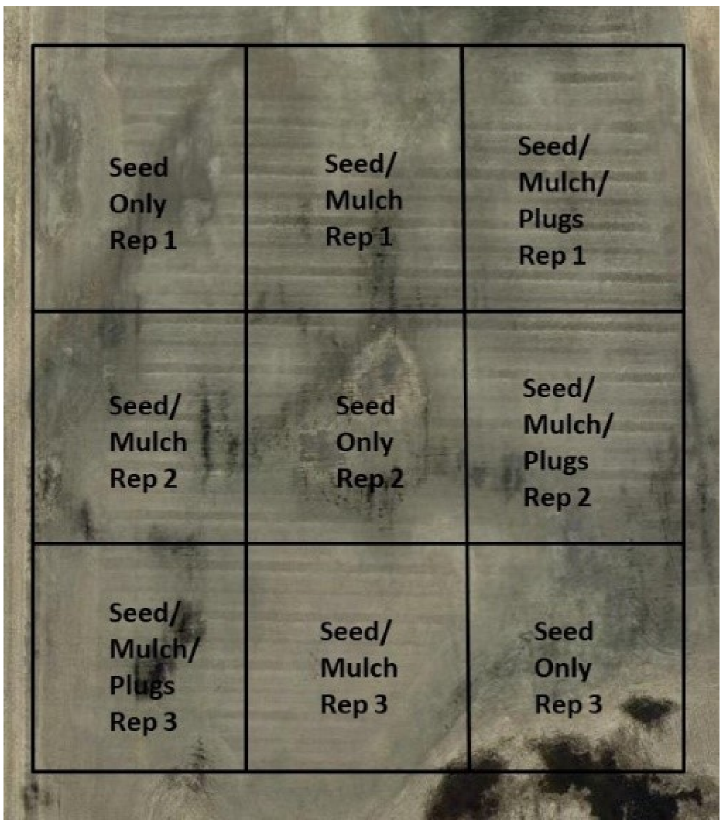

Figure 1.1. Treatment Design for Wetland Restoration Trial in Ransom County, North Dakota.

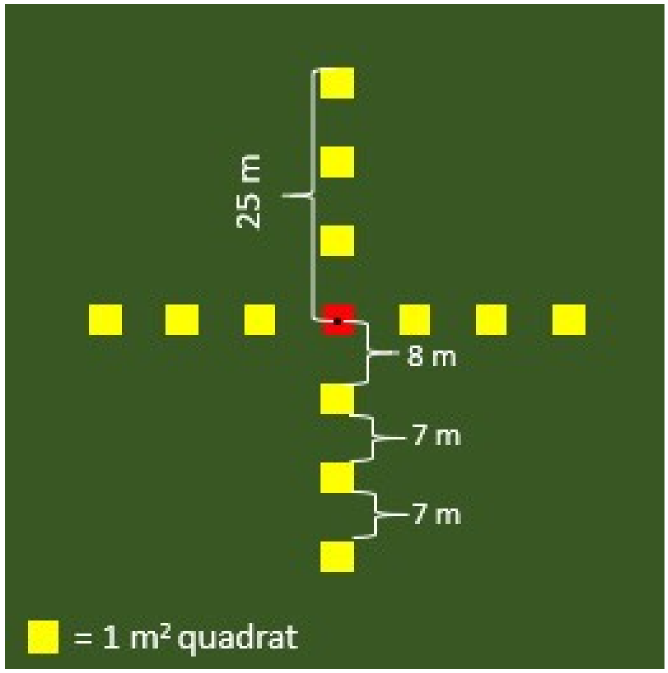

Figure 1.2. Sampling Layout for Vegetation Transects.

Another experiment was developed to assess the effectiveness of plugs versus traditional seeding methods, and also the effectiveness of plug configuration. Plugs used for this experiment were gathered from throughout North Dakota, including: the Sheyenne National Grassland, Eddy County, Stutsman County with the focus being on native graminoids like native sedge (*Carex*) species, northern reedgrass (*Calamagrostis stricta*), and prairie cordgrass (*Spartina pectinata*). Plugs were gathered from native wet meadows utilizing a standard golf cup cutter. The cutter would be placed over an existing graminoid and then cut. Each plug had a dimension of approximately 10 cm diameter and 15 cm in depth (Strehlow 2015).

The plug configuration study site was initially tilled to bare soil to free the space of competing vegetation. Three different hydrology sites were identified: shallow (349.33 m elevation), medium (349.00 m), and deep hydrology (348.67 m). Within each of these three hydrology sites, the area was further divided into three treatments with three replicates of each for a total nine 3m x 3m plots. In each replication row, a plot was randomly assigned with one of three treatments: Twenty-five plugs dispersed evenly as a grid, twenty-five plugs clumped into groups of five (Figure 1.3) and seeding. The assignments and layout of the experiment can be seen in Figure 1.4. No hydrology was present throughout the time of observation.

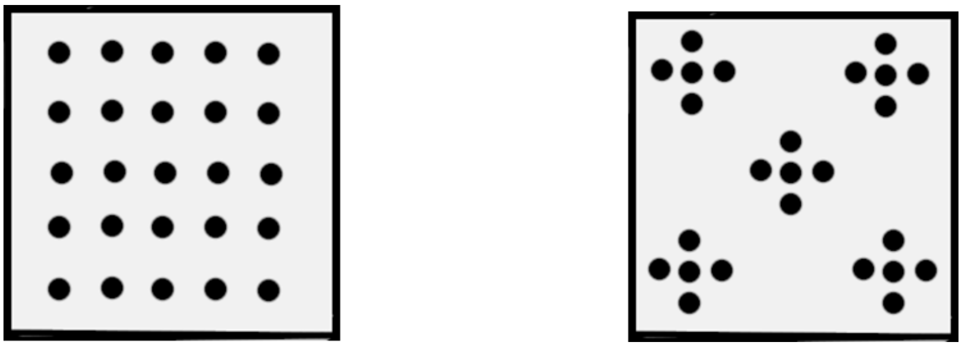

Figure 1.3. Grid and Clumped Configurations of Vegetation Plugs.

Shallow Hydrology

Seeds / Rep 1	Dispersed Plugs / Rep 1	Clumped Plugs / Rep 1
Clumped Plugs / Rep 2	Dispersed Plugs / Rep 2	Seeds / Rep 2
Dispersed Plugs / Rep 3	Seeds / Rep 3	Clumped Plugs / Rep 3

Medium Hydrology

Seeds / Rep 1	Clumped Plugs / Rep 1	Dispersed Plugs / Rep 1
Dispersed Plugs / Rep 2	Clumped Plugs / Rep 2	Seeds / Rep 2
Dispersed Plugs / Rep 3	Seeds / Rep 3	Clumped Plugs / Rep 3

Deep Hydrology

Seeds / Rep 1	Clumped Plugs / Rep 1	Dispersed Plugs / Rep 1
Clumped Plugs / Rep 2	Seeds / Rep 2	Dispersed Plugs / Rep 2
Seeds / Rep 3	Dispersed Plugs / Rep 3	Clumped Plugs / Rep 3

Figure 1.4. Treatment and Replication Layout for Plug Configuration Analysis for Shallow, Medium, and Deep Hydrology.

These plugs were planted on September 1, 2017 and seeding in mid-May 2018. The seeds were a blend of native wetland and upland plant species (Table 1). Clumping plugs could provide substantial success in wetland restoration without the need of placing additional resources or money into the project (Silliman et al. 2015).

A plant survey was conducted September 2019 to measure how much coverage each treatment provided for each hydrologic site. Live plant material, litter, and bare ground were estimated to the nearest percent utilizing three 0.25 m^2 quadrats place approximately 0.5 m from the center of each replication and equally spaced in three random compass directions

Table 1.1. List of native species included in seed mix in plug configuration analysis.

Species	**Latin Name**	**% in Mix**
Variety Not Stated Prairie Coneflower	*Ratibida columnifera*	0.20
MN Native Purple Prairie Clover	*Dalea purpurea*	0.19
MN Native Big Bluestem	*Andropogon gerardi*	4.75
Bad River Blue Gramma	*Bouteloua gracilis*	5.50
K Native Canada Bluejoint	*Calamagrostis canadensis*	0.14
Mandan Canada Wildrye	*Elymus canadensis*	4.09
Lodonn Green Needlegrass	*Nassella viridula*	2.62
Itasca Little Bluestem	*Schizachyrium scoparium*	5.33
Needle-and-Thread Grass	*Hesperostipa comata*	3.63
Red River Prairie Cordgrass	*Spartina pectinata*	5.53
Prairie Junegrass	*Koeleria macrantha*	3.08
Certified Cochen Prairie Sandreed	*Calamovilfa longifolia*	7.11
MN Native Sideoats Grama	*Bouteloua curtipendula*	9.38
Certified First Strike Slender Wheatgrass	*Elymus trachycaulus*	4.35
Cacotah Switchgrass	*Panicum virgatum*	6.53
Certified Rosana Western Wheatgrass	*Pascopyrum smithii*	4.01
IA Native Brown Fox Sedge	*Carex vulpinoidea*	0.13
IA Native Plains Oval Sedge	*Carex brevior*	0.27
MN Native Big Bluestem	*Andropogon gerardi*	12.09
MN Native Indiangrass	*Sorghastrum nutans*	7.87

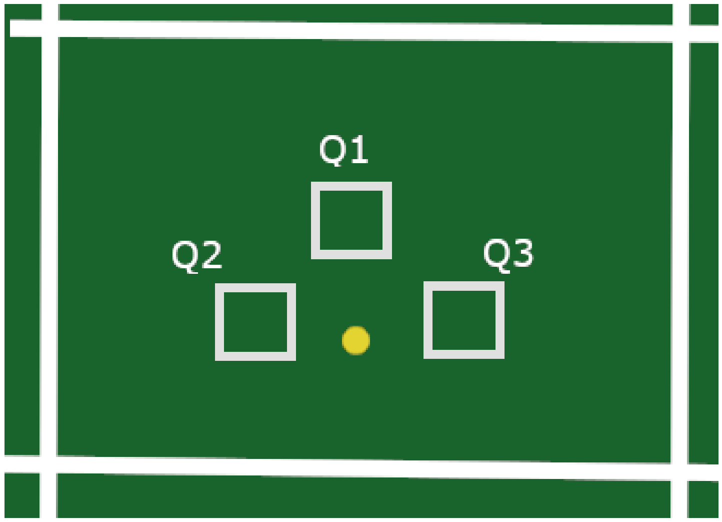

Figure 1.5. Placement of Quadrats for Each Treatment in the Plug Configuration Wetland Restoration Trial.

Results

Comparing Restoration Methods: Seedling, Seed + Mulch, Seed + Mulch + Plugs

There were no differences in the number of native species ($p = 0.11$) (Figure 1.6) or introduced species ($p = 0.24$) (Figure 1.7) among restoration treatments from 2015 to 2019.

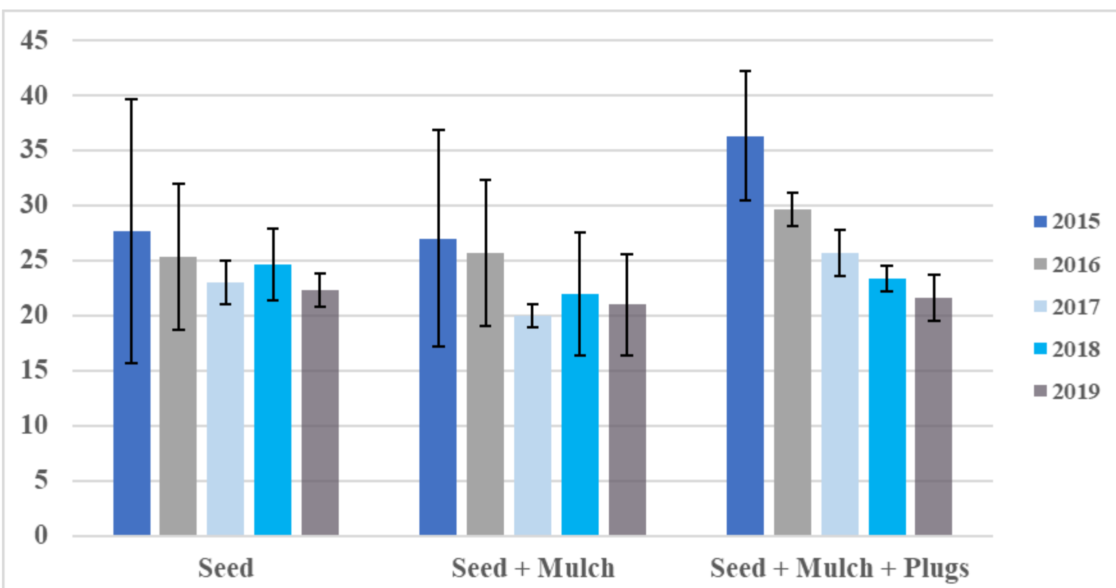

Figure 1.6. Mean Number of Native Species (± Standard Deviation) for Each Restoration Treatment from 2015 through 2019.

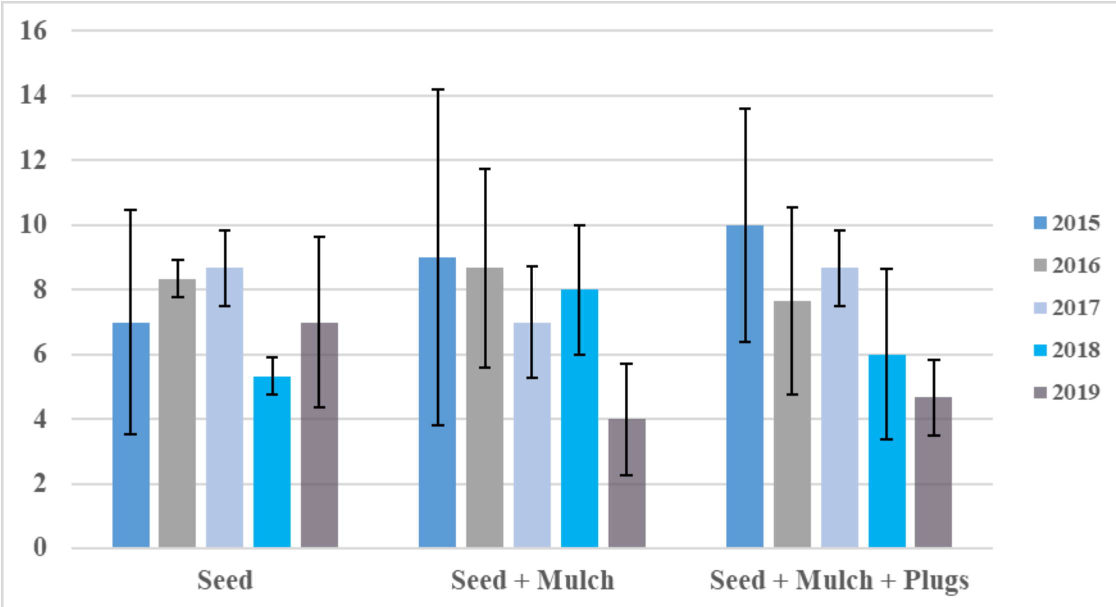

Figure 1.7. Mean Number of Introduced Species (± Standard Deviation) for Each Restoration Treatment from 2015 through 2019.

The ANOVA showed there were differences in the relative cover of native species among our restoration treatments between 2015 and 2019 ($p = 0.04$). Native relative cover was affected by year ($p = 0.001$). Averaging across the restoration treatments, native relative cover was lower in 2017 ($\bar{x} = 0.47$) than 2015 ($\bar{x} = 0.82$) and 2016 ($\bar{x} = 0.70$) (Figure 1.8). Restoration treatment ($p = 0.31$) and the interaction of restoration treatment and year ($p = 0.80$) (Figure 1.9) did not affect native relative cover.

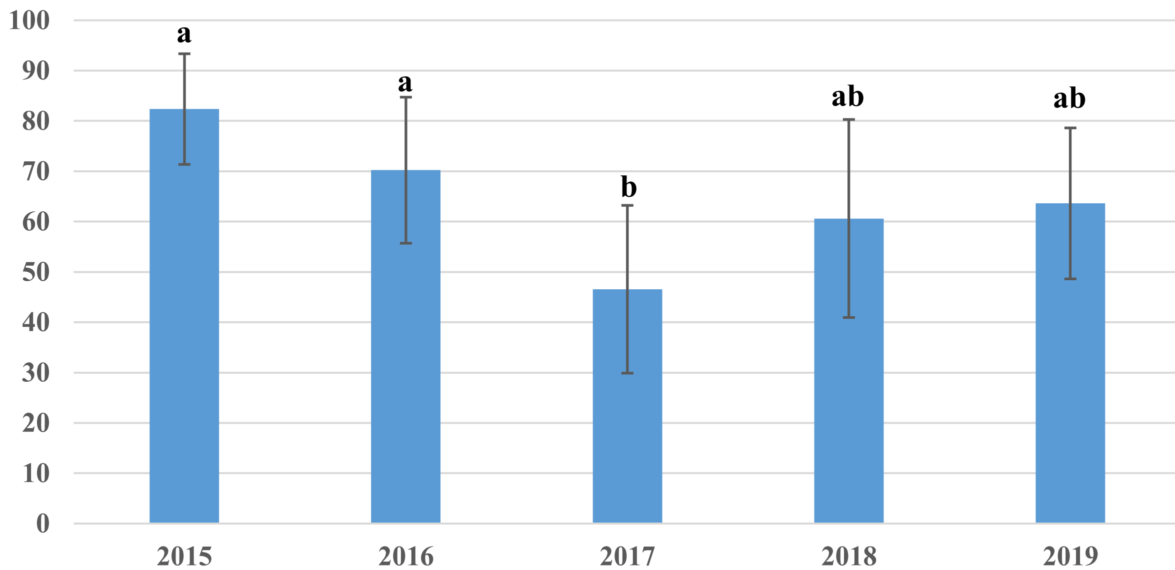

Figure 1.8. Mean Relative Cover of Native Species (± Standard Deviation) from 2015 through 2019.

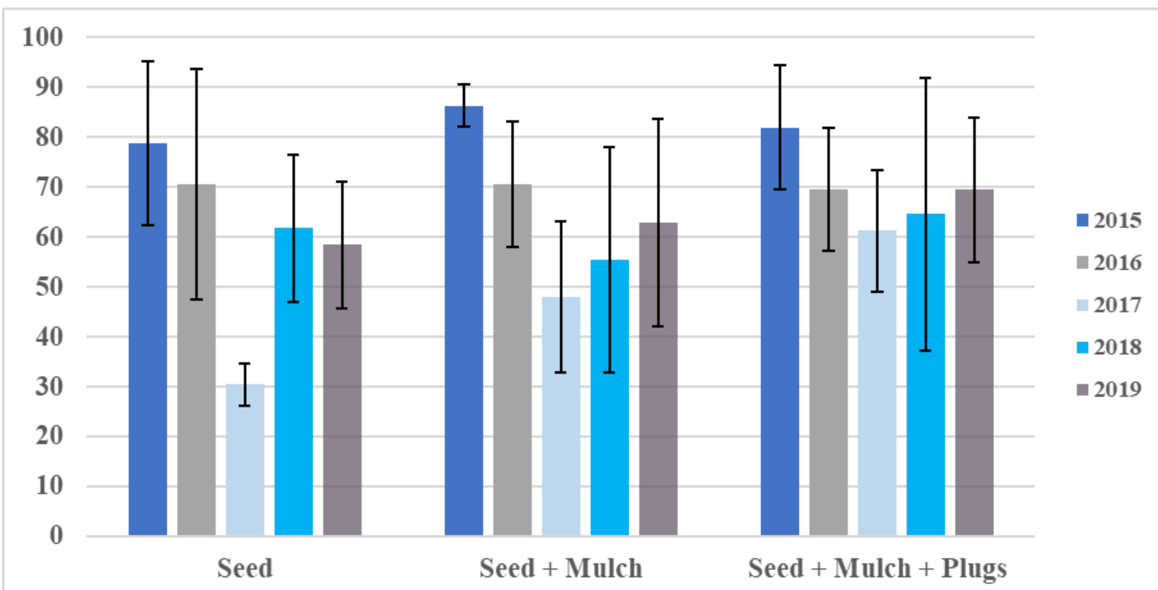

Figure 1.9. Mean Relative Cover of Native Species (± Standard Deviation) for Each Restoration Treatment from 2015 through 2019.

For the experiment examining the effectiveness of plug configurations versus seeding alone (treatment) by different hydrology type, ANOVA indicated differences in total plant cover ($F = 6.39$, $p = 0.0005$) among our treatments. Plant cover was affected by hydrology ($p = 0.0012$)

and treatment (p = 0.0003), but not the interaction of hydrology and treatment (p = 0.4272). Plant cover was lower in the deep hydrology ($\bar{x}$ = 0.37) than the shallow ($\bar{x}$ = 0.58, p = 0.0038) and middle hydrology ($\bar{x}$ = 0.60, p = 0.0023) (Figure 1.10). Plant cover was higher in the seeded plots ($\bar{x}$ = 0.67) than in the grid configuration of plugs ($\bar{x}$ = 0.49, p = 0.0135) and the grouped configuration of plugs ($\bar{x}$ = 0.3, p = 0.0002). (Figure 1.11).

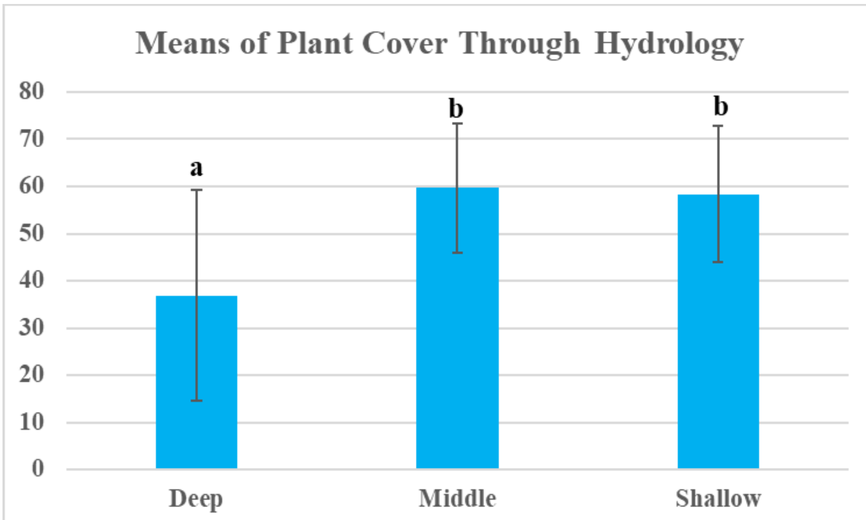

Figure 1.10. Mean Plant Cover (± Standard Deviation) for Each Hydrology Level.

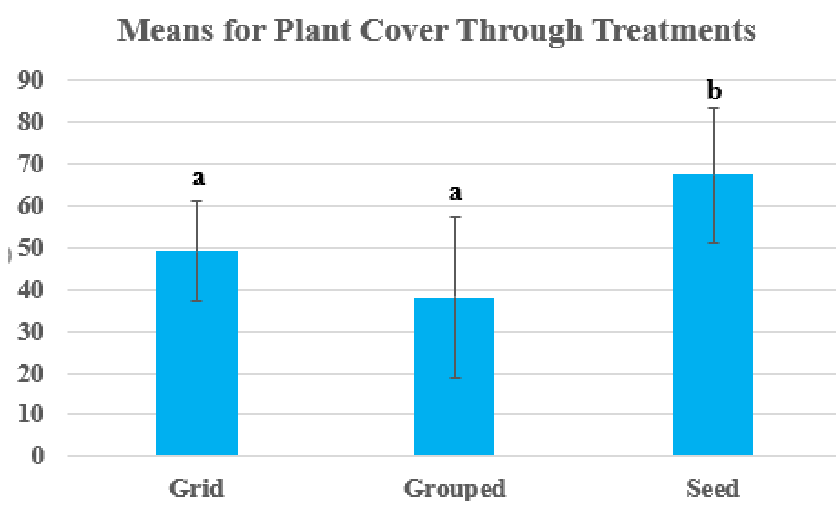

Figure 1.11. Mean Plant Cover (± Standard Deviation) for Each Restoration Treatment.

Discussion

Actively maintaining re-established plant communities will require extended periods of time and management. As active restoration management continues, features of the original wetland plant communities continue to develop. Observations of returning wetland hydrology have shown that passive wetland restoration was ineffective in re-establishing wetland function (Galatowitsch & Van der Valk 1996a, 1996b; Seabloom & Van der Valk 2003; Mulhouse & Galatowitsch 2003; Aronson & Galatowitsch 2008; Zedler & Kercher 2010). The composition of restored wetland plant communities was shown to be distinctly different from those of intact wetlands, which not only limits the ecosystem's diversity but its abilities to contribute to water quality and a region's sustainability for wildlife and people. For example, invasive plant species have changed the physical and chemical make-up of restored wetlands, making them unable to function optimally. Not only that, but invasive plants have also altered the wetlands' composition to favor them over native species, further perpetuating the problem (Zedler & Kercher 2010; Sheley et al. 2006; Vinton & Goergen 2006).

In our study, simply applying native seed mixes appears to be as effective in re-establishing native species and controlling invasive weeds as the more labor-intensive restoration methods involving mulch and plugs. Over the six years of observation, the number of native species and native species relative cover varied between the three treatments. There was no clear trend that signified one treatment being more successful than the others. We found no differences among treatments nor any interaction between treatments and years concerning native species emergence and decrease of invasive plants.

There was a difference when comparing the native relative cover throughout the years ($p = 0.0013$). Mean native relative cover was lower in 2017 compared to 2015 and 2016. We believe this difference was a function of climate, as the growing season in 2017 was drier than the long-term average. Yearly precipitation for the area was 39.11 cm in 2017, which has been the driest year since 2012 (38.46 cm). Annual precipitation in 2013, 2014, 2018 and 2019 ranged from 43.94 to 53.09 cm (NDAWN 2019). The predominant vegetative was reed canarygrass in 2017, which responded positively to the drier conditions (See Figure 1.12).

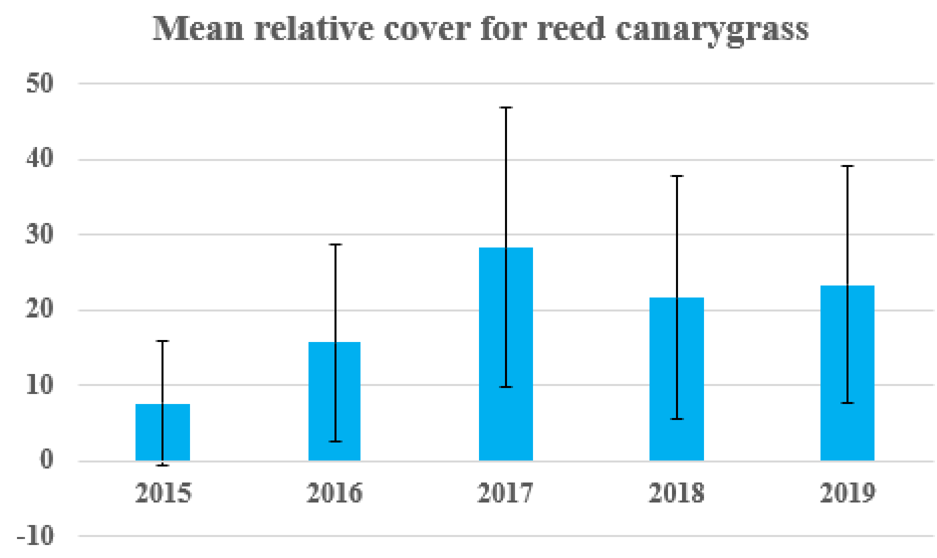

Figure 1.12. Mean Relative Cover of Reed Canarygrass (± Standard Deviation) from 2015 through 2019.

There was also a wildfire on May 9, 2017 on the entire site. The site was grazed early in 2017 with cow/calf pairs with the hope reed canarygrass would decrease using a fire and grazing interaction. All these factors may have played a role in the decreased cover of natives and the increased cover of reed canarygrass in 2017.

The differences across the years suggest that climate and management play a role in determining the actual cover of native species more than which restoration method was used. It should be noted, research shows there are cumulative effects in restoration, which are the net effects of the past, present, and future circumstances upon a landscape (Duinker & Beanlands

1986, Preston & Bedford 1988). Though individual actions only have a minor effect, they can cumulate into a large enough force to change the wetlands' plant communities. The degradation and change of wetland ecosystems are a result of cumulative effects. It took time to lose wetlands and their functions, and it will take a considerable amount of time to restore what was lost (Bedford et al. 1999).

Mitsch & Wilson (1996) identified time being one of the main determining factors whether wetland restoration is successful or not. The need for extended periods of observation is exemplified by a tidal wetland created in western Virginia. The wetland was initially observed from 1978 to 1982 and was deemed successful due to its highly diverse plant communities containing natives of the area. However, a flooding event in 1986 submerged and destroyed the plant communities. The project was considered an ecological failure sixteen years post-creation. Long-term survival of the wetland is vital in restoring their valuable functions, and the unpredictability of nature proves that the common five-year observation is too short.

Concerning wetlands like those found in the PPR, a study focused on the succession of eight emergent marshes was conducted in Ohio. After 14years, the wetlands had equal species richness as intact sites (Gutrich et al. 2009). However, after the first five years of that study, species richness sharply declined by nearly fifty percent, solidifying the need for longer monitoring periods.

Another study of restored wetlands indicated that at least ten years is needed to have 31 to 93% of services provided like those of intact prairies (Dodds et al. 2008). Longer periods of management are needed to prevent widespread failure (Klimkowska et al. 2007, Fagan et al. 2008, Balletine & Schneider 2009, Matthews & Spyreas 2010). Trajectories of ecological function typically show that restorations are a slow process and may never match that of reference sites (Bullock et al. 2012).

Despite the lack of difference between treatments, the consistent means of native relative cover show that active restoration efforts are necessary for success to be possible. The study area was idle (i.e. fallow) cropland prior to restoration efforts. There has been substantial improvement in native plant cover after only six years since application of treatments. However, based on the research pointed out above, active management needs to continue. Invasive species have been a constant obstacle for wetlands and their functions throughout the decades and will continue to persist into the future. Passive management will allow these invasions to return and impair restored areas (Galatowitsch & Van der Valk 1996a, 1996b; Seabloom & Van der Valk 2003; Mulhouse & Galatowitsch 2003; Aronson & Galatowitsch 2008; Zedler & Kercher 2010). The need for active management is further confirmed upon looking at the study area's transformation in only six years. From what was once a cornfield, native plant species quickly returned after seeding, whereas it would be debatable for an idle wetland to have such success. With wetland degradation rising rapidly, these findings show a hopeful possibility in countering these losses in a more reasonable amount of time.

As stated before, restoration efforts are expensive. Costs were analyzed in the three methods utilized in this study, with results that showed direct and associated costs were estimated to be $1,143 per hectare for native seeding only, an additional $379 per hectare to acquire and apply hay mulch, and still additional $2,784 per hectare for extracting, transporting and applying vegetation plugs (Strehlow et al. 2017). Using seed, hay mulch, and vegetation plugs is the most expensive treatment in our study, yet others have shown vegetation plugs to be an effective method in increasing species diversity, richness, re-establishing original wetland functions (Middleton et al. 2010). Finding ways to guarantee this restoration method's success without additional cost would ensure future applications of plugs, thus ensuring the vast benefits healthy wetlands provide. However, based on our current findings and that by Strehlow et al. (2017), the additional costs beyond seeding alone may not be worth the costs.

In our second experiment, we investigated the effects of plug configuration and hydrology on the re-establishment of plant cover. Silliman et al. (2015) found that simply changing the configuration of plugs could increase cover of native plants by an average of 107%. These results, however, come from coastal wetlands along the Atlantic states and Netherlands, which have different salinity and environmental conditions than depressional wetlands of the PPR. Compared to Silliman et al. (2015), our study using plug configurations were not different between configurations after two years. While the ecological differences between coastal and depressional wetlands could affect how the plugs survived and propagated, other factors such as the site's hydrology, run-off, and management could explain the differences.

The lower mean of the deep hydrology could be influenced by the deposition of sand from the shallower treatments. The sand could have impeded the growth and spread of target native species, thus resulting in lower plant cover. The lower means for both grid plugs and grouped plugs could be explained by management of the study area. Weeding of the plots containing these plugs decreased the overall plant cover, while plots containing only native seeds were left idle. The lack of significant difference between grid and grouped plugs show that neither configuration affects the success of native species establishment. However, these results were collected only within a span of two years and require more time and data collection to truly see whether grouped plugs are more successful than grid plugs.

Allowing a minimum of six years to collect data could show a positive picture regarding the configuration of plugs, though having longer periods of time would reveal more accurate effects of treatment and hydrology. Supplementing areas with seeds, mulch, and plugs have a positive effect on the wetland plant communities, though more observation is needed to determine if there are any differences between the three methods.

Similar studies focused on the effectiveness of vegetation plugs revealed a general increase of survivorship and plant cover containing *Spartina pectinata* and *Eleocharis macrostachya*, which showed exponential increase over time (Fraser & Kindscher 2001). The differences in plug response could be attributed to the amount of time and possibly the study sites, as the Santa Fe wetland is within Lawrence, Kansas, which has warmer, more humid conditions than the Ekre project in southeastern North Dakota. The wetland in the Kansas study also had clay soils, which can retain water more efficiently than the sandier soils of southeastern North Dakota. The permeability of the sandier soils could cause decreases of mean area for the treatments involving plugs due to shortened water permanence. The method of extracting plugs could also be a factor, since the plugs for the Santa Fe restoration were removed with the use of tree spades. The plugs extracted for the Ekre restoration were manually removed with the use of augers, which proved difficult when extracting from tightly packed, muddy soil. These limitations could have affected the effectiveness of the plugs, as some of the plants' root systems could have been severely damaged during removal. Fraser & Kindscher (2001) conclude that the use of tree spades aided in the plugs' continued increase of area and clonal growth.

It should be noted that consistent, active management in restoration could slow, or even reverse, damages upon the wetlands' ecological functions from major disturbances like cultivation, which can happen in just as short amount of time. Restoring and preserving the wetlands' highly specific water regimes and plant communities provide protection to many other organisms like waterfowl (Faulkner et al. 2011). Preservation of waterfowl and their habitat in turn could preserve small, mid-western towns' ability to maintain economic stability and livability. Another potential example would be a wetland's ability to effectively detoxify water that frequently runs off from fertilized cropland and pasture when native wetland plants are re-established versus invasive plants. Mitsch and Gosselink (2015) note the remarkable ability of species native to wetlands at filtering and locking away excess nutrients and chemical compounds commonly used in agriculture-dominated areas such as southeastern North Dakota. The loss of these species has allowed nitrates and pesticides to flow freely into streams and groundwater reservoirs (Mitsch & Day 2006, Marton et al. 2014). Time in bringing back this function is vital as the health and well-being of communities depends on accessibility to clean water (Mulhouse & Galatowitsh 2003). Overall, it is important to restore not only hydrology, but also the native plant community of a wetland. It will be essential that management toward a diverse native plant community continue on this site into the future to benefit the ecosystem, and the regional community.

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CHAPTER 2. DEGRADATION OF UPLANDS AND IMPACTS INVASIVE SPECIES HAVE ON RESTORATION EFFORTS[1]

Introduction

Waterfowl depend on the Prairie Pothole Region (PPR), as it provides uplands as nesting grounds which are alongside their preferred foraging and breeding grounds (Batt et al. 1989). The vegetation found in the uplands provide cover for multiple species of birds, invertebrates (insects, arachnids), small reptiles, and large mammals such as the whitetail deer (*Odocoileus virginianus*). This vegetation also provides ample food sources for the wildlife mentioned above (Krausman et al. 2009) and grazing livestock. Grasslands surrounding potholes also help ecosystem productivity as plants found in the uplands have a similar function of locking away carbon (Zhang et al. 2011). Not only do wetlands and grasslands sustain biodiversity by providing necessities such as shelter and energy, but through a number of functions maintain the ecosystem's health. Interestingly, Paradeis et al. (2010) found that a wetlands' ability to sustain biodiversity could be attributed to the surrounding grasslands (uplands), as they are linked together. Disturbances that occur in the uplands affect a wetland's ability to provide its ecological services.

Another study from 2009 to 2013 showed losses of grassland has increased (Hossler et al. 2011). The total loss of grassland to cultivation, known as the Plow Print, increased to 10%, an increase of previous years with rates of grassland to cultivated land being 1%-5% a year. Hossler

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1 The material in this chapter was co-authored by Cheyenne Durant, Dr. Shawn DeKeyser, and Breanna Kobiela. Cheyenne had primary responsibility for conducting surveys in the field and analyzing the data collected from these surveys. Cheyenne was the primary developer of the conclusions that are advanced here. Cheyenne also drafted and revised all versions of this chapter. Dr. DeKeyser served as proofreader and Breanna checked the math in the statistical analysis conducted by Cheyenne.

et al. (2011) bring attention to the socio-economic Quinclorac of this issue. With these wetlands and grasslands being altered or lost, waterfowl species may struggle to survive, resulting in not just a loss of biodiversity but a negative impact to humans. Recreational activities, such as duck hunting and bird watching, depend on these grasslands' and wetlands' resilience and ability to provide for wildlife.

Invasive Species

Uplands have endured long-term impacts due to extensive agricultural uses, and it is because of these uses uplands are vulnerable to invasion (Hobbs & Huenneke 1992). Land conversion for agriculture has led to the removal of regular prairie fires, which were a defense against the spread of some invasive plant species. Not only has that, but having cattle confined in fenced pastures encouraged over-grazing, which led to further degradation. The resulting degradation freed valuable, nutrient-rich land for invasive species, and their aggressive nature further isolated native species in the fragmented habitat. Furthermore, fragmentation impedes the native plants' ability to reproduce, threatening not only their numbers but also their genetics and their ability to compete and adapt to the changing environment (Rabinowitz and Rapp 1980; Poiani and Johnson 1991; Guertin et al. 1997; Higgins et al. 2002). Foreign species invading uplands have shown to alter soil conditions and hydrology, making them even more resistant to competition (Vitousek et al. 1987; Ehrenfeld 2003; Jordan et al. 2008; Schmidt et al. 2008). Due to these complications, more involved methods of restoration are vital in conserving threatened prairies.

Smooth Brome

Smooth brome (*Bromus inermis*) is capable of cloning through rhizomatous roots. Otfinowski & Kenkel (2008) found the connection between ramets and mother clones maintain density and dominance over areas where resources are strained. The network of ramets allow wider availability to limited resources and ability to share these resources among the smooth brome clones. In enriched areas, the proliferation of smooth brome increased, leading to monocultural patches.

Ott et al. (2016) explain what further complicates control of smooth brome's spread is its ability to withstand and propagate under a variety of conditions. Smooth brome has a higher bud count than observed native species despite the changes of soil temperature, ambient temperature, and different watering levels (Ott et al. 2016).

Smooth brome's ability to endure infrequent moisture has become an adaptation in the PPR as the ecosystem's climate varies wildly. Galatowitsch et al. (2009) predicts that the already shifting climate of the continental prairies will become warmer, have increased seasonal shifts, and an even more infrequent rate of precipitation. Future changes of the Midwest's climate would continue to favor the production and spread of invasive species such as smooth brome.

Grant et al. (2009) found in a study on grass communities on private land and United States Fish and Wildlife Service (USFWS) land that USFWS land in South and North Dakota have forty-five to forty-nine percent of the vegetation taken over by smooth brome. These observations also report that Kentucky bluegrass makes up twenty-seven to thirty-six percent of the total cover (Grant et al. 2009). The predominant land management on the USFWS land was rest, while grazing was the dominant management on private lands.

Herbicides, such as atrazine, imazapic, imazapyr, and sulfometuron, have been used to control smooth brome. Despite its success on cool-season invasive grasses, atrazine is not recommended for non-crop application. There is also concerns involving the popularly used herbicide, glyphosate (Roundup®), as its wide range of targets could cause more damage to the native grasses if applied (Anderson 1994; Barnes 2007). The herbicides, sulfosulfuron, imazapyr, and imazapic+ sulfosulfuron have some success in decreasing smooth brome cover (Bahm et al. 2011). The decrease was not long-term, however, as the differences of smooth brome growth between treated and control sites decreased during the second growing season. Smooth brome cover increased by the third growing season since application.

Due to these limitations, other methods of smooth brome control have been tested. Clipping during April or May, when smooth brome has exhausted its carbohydrate resources to tiller elongation, has some success in control. Implementing this method after a spring burn showed even more hopeful results in causing significant decreases in smooth brome density (Old 1969; Kirsch & Kruse 1973; Willson 1991). When using burning alone for smooth brome control, it has been shown to be effective when burning takes place at tiller elongation (Willson and Stubbendieck 1997). Grazing proved to be even more effective than herbicides and burning, as it doesn't put native species at further risk (Murphy & Grant 2005).

Kentucky Bluegrass

While grazing appears effective against smooth brome, season-long grazing has encouraged Kentucky bluegrass (*Poa pratensis*) growth. This species withstands frequent grazing well, and usually dominates grasslands that are overgrazed (Murphy & Grant 2005; Patton et al. 2010). However, recent studies showed promising results when alternative grazing methods, such as early intensive grazing, may reduce Kentucky bluegrass (Dornbusch et al. 2020). Kentucky bluegrass was commonly used as a turf grass and soil stabilizer (DeKeyser et al. 2015). This species' popularity also comes from its previous use for hay and grazing due to livestock's preference of the grass. Widespread use of Kentucky bluegrass for pastures in Eurasia has encouraged anthropomorphic spread to the United States as early as the 1600's. However, it has spread beyond intended areas and added to the negative impacts exotic species have on North American grasslands (Toledo 2014).

This invasive cool-season grass grows in early spring, starting before many of the native cool-season species. While it is a short turf grass, Kentucky bluegrass has shown to dominate prairies by forming thick root mats and spreading rapidly through rhizomatous tillers (Bonos & Murphy 1999). Other factors that allow Kentucky bluegrass to proliferate and form monocultures are its ability to cause allelopathic effects from its litter, potential to alter light availability and temperature of the area, and nutrient cycling (Weaver & Rowland 1952; Bosy & Reader 1995; Hamilton & Frank 2001). Kentucky bluegrass has been reported to alter nitrogen cycling of mixed grass prairie in the PPR (Toledo 2014). While most native plant species are acclimated to lower nitrogen levels in the soil, Kentucky bluegrass' low carbon to nitrogen ratio causes an excess of nitrogen to occur. The increase of nitrogen also increases production in the plant community, native plants struggle to adapt and thus lose their ability to compete against Kentucky bluegrass. The increase of nitrogen from fertilizer pollution can also contribute to the reduction in native species and proliferation of invasive species like Kentucky bluegrass.

Like smooth brome, burning has some effect in decreasing Kentucky bluegrass. If growing along warm-season native grasses, prescribed spring burning can impact Kentucky bluegrass enough to give the natives a competitive edge (Anderson 1965; Owensby & Smith 1973; Towne & Owensby 1984). Seasonal prescribed burning prevents Kentucky bluegrass' ability to overtake native plant communities and encourages species diversity (Kral et al. 2018). The use of imazapic in combination with glyphosate successfully wiped the invasive grass from study sites in Kentucky (Adkins 2007). Treatment with the three herbicides mentioned in the smooth brome section, however, showed not to have long-term effects in controlling Kentucky bluegrass (Bahm et al. 2011). Results for this study also showed the need for reapplication in as little as two growing seasons.

Leafy Spurge

Bourchier et al. (2006) describes leafy spurge (*Euphorbia esula*) as a deeply rooted forb found originally in Europe and Asia. It was first reported on U.S. soil in Massachusetts in 1827 and believed to have been brought unintentionally within contaminated soil in ship ballasts. When the water in these ballasts were drained, it washed the leafy spurge contaminated soil onto the shores, beginning an infestation that spread westward into the Midwest, western states, and Prairie Provinces in Canada (Bourchier et al. 2006). Leitch et al. (1996) report that 637,000 hectares of land in Montana, North Dakota, South Dakota, and Wyoming reported infestations of the weed; six percent of North Dakota's untilled land, including abandoned cropland and idle restoration sites, have severe leafy spurge invasions. A more recent estimate shows two million hectares of the Great Plains, Rocky Mountains, and Canada are infested (Bourchier et al. 2006).

Leafy spurge's success in prairies can be attributed by the rich environment and the plant's biology. The plant grows and spreads rapidly through the growing season and disperses as seeds and through the buds at its woody roots. The rate leafy spurge reproduces by root buds can be as quick as seven to ten days after the plant's emergence (Lym 1998). Leafy spurge also has an adaptation that protects it from grazing. Within the plant is a sticky latex that seals any wounds it may endure. This latex contains the chemical compound, *ingenol*, which causes irritation and vomiting if ingested (Lym 1998).

Leistritz et al. (2004) and Bourchier (2006) found that grazing animals such as cattle avoid leafy spurge. This is because grazing on leafy spurge causes mouth sores, weakness, and death. This deterrent harm the native grasses even more, as those present will be more preferred and thus heavily grazed until they are eliminated from the area. The displacement of native grasses by leafy spurge leads to the restriction of available foliage for cattle and other animals, impacting not just the biodiversity but the economic value of livestock. An estimated 120 million dollars were lost due to leafy spurge and the damage caused by its spread (Leistritz et al. 2004; Bourchier 2006).

Not only does the presence of leafy spurge place more foraging pressure on other grasses (Lym and Kirby, 1987), several studies throughout the years show that not only does the latex in the plant deter livestock, but also phytotoxins kill different plant species nearby, which increases its spread (Tanveer et al. 2013). The roots of the plant deposit allopathic chemicals deep in the soil, and over time, the accumulation of these chemicals prevents seedlings of both crop plants and forage plants to survive.

Several control methods have been used to control leafy spurge numbers in the Northern Great Plains. The introduction of flea beetles into infested areas theoretically provided some promise. North Dakota's early and long winters, however, have limited the spread of flea beetles, thus making them less of a long-term solution in certain areas (Joshi et al. 2009). Previous attempts to introduce flea beetles showed little to no success as leafy spurge control is slow and doesn't encourage native grass establishment (Lym 1998). In contrast, goats seem like a more feasible biological control in certain regions of North Dakota. While sheep are also able to utilize leafy spurge, goats have a stronger preference to forbs over grasses, and have been popular biological controls during the 1980's and 1990's (Hanson 1994, Olson and Lacey 1994). Though goats successfully decrease the cover of leafy spurge, the weed's resilience allows them to readily regrow once the grazing ceases (Lym et al. 1997).

Unlike both smooth brome and Kentucky bluegrass, herbicides appear to be the most effective method in controlling leafy spurge. A study in North Dakota showed that the combination dicamba + 2,4- D has been most effective at control than dicamba or 2,4-D alone (Lym & Messersmith 1985). After two years of biannual application, dicamba + 2, 4-D decreased average leafy spurge cover by seventy percent after twenty-seven months, while dicamba alone only decreased it by thirty-three percent and 2,4-D decreased it by fifty-five percent. Picloram, another herbicide tested, had an average success rate at eighty-two to eighty-six percent within that same timeframe. Glyphosate is most effective when applied once during autumn, however, it requires an application of 2, 4-D in the spring. Though having a better success rate than grazing, the use of one herbicide is not an effective method in controlling leafy spurge. Other experiments have confirmed Quinclorac's ability to decrease the presence of leafy spurge, becoming especially effective when added with diflufenzopyr (Dicamba) (Lym & Deibert 2005).

Though Kentucky bluegrass and smooth brome are a concern, my upland study site has a larger prevalence of leafy spurge. These species are aggressive in proliferation; however, the use of herbicides and prescribed burning could have a greater effect on these species. With burning causing the most impact to other upland species of concern, smooth brome and Kentucky bluegrass, the question on whether it could affect leafy spurge has yet to be asked. Using a combination of burning and herbicides known to successfully control leafy spurge could provide an economical solution.

Methods

A study was designed that used prescribed burning and herbicides at the Albert Ekre Grassland Preserve (46.54000, -97.14100) in southeastern North Dakota. The burning treatment took place on May 9, 2017, and the herbicides Quinclorac (Facet®) + Methylated Seed Oil (MSO), Quinclorac + Sodium Salt of Dilufenzopyr and Dicamba (Overdrive®) + MSO, and Glyphosate (Roundup) were used to treat the study sites. Figure 2.1 shows the layout of each of these treatments.

The Albert Ekre Grassland Preserve is approximately fifteen kilometers southwest of Kindred in Richland County, North Dakota. The study site was dominated by tame grasses such as Kentucky bluegrass and smooth brome, as well as having a prevalence of leafy spurge. Six hectares of this land were divided into treatment areas with a blocking design, with randomly assigned four treatments, replicated 3 times on burned and unburned sites. The treatments were: (1) interseeded control (no chemical controls), (2) interseeded with application of Quinclorac+MSO (Quinclorac = 4.68 l/ha; MSO = 1.75 l/ha), (3) interseeded with application of Quinclorac + Sodium Salt of Dilufenzopyr +dicamba +MSO (Quinclorac = 2.34 l/ha; Sodium salt of dilufenzopyr +dicamba = 420.32 g/ha; MSO = 1.75 l/ha), (4) interseeded with application of Glyphosate (2.34 l /ha; MSO = 1.75 l/ha). Each treatment replication was a plot 40 x 100 meters. All chemical treatments occurred on June 12, 2017. Seeding occurred in July 2018. These dates were selected due to these times being logistically best for application and previous studies showing positive results (Link et al. 2017).

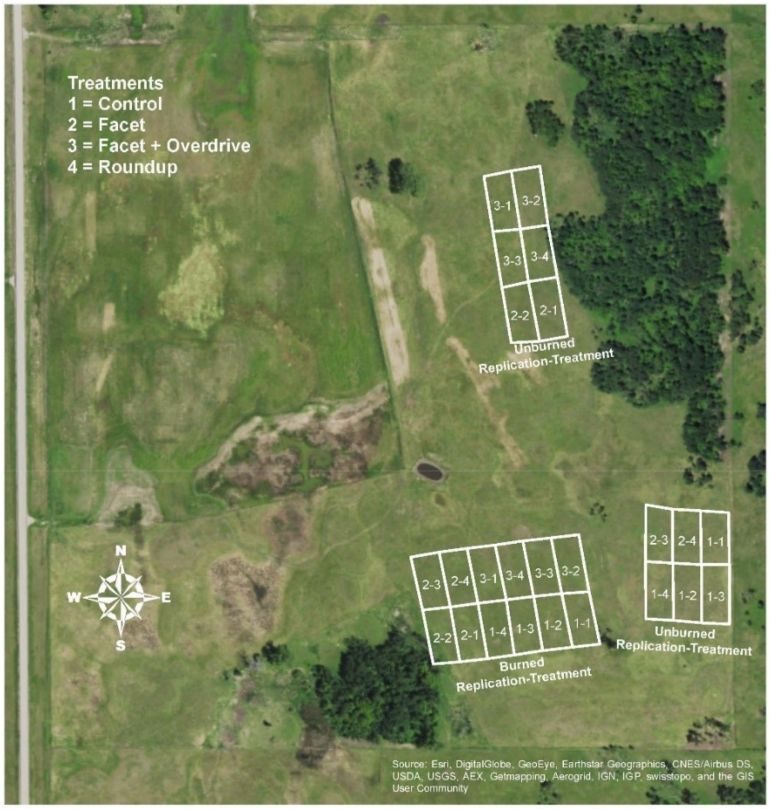

Figure 2.1. Treatment Layout for Leafy Spurge Control on Burned and Unburned Sites in Interseeded Restoration.

Surveys were conducted to count adult leafy spurge stems by treatment and replicate on12 June 2018, 3 -10 June 2019, and 9 September 2019. These adult stem counts were done by hand with fifteen ¼ m^2 quadrats per replication. Grass coverage surveys were also collected as a percent cover the summer of 2019 to account for emergence of seeded species. The coverage surveys were done by identifying each species within a ¼ m^2 quadrat and estimated each species' cover by the nearest percent, with ten total quadrats being estimated per replication.

The number of adult stems and percent coverage were recorded and analyzed using a one-way ANOVA in SAS Enterprise Guide 7.1. The means of leafy spurge stem counts and grass coverage and standard deviations were taken from the blocking analysis results, then compared and graphed on Microsoft Excel 2016.

Due to low seedling numbers in the treated replications, additional areas located on top of three hills west of the study area were also surveyed for comparison purposes during early June 2019. These areas were burned during May 9, 2017 and treated with Glyphosate during June 12, 2017. The transect for the surveys have a bearing of 160 degrees north and were observed atop of each of the three hills. The results for these species surveys are compared to those of our upland study area in the discussion section. These additional areas were compared due to the study sites variability, low seedling emergence, and were within the similar area and received the same treatments as burned glyphosate-applied replications. It was visually evident these additional areas had better seedling emergence and growth.

Results

Herbicide treatments affected leafy spurge stem densities in June 2019 ($p = 0.0021$) in the burned plots 24 months post-treatment. In June 2019, the average spurge counts were higher in the burned plots treated with glyphosate ($\bar{x} = 36.18/m^2$) than in the burned plots treated with quinclorac ($\bar{x} = 1.87/m^2$) and quinclorac + dicamba ($\bar{x} = 2.67/m^2$) (Figure 2.2). The spurge counts in the burned control plots ($\bar{x} = 16.53/m^2$) were not different from any of the herbicide treatments in June 2019, 24 months post-treatment.

Herbicide treatments also affected mean leafy spurge counts in burned plots as measured in September 2019 ($p = 0.0497$), 26 months post-treatment. The mean leafy spurge counts in the burned plots treated with glyphosate ($\bar{x} = 74.93/m^2$) were higher than burned plots treated with Quinclorac ($\bar{x} = 4.98/m^2$). The mean leafy spurge counts in the burned plots treated with quinclorac + dicamba ($\bar{x} = 24.8/m^2$) and the control plots ($\bar{x} = 38.58/m^2$) were not different from either glyphosate or quinclorac burned plots (Figure 2.3).

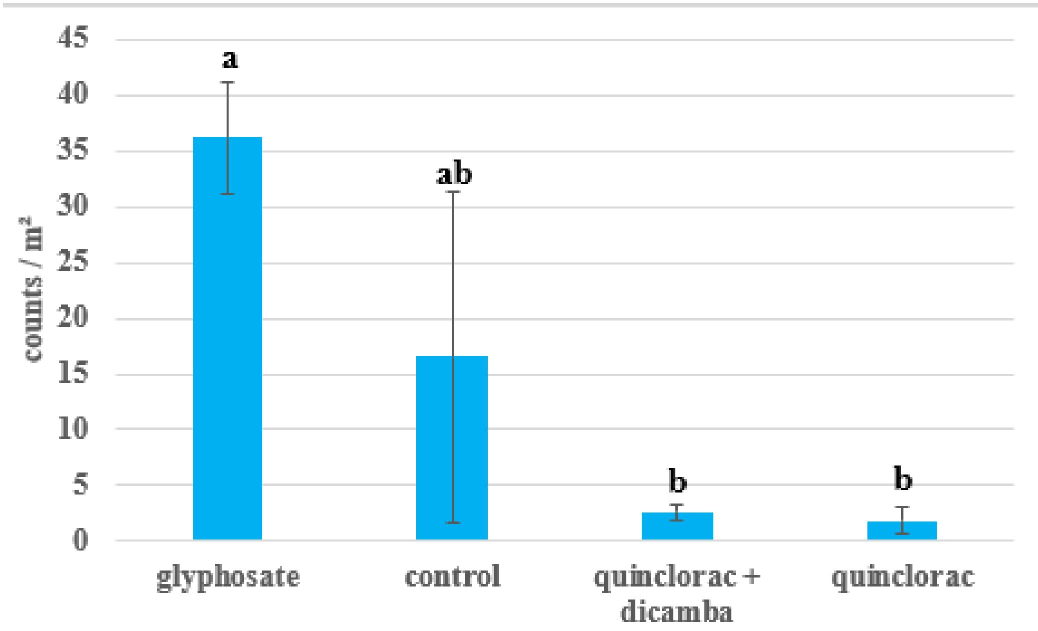

Figure 2.2. June 2019 Mean Leafy Spurge Count Per Square Meter (± Standard Deviation) in Burned Plots for Each Herbicide Treatment. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

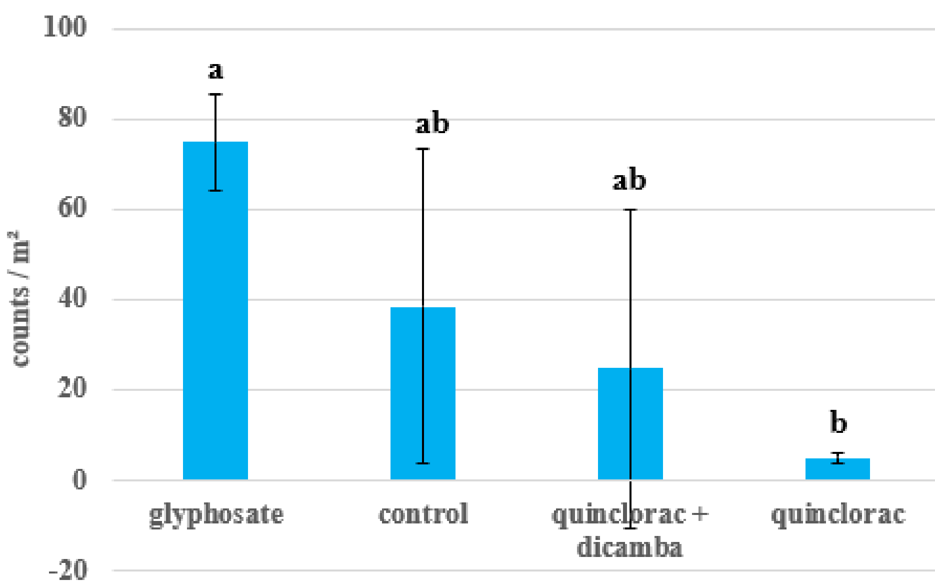

Figure 2.3. Mean Counts of Leafy Spurge Per Square Meter (± Standard Deviation) on Burned Plots for Each Herbicide Treatment During September 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

Mean leafy spurge counts in the unburned plots were also affected by herbicide treatments ($p = 0.0475$) 24 months after treatment. However, pairwise comparisons (Tukey's HSD) did not indicate differences between any herbicide treatments in the unburned plots. Mean leafy spurge counts were 124.44/m^2 in the unburned plots treated with Glyphosate. Unburned control plots had mean leafy spurge counts of 76.44/m^2, and the unburned plots treated with Quinclorac + Dicamba and Quinclorac alone had mean leafy spurge counts of 2.76/m^2 and 2.31/m^2, respectively (Figure 2.4).

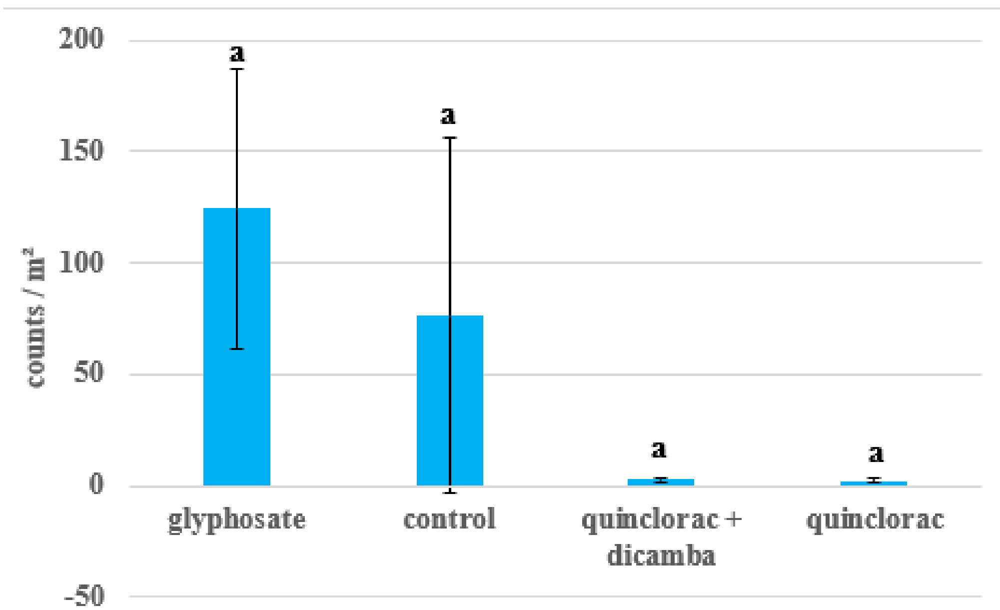

Figure 2.4. Mean Counts of Leafy Spurge Per Square Meter (± Standard Deviation) on Unburned Plots for Each Herbicide Treatment During June 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

The mean leafy spurge stem density counts in the unburned plots as measured 26 months after treatment were not affected by herbicide treatment ($p = 0.18$). The mean leafy spurge stem density counts for unburned plots treated with Glyphosate were 29.16/m^2 26 months after treatment. Unburned control plots had a mean leafy spurge stem density count of 19.91/m^2, and the unburned plots treated with Quinclorac + Dicamba and Quinclorac alone had a mean leafy spurge stem density of 3.38/m^2 and 12.8/m^2, respectively (Figure 2.5).

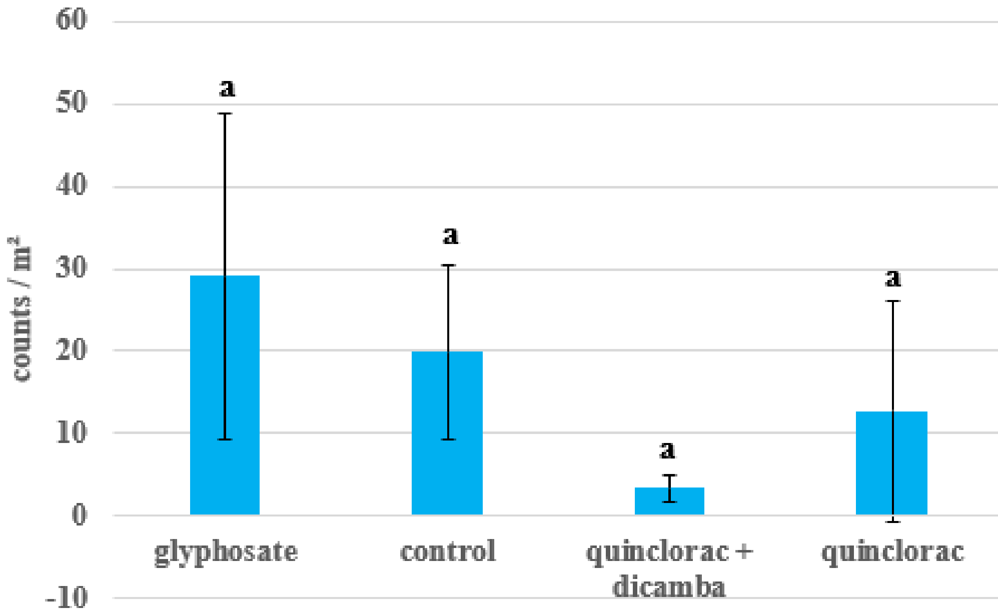

Figure 2.5. Mean Counts of Leafy Spurge Per Square Meter (± Standard Deviation) on Unburned Plots for Each Herbicide Treatment During September 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

Mean relative cover for leafy spurge in burned plots was also affected by herbicide treatment ($p = 0.0047$) 24 months after treatment. Mean leafy spurge cover was higher in the control burned plots ($\bar{x} = 0.54$) and burned Glyphosate plots ($\bar{x} = 0.42$) than in burned plots treated with Quinclorac ($\bar{x} = 0.02$) and Quinclorac + Dicamba ($\bar{x} = 0.03$) (Figure 2.6). Mean relative cover of native species in burned plots was affected by herbicide treatment ($p = 0.0248$). Mean native relative cover was higher in burned plots treated with Glyphosate ($\bar{x} = 0.38$) than in burned control plots ($\bar{x} = 0.06$) and plots treated with Quinclorac + Dicamba ($\bar{x} = 0.05$) (Figure 2.7). Mean native relative cover in the burned plots treated with Quinclorac ($\bar{x} = 0.24$) was not different from the burned control plots, Glyphosate plots, or Quinclorac + Dicamba plots (Figure 2.7).

Herbicide treatment also affected the mean relative cover for leafy spurge on unburned plots (p = 0.014) 24 months after treatment. Mean relative cover of leafy spurge was higher in the unburned control plots ($\bar{x}$ = 0.31) than in the unburned plots treated with Glyphosate ($\bar{x}$ = 0.06) (Figure 2.8). Mean relative cover of leafy spurge in the unburned plots treated with Quinclorac and Quinclorac + Dicamba was 0.25 and 0.16, respectively. Similarly, mean relative cover of native species for unburned plots was affected by herbicide treatments (p = 0.0295). Mean relative cover of native species was higher in the unburned plots treated with Glyphosate ($\bar{x}$ = 0.55) than the control plots ($\bar{x}$ = 0.24), while no difference was found on the unburned plots treated with Quinclorac ($\bar{x}$ = 0.29) or Quinclorac + Dicamba ($\bar{x}$ = 0.38) (Figure 2.9).

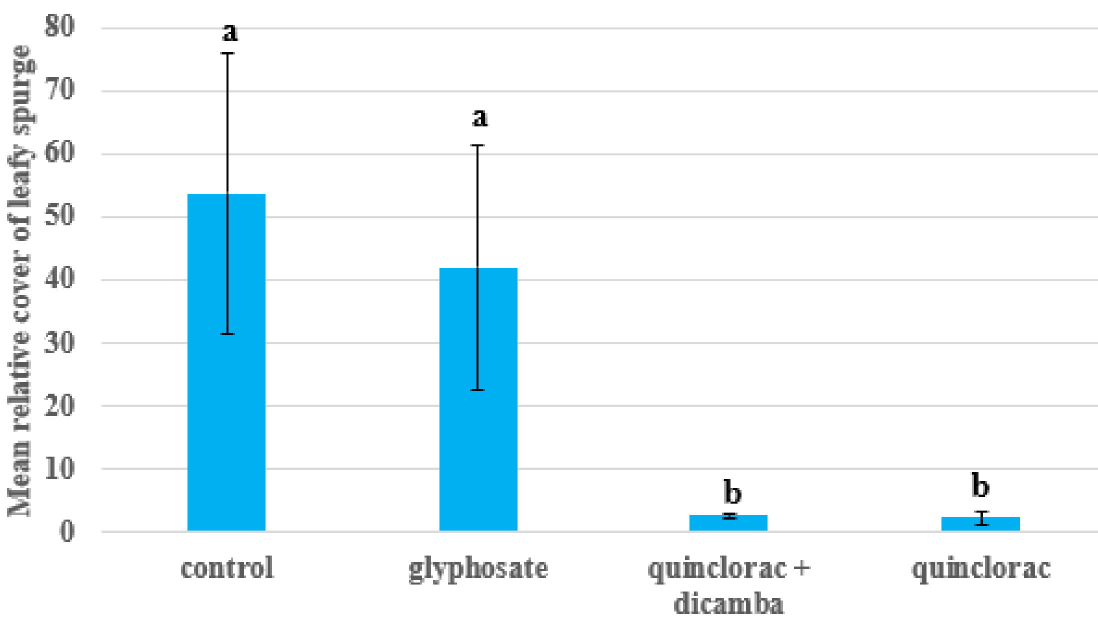

Figure 2.6. Mean Relative Cover of Leafy Spurge (± Standard Deviation) on Burned Plots for Each Herbicide Treatment During June 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

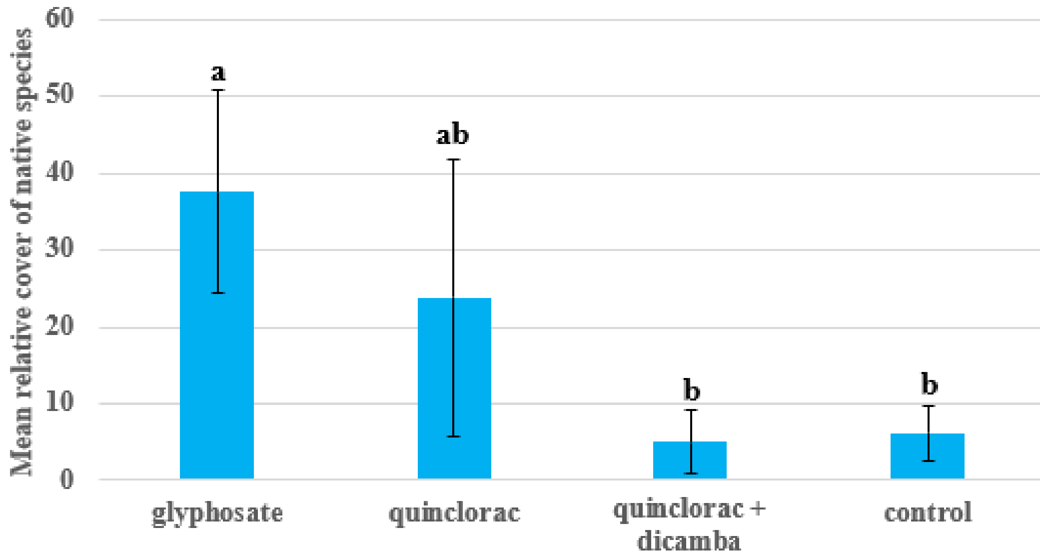

Figure 2.7. Mean Relative Cover of Native Species (± Standard Deviation) on Burned Plots for Each Herbicide Treatment During June 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

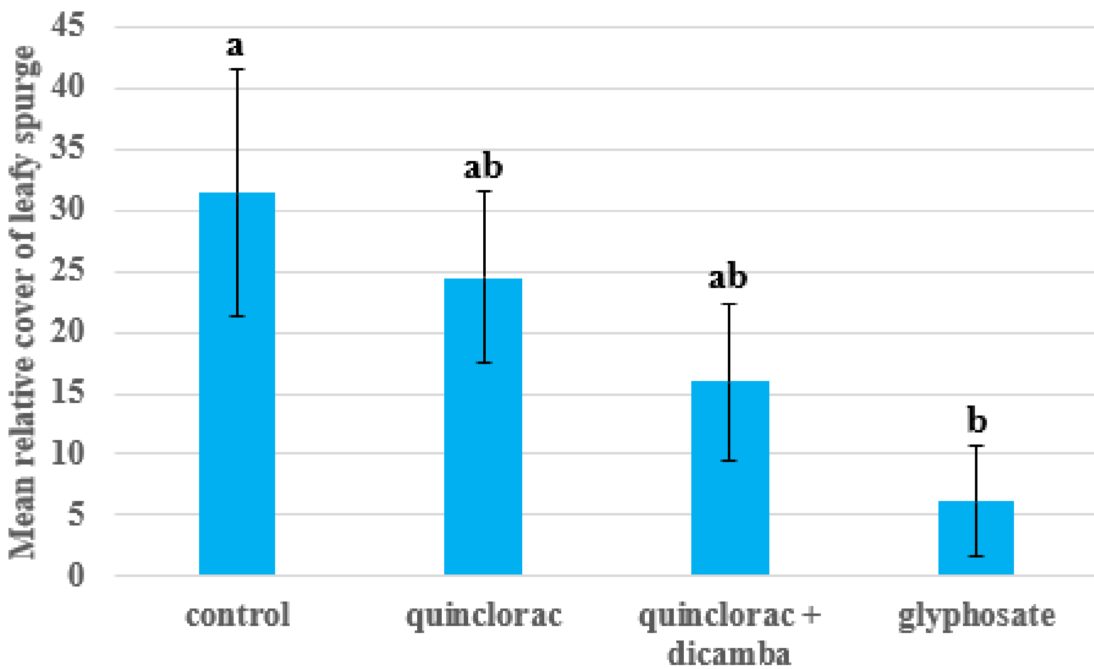

Figure 2.8. Mean Relative Cover of Leafy Spurge (± Standard Deviation) on Unburned Plots for Each Herbicide Treatment During June 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

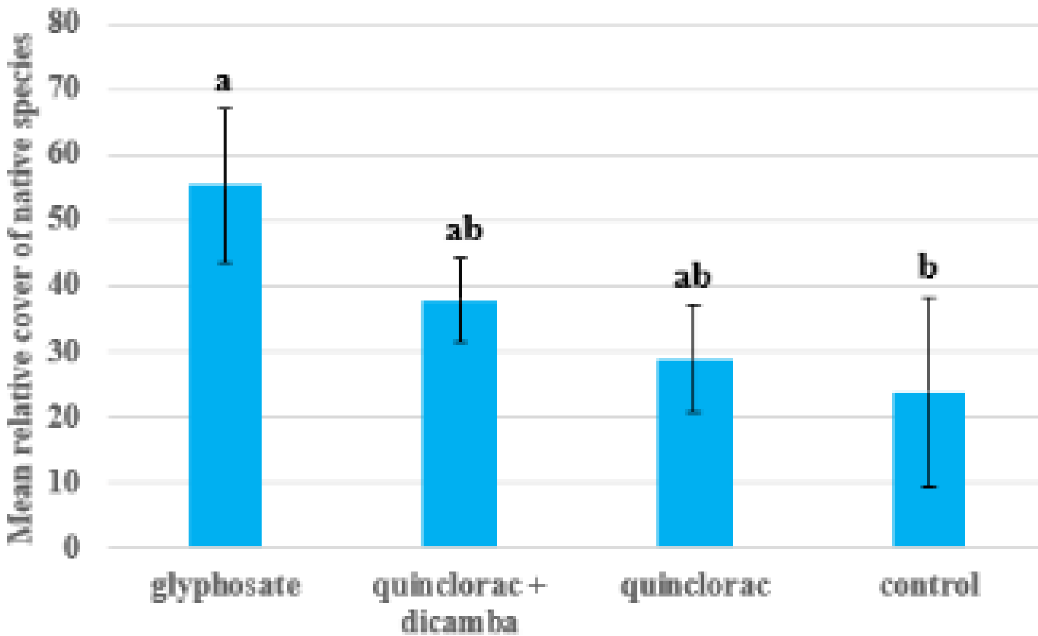

Figure 2.9. Mean Relative Cover of Native Species (± Standard Deviation) on Unburned Plots for Each Herbicide Treatment During June 2019. Significant Differences ($p < 0.05$) are Indicated by Different Letters.

Discussion

Mechanical and sampling errors and site selection may have caused the wide variation found in the data, particularly that of leafy spurge. There is the possibility that the drill did not apply seeds correctly during the inter-seeding process. Leafy spurge counts could also be inaccurate due to the potential of some stems being counted multiple times or missed. This was a concern for areas that have very high concentrations of leafy spurge. Due to the spring burn, there are fire lines that have left dense patches of leafy spurge. These areas might have mistakenly been sampled during the surveys, leading to the inclusion of outliers in the data. While herbicide seems to be the most effective control method for leafy spurge and herbicide did impact the leafy spurge counts measured on June 2019, twenty-four months after treatment, there is only a difference of stem density counts between Glyphosate and Quinclorac and Quinclorac +

Dicamba. Burned plots treated with Glyphosate had greater leafy spurge stem densities than Quinclorac + Dicamba and Quinclorac alone. The higher counts could have been influenced by Glyphosate's broad range of plant control. This led to all plants in these plots to die off and leave available space for leafy spurge to emerge from the seed bank and spread, though only matured individuals were counted and recorded. Leafy spurge has large carbohydrate reserves within their extensive roots, which allow the plant to withstand damage by herbicides and grazing (Raju et al. 1963; Marrow 1979). Lym and Messersmith (1985) noted glyphosate is most effective when applied once during autumn, followed by an application of 2, 4-D in the spring. We applied Glyphosate in the spring and did not have any other chemical applications. This could explain why leafy spurge survived the Glyphosate treatment.

Leafy spurge can reproduce by buds via their deep roots, which can reach as far as the water table. Leafy spurge seeds are also durable and can remain dormant in inhospitable conditions for as long as eight years (Raju et al. 1963; Marrow 1979; Lym 1998). These adaptations could have allowed the weed's new growth to evade Glyphosate's effects. The Quinclorac herbicide combinations have narrower ranges of targets, particularly annual grasses and broadleaf weeds. Research has proven Quinclorac's potential as an effective control in restoration areas. One such study revealed that Quinclorac (Quinclorac) effectively decreased leafy spurge yet did not harm native prairie plants such as the endangered Prairie Fringed Orchid (Erikson et al. 2006). Quinclorac is not only absorbed readily through leafy spurge's leaves, but also becomes affixed to the soil. The continued presence in the soil may have kept conditions unfavorable for dormant seeds to germinate (Lamoreux & Rusness 1995). Their formulations have killed leafy spurge and allowed a wide variety of native species to survive, resulting in higher competition for an area.

Burned plots after 26 months of treatment continue to be influenced by herbicides; however, the difference is seen only between Glyphosate and Quinclorac alone. It appears that Quinclorac alone also has a longer period of effectiveness than the combination of Quinclorac + Dicamba, with pure Quinclorac having a mean adult stem count of 4.98/m^2 and Quinclorac + Dicamba's mean count of 24.8/m^2. These findings generally differ from Lym and Deibert (2005) that the combination being more effective on leafy spurge. However, there is no difference of control between Quinclorac + Dicamba and Quinclorac, which remains consistent with our results. This difference is possibly explained by herbicides drifted from the sprayer and along the wind, or human error in sampling. Difficulty of drilling native seeds through Kentucky bluegrass thatches could have also influenced this variability. Though these burned plots were observed for a short amount of time, applying pure Quinclorac appears to be the most cost-effective in controlling this noxious weed in our study.

While herbicide still affects the mean cover on burned plots, control plots had the highest relative leafy spurge cover ($\bar{x}$ = 0.54), followed by Glyphosate ($\bar{x}$ = 0.42). The effectiveness of Quinclorac ($\bar{x}$ = 0.02) and Quinclorac + Dicamba ($\bar{x}$ = 0.03) against leafy spurge is further supported by having the lowest relative leafy spurge cover. Mean native relative cover differs as Glyphosate has the highest mean ($\bar{x}$ = 0.38). Though Quinclorac has the second highest relative cover for native species ($\bar{x}$ = 0.24), it is not different from the control ($\bar{x}$ = 0.06) and Quinclorac + Dicamba ($\bar{x}$ = 0.05). The conflicting results between mean leafy spurge counts and mean relative native cover for the Quinclorac treatments could mean that treating with Glyphosate is more beneficial in maintaining and possibly re-establishment of native species. While the plots treated with Quinclorac and Quinclorac + Dicamba decreased leafy spurge, other invasive species, such as smooth brome and Kentucky bluegrass, invaded and became the most dominant invasive species. Although not analyzed, these plots were observed to have extensive spread of smooth brome.

The herbicide treatments on the unburned plots impacted leafy spurge stem density counts 24 months after treatment. but where not statistically different among treatments. The unburned plots show that Quinclorac and Quinclorac + Dicamba have the most success in decreasing mean leafy spurge counts. However, data measured on September 2019 showed this effect decreased. This shows the three herbicide treatments decrease leafy spurge for only a short amount of time. Though Quinclorac first appeared to be the most effective in weed control, data from September 2019 show that the addition of Dicamba keep the counts lower with 3.38/m^2 compared to pure Quinclorac's 12.8/m^2 and Glyphosate's 29.16/m^2. While Quinclorac + Dicamba doesn't decrease leafy spurge counts during June, its effectiveness shows as it's able to keep counts low throughout the summer.

Mean relative cover of leafy spurge in unburned plots was affected by herbicide treatments. Control plots had the highest mean relative cover ($\bar{x}$ = 0.31), followed by Quinclorac ($\bar{x}$ = 0.25), Quinclorac + Dicamba ($\bar{x}$ = 0.16), and Glyphosate ($\bar{x}$ = 0.06). Burning did not take place in these areas, which could have led to untreated plots having the highest relative cover of leafy spurge. For mean relative cover of native species, Glyphosate had the highest mean ($\bar{x}$ = 0.55), followed by Quinclorac + Dicamba ($\bar{x}$ = 0.38), Quinclorac ($\bar{x}$ = 0.29), then control ($\bar{x}$ = 0.24). The relative native cover of Quinclorac + Dicamba, Quinclorac, and control plots were not different from one another. Not being burned, the plots likely had extensive infestations of weeds and/or invasive species, even more so than seen in burned plots. Quinclorac's and Quinclorac + Dicamba's narrow target range may have a negative impact on these areas as they may not have been able to kill invasive species such as smooth brome and Kentucky bluegrass. Burning has been shown to control these invasive plants (Owensby & Smith 1973; Towne & Owensby 1984).

Quinclorac's effects on seed emergence could also be impacting the cover and spread of native species. Boydston et al. (2010) investigated quinclorac's impacts on a valuable native species Switchgrass (*Panicum virgatum*). Throughout the observed years of 2005 and 2006, quinclorac caused injury to established switchgrass stands. While quinclorac was the least harmful of the three tested herbicides, it still negatively impacted switchgrass yield in 2005. During the first year of the grass' establishment, quinclorac reduced switchgrass biomass by sixteen percent.

Since establishing switchgrass requires long periods of time with consistent, active management, the risk Quinclorac poses for injury and subsequent failure to spread may be costly (Kering et al. 2013). More recent studies solidify the concerning effects on switchgrass as stands were poor after application of herbicides, including quinclorac. Switchgrass needs to cover at least 40% to be considered successfully established (Schmer et al. 2006). Though that is a concern, the timing in which inter-seeding took place may have also influenced the wide variability in the data. July is not a typical time for native species, such as switchgrass, to emerge. Application of herbicide combinations including quinclorac during the first growing season resulted in 13-26% coverage. This is far below the minimum coverage needed to be defined as self-sustaining (Kering et al. 2013). Since quinclorac causes a negative impact on switchgrass' ability to establish and spread, the possibility that it could also reduce yield of other native grass species is a real concern for restoration efforts. Application of herbicides alone is not an adequate solution in encouraging survival and growth of native grasses such as switchgrass.

When applying these herbicides, allowing a prescribed burn before application may be required to not only keep leafy spurge at low numbers but also to maintain native relative cover. Applying herbicide treatments multiple times is also recommended as the strong effects of Quinclorac against leafy spurge counts is short-term. Adding Dicamba with Quinclorac does have a more lasting effect in control, despite having higher leafy spurge counts in the earlier June 2019 measurement.

Due to the low emergence of seedlings on the treatment areas with high leafy spurge density, we surveyed an adjacent planting which obviously had better germination of the planted seeds. The three hilltops were treated with the same rate application of Glyphosate + MSO, and a spring prescribed burn at the same time we established our treatments, showed more promise for native species establishment than any of the surveyed treatment plots. Mean relative cover for leafy spurge ranged between only 0.01 to 0.04 (Figure 2.10) and mean relative cover for native species ranged from 0.95 to 0.99 (Figure 2.11). This is obviously different from the burned plots surveyed for this study. We believe this data supports our theory of some sort of mechanical malfunction in our seed drill within the treatment areas.

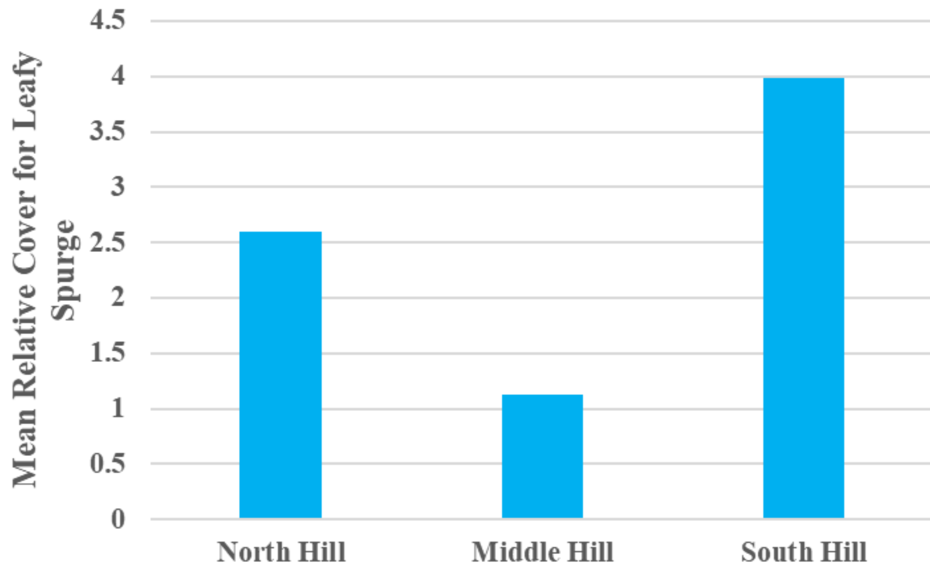

Figure 2.10. Mean Relative Cover of Leafy Spurge on Burned Hilltop Plots for Glyphosate Treatment During June 2019.

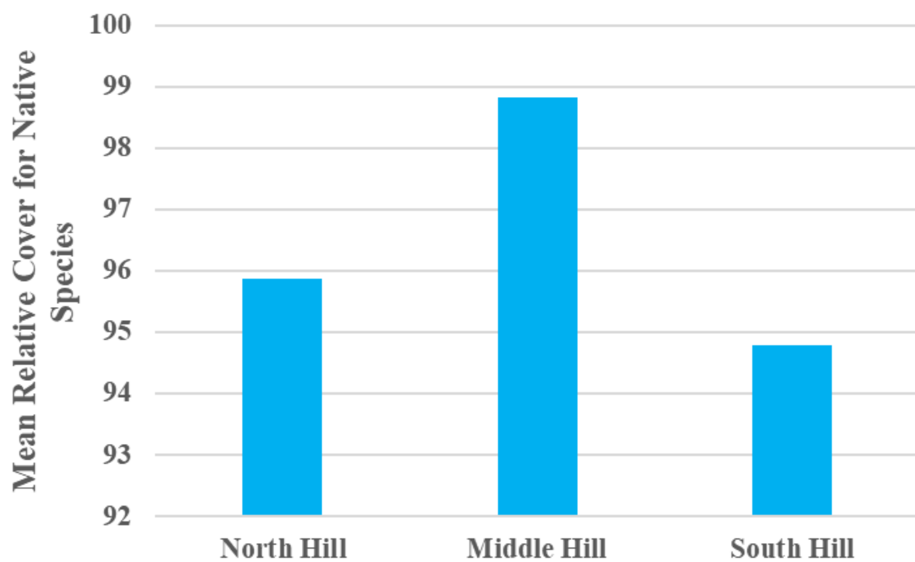

Figure 2.11. Mean Relative Cover of Native Species on Burned Hilltop Plots for Glyphosate Treatment During June 2019.

Summary

Invasive plant species have complicated restoration efforts by altering prairies and wetlands into new ecosystems. More active restoration methods are required to both control invasive plants and re-establish natives. Based on our experiment regarding methods: seeding, seed + mulch, and seed + mulch + plugs; seeding appears to be just as effective compared to the more involved methods. While the experiment on plug arrangement showed that seeding alone had the highest plant cover, this may be due to the plug-treated areas being weeded during the summer months and the area's hydrology.

Quinclorac appears to be the most effective at controlling leafy spurge numbers in our experiments involving spring-prescribed burn and without burning. Other invasive upland species, however, become dominant in areas treated with this herbicide. Difficulties with inter-seeding and surveying, herbicide drift, and widespread thatches of Kentucky bluegrass may have influenced the wide variation in our data. The additional sites west of the study area show stronger results for both the spring-prescribed burn and glyphosate use in re-establishing native plant communities and decreasing leafy spurge prevalence.

Management Implications

Six years is not enough time to gauge the actual success rate of native re-establishment between restoration treatments. More time, a minimum of ten years, is required to determine if seeding is truly as effective as adding native hay mulch and vegetative plugs. The experiment involving grid plugs versus grouped plugs also needs more time and observation. Initial findings indicate there might not be a difference in spread of plugs, and seeding is the most effective in initially establishing plant cover. Consistent management involving these methods over the subsequent decades are needed to ensure restoration efforts continue to be successful in bring back native wetland communities. Extensive training in identifying areas to survey and avoid may help limit the variability of data. Keeping a record of weather, specifically wind speed and direction, on days of herbicide application may also help determine if drift may have occurred.

Quinclorac and quinclorac + dicamba are most effective in the short-term. Reapplication of herbicide is needed to keep invasive species numbers low. The hilltops west of the study site showed that glyphosate after a spring-prescribed burn had the greatest success in limiting leafy spurge and re-establishing natives. Taking note of the differing cover (thick thatches of Kentucky bluegrass on Quinclorac-treated areas, or looser sandier soils) may be beneficial in determining which combination of herbicide and burning works best. Adjusting treatments depending on the soil and current plant cover, such as tilling to break up Kentucky bluegrass or alternative grazing methods, may prevent variability of results.

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