RIPARIAN GRAMINOID SPECIES RESPONSES AND PRODUCTIVITY IN COMPROMISED ENVIRONMENTAL AND SOIL CONDITIONS

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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

Riparian buffers have been created as a sustainable and effective way to combat the harmful effects of excess nitrogen and soil salinity in riparian settings. The goal of this research was to determine what species will I) germinate in saline environments and II) establish and produce sufficient biomass while being exposed to increased nitrogen. Incubation of eight native riparian graminoid species were evaluated for their ability to germinate in $MgSO_4$-induced salinity. In a greenhouse study, seven riparian graminoid species were evaluated to quantify their ability to survive and take up nitrogen, mimicking buffer strips exposed to high inputs of runoff nitrogen. Slender wheatgrass and green needlegrass were able to germinate successfully when exposed to $MgSO_4$ with EC levels up to 16 dS m^{-1} and 8 dS m^{-1}, respectively. Of the graminoid species tested, smooth brome yielded sufficient biomass and nitrogen uptake percentages in a controlled setting.

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GENERAL INTRODUCTION

Riparian graminoids are critical to the proper functioning of riparian systems in the northern Great Plains. These species provide vital ecosystem services including, but not limited to, enhancing water quality by trapping sediments before entering the system and slowing the process of stream bank erosion (Brevik et al. 2015; Cao et al. 2019). Major focus has recently been given to implementing buffer strip laws. All fifty states within the United States (U.S.) have some sort of guidelines in place for minimum riparian management zones (RMZ) (Blinn and Kilgore 2001). However, these guidelines only include a certain width required for RMZ for the protection of perennial and intermittent streams, lakes, wetlands, ponds, and domestic water supplies (Blinn and Kilgore 2001). The guidelines do not specify what species to plant within the riparian buffer zone.

Although all states within the U.S. have guidelines, Minnesota is the only state with an active law in place. The Minnesota Buffer law was signed into law by Governor Dayton in June 2015 (Minnesota Board of Water and Soil Resources 2017). The law states that public waterways are to have an average width of 15.2 meters with a 9.1 meter minimum width of continuous buffer along the public waterway. Public waterways include lakes, reservoirs, wetlands, rivers, and streams. Ditches within Minnesota are to have a 5-meter-wide buffer and the vegetation must not interfere with future ditch maintenance. Landowners with property adjacent to a water body whose property is used for crop production, must comply with the buffer strip law (Minnesota Board of Water and Soil Resources 2017). The goal of this law is to mitigate non-point source pollution from agricultural fields by planting and establishing riparian buffers.

Native riparian graminoids have deep roots that keep stream banks from eroding (Weaver and Darland 1949). Soil and riparian vegetation filter out contaminants before entering the water system (Brevik et al. 2015; Cao et al. 2019). They also act together in increasing infiltration rates of water. Overland flow is then hindered and, in turn, flood damage to humans can be mitigated (Nielsen et al. 2009). All of this is vital in providing stream bank protection and maintaining a healthy riparian ecosystem (Kronvang et al. 2005). These vital ecosystem services are being threatened by increases in soil salinity (Nielsen et al. 2009). The ecosystem services riparian graminoids possess are vast and soil salinity is a growing issue in the northern Great Plains, specifically the Red River Valley (Brevik et al. 2015; Franzen 2007). Despite recent evidence to the potential severity of this problem, additional research is needed to understand how individual species respond to soil salinity and can therefore be used to combat this environmental concern. Salinity is a threat to these species and the ecosystem services they provide (Bernstein 1975). Riparian graminoids are unable to function properly when flushing of wetlands is reduced by flooding (Nielsen et al. 2009; Cao et al. 2019). Flooding extends the time water is on the landscape and when soils are saline, wetland vegetation is exposed to such conditions (Nielsen et al. 2009).

Soil salinity has become an overwhelming issue in much of the world (Bernstein 1975). Increasing soil salinization has impacted crops and restoration work, severely hindering food production and ecological management solutions (Akbarimoghaddam et al. 2011). Ecological management solutions cannot be implemented when plant physiology is affected (Onkware 2000). Physiology is affected when salinity creates an external osmotic potential around the root within the soil. In turn, this osmotic potential inhibits, and eventually prevents water uptake (Akbarimoghaddam et al. 2011; Naz et al. 2010; Onkware 2000). Research has been done on how plants are affected by salinity (Naz et al. 2010), but information on the ability of a seed to uptake water during the process of germination under saline conditions is limited (Schmer et al. 2012).

High water tables and capillary rise in conjunction with irregular topography are contributing factors to much of the salinity in the northern Great Plains (Black et al. 1981; Franzen 2007; Lobell et al. 2010). As a result of salinity, root zones of native plants and crops are negatively affected (Akbarimoghaddam et al. 2011; Guha and Panday 2012). Extensive research has been done to identify the salinity tolerance of crop species (Akbarimoghaddam et al. 2011); however, the information on the response of riparian plants is limited. One method to assess the effect of salinity on the germination success of riparian graminoids is to expose seeds to the salt directly (Schmer et al. 2012). This method introduces the seed to the salt without any confounding influence from the soil.

A broad number of species under different types of salinity need to be assessed for water resource specialists to be able to incorporate these particular graminoids in restoration planning. The three species required in the Minnesota Buffer Strip seed mix (CP21 Buffer Strip Mix – meets NRCS CRP guidelines) are smooth brome (*Bromus inermus*), Timothy (*Phleum pratense* L.), and perennial ryegrass (*Lolium perenne* L.) (Smith, Margaret. Personal communication, 2019). In addition to the above-mentioned species, Schmer et al. (2012) assessed four riparian grass species: Big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), Indiangrass (*Sorghastrum nutans*), and prairie cordgrass' (*Spartina pectinata*) ability to germinate when exposed to NaCl and determined that switchgrass had the greatest germination success.

Switchgrass, big bluestem, and prairie cordgrass are species typically found in riparian environments in the northern Great Plains and served as the study's tall warm season grass species. Slender wheatgrass and green needlegrass are cool season grasses and represented such in the study. Kentucky bluegrass is a cool season grass but is not native and is typically found in invaded/native locations. The native sedge, Woolly sedge, is a very common wetland obligate species in much of the Red River Valley, therefore, it represented the grass-like species in this study [USDA Natural Resources Conservation Service Ecological Site Information System (USDA-NRCS ESIS) 2012]. Variation within cool and warm season grasses was represented in the study to promote ground coverage throughout an entire growing season in future riparian reclamation projects.

The vast majority of saline soils within the state of North Dakota are influenced by $MgSO_4$ (Seeling 2000). Currently, there is limited to no research on the ability of the plant species to germinate in $MgSO_4$ induced salinity within riparian ecosystems. The lack of information concerning the germination response of riparian plant species in the northern Great Plains has created uncertainty for environmental planners restoring riparian ecosystems and landowners establishing riparian buffers in determining I) what species to plant? II) what level of salinity seeds can tolerate? and III) what seeding rate is needed for successful establishment? The objective of study one was to determine final germination of seven graminoid species commonly found in riparian areas across increasing electrical conductivity (EC) levels of $MgSO_4$ induced salinity. The objective of study two was to identify riparian graminoid species with the potential to mitigate the impacts of excess N within riparian ecosystems. The results from this research can be used to determine the salinity and N thresholds at which the selected species can successfully germinate and identify the plant species that would be best suited for riparian restoration and buffer establishment.

Thesis Organization

This thesis consists of three chapters and is organized in a manuscript format. Chapter 1 is a literature review regarding riparian graminoid species and the known impacts of increased soil salinity and excess nitrogen and is titled "Species Specific Responses to Environmental Conditions: A Review of Riparian Ecosystems." The review summarizes the known effects of soil salinity and how it can impact plant growth, establishment, and productivity within riparian ecosystems as well as how nitrogen is known to affect those same species. Chapter 2 is titled "Germination Thresholds of Riparian Graminoid Species in Response to $MgSO_4$ Induced Salinity" and focuses on the germination response of the species tested when exposed to increased salinity levels. Chapter 3 is titled "Determination of Best Vegetation for Riparian Buffer Sites Exposed to Excess Nitrogen from Field Runoff" and highlights our greenhouse study which was geared more towards field-related parameters.

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CHAPTER 1. SPECIES SPECIFIC RESPONSES TO ENVIRONMENTAL CONDITIONS: A REVIEW OF RIPARIAN ECOSYSTEMS

Introduction

Riparian ecosystems are locations that unite land and water ecosystems. These environments play crucial roles in nutrient removal from nonpoint source (NPS) pollution and slow the process of stream bank erosion (Cao et al. 2019). Riparian buffer strips have been implemented in riparian ecosystems to mitigate the transport of excessive nutrients from NPS pollution in order to slow or cease the process of eutrophication in freshwater systems (Kronvang et al. 2005). The most effective types of vegetation utilized in these buffer strips are grasses, woody vegetation, and forested species. These species have proven to be most effective at reducing nitrates and phosphorus contaminants from subsurface flow (Kyehan et al. 2000; Zhang et al. 2010; Izydorczyk et al. 2018). In addition to the above-mentioned ecosystem services, riparian vegetation as a whole acts as a major streambank stabilizer due to the deep root systems associated with these particular plant species (Hagerty et al. 1981; Henderson 1986; Medina 1995; Wynn et al. 2004; Brevik et al. 2015).

In order to protect riparian ecosystems, all states within the United States have focused on implementing buffer strip laws. Most of these states have guidelines to manage riparian zones but they only include widths necessary for land managers to follow (Blinn and Kilgore 2001). The guidelines do not specify what species to plant within the riparian buffers. The only state with an active law in place is Minnesota. Governor Dayton signed the Minnesota Buffer law into action in June 2015 (Minnesota Board of Water and Soil Resources 2017). The law includes the proper width required for buffer strips, who needs to adhere to the law, and what types of waterways require buffers. A buffer strip seed mix has been created to help landowners utilize the correct riparian species to plant within their buffers. The Minnesota Buffer Strip seed mix is comprised of (CP21 Buffer Strip Mix – meets NRCS CRP guidelines) smooth brome (*Bromus inermus*), Timothy (*Phleum pratense* L.), and perennial ryegrass (*Lolium perenne* L.) (Smith, Margaret. Personal interview 2019).

Riparian buffer strips provide stream bank stabilization and help to maintain healthy aquatic ecosystems. Stream banks are stabilized by the presence of deep-rooted vegetation in the form of grasses or sedges (Hagerty et al. 1981; Henderson 1986; Medina 1995; Wynn et al. 2004; Brevik et al. 2015). These species are referred to as riparian graminoids. Riparian graminoids are critical to the proper functioning of riparian systems in the northern Great Plains (Dodds et al. 2004; Lichvar et al. 2012; Brooks et al. 2013).When riparian graminoids establish successfully, major ecosystem services are provided. These species provide vital ecosystem services including, but not limited to, enhancing water quality by trapping sediments before entering the system and slowing the process of stream bank erosion (Petersen 1986; Winward 1994, 2000; Brevik et al. 2015). These plants increase water infiltration rates which, in turn, slows overland flow in major flooding events (Hagerty et al. 1981; Henderson 1986; Medina 1995; Wynn et al. 2004; Brevik et al. 2015). When overland flow is slowed, flood damage to humans is mitigated. In addition to mitigating flood damage, riparian graminoids uptake nitrogen (N) which inhibits the negative environmental impact excess N can cause in aquatic ecosystems. With all of the above-mentioned, riparian buffer strips have been created as a natural and sustainable way to mitigate these harmful environmental impacts.

A wide range of riparian-related literature reviews have been conducted. Of those, forested riparian ecosystems are most heavily reviewed (Fisher & Acreman 2004). In addition to forested ecosystems, reviews have focused on the type of wetland buffer strips (e.g. crop, forest, or grassland) that are most effective in reducing N or phosphorus inputs. Buffer strips have also been studied with regards to how effective various buffer widths may be in removing nutrient inputs (Poff et al. 2011). Overall, it is well documented from previous research as to which buffer zone types may be best in reducing nutrient loading from nitrogen and phosphorus (Fisher & Acreman 2004). However, there is a need for synthesis of literature that focuses on the ability of riparian plant species to establish successfully under saline conditions in order to uptake N.

The purpose of this literature review is to synthesize literature on the ability of riparian species to establish and provide ecosystem services in environments with high salinity and N. These environmental conditions were selected because they are often common in riparian ecosystems and can cause the degradation of riparian vegetation health, which plays a crucial role in maintaining healthy water resources. Knowing how increased soil salinity impacts the establishment of riparian graminoids and the threat nitrogen poses to water resources when they are exposed to this nutrient in abundance, has created a need for synthesis of literature on what species may be best to plant in riparian buffers to uptake N and combat its negative environmental impacts.

Effects of Salinity on Riparian Ecosystems

Riparian species have unique ecological adaptations and degrees of tolerance to withstand many types of environmental conditions. A main concern for natural resource professionals is soil salinity within riparian ecosystems and the significant impact it can have on species distribution and community composition (Nucci et al. 2012; Walbridge 1994). In native grasslands, soil salinity can affect the plant's reproductive structure, thus, hindering the rate at which a plant community can establish (Onkware 2000). In addition to establishment, groundwater salinity influences plant species composition in riparian ecosystems (Guha and

Panday 2012). Haiyang et al. (2016) found that groundwater salinity affected plant communities more than soil salinity within riparian ecosystems. The relationships between soil salinity and plant species structure also has an impact on the above-mentioned factors influencing the survivorship of riparian vegetation (Naz et al. 2010). Because of changing habitats, the distribution of plant species within riparian ecosystems can be greatly affected by soil salinity. Furthermore, saline conditions may weaken soil aggregate stability and increase levels of bare ground which puts these fragile ecosystems at a high risk for erosion and streambank degradation (Mamedov et al. 2002; Hecker et al. 2018).

The ecosystem services that riparian graminoids provide are extensive and increased soil salinity compromises those services by inhibiting establishment (Bernstein 1975) (Figure 1.1). Soil salinity creates an external osmotic potential around cells within plant roots (Seelig 2000; Akbarimoghaddam et al. 2011). Plant roots then struggle to extract water from the soil and drought-like symptoms are exhibited by the plant and as a result, growth is reduced or the plant dies (Seelig 2000).

Riparian graminoids are unable to function properly when flushing of wetlands is reduced; due to flooding (Nielsen et al. 2009). Flooding extends the time water is on the landscape and when soils are saline, wetland vegetation is exposed to such conditions (Nielsen et al. 2009). Despite recent evidence to the potential severity of this problem, additional research is needed to understand how individual species respond to soil salinity and can therefore be used to combat this issue.

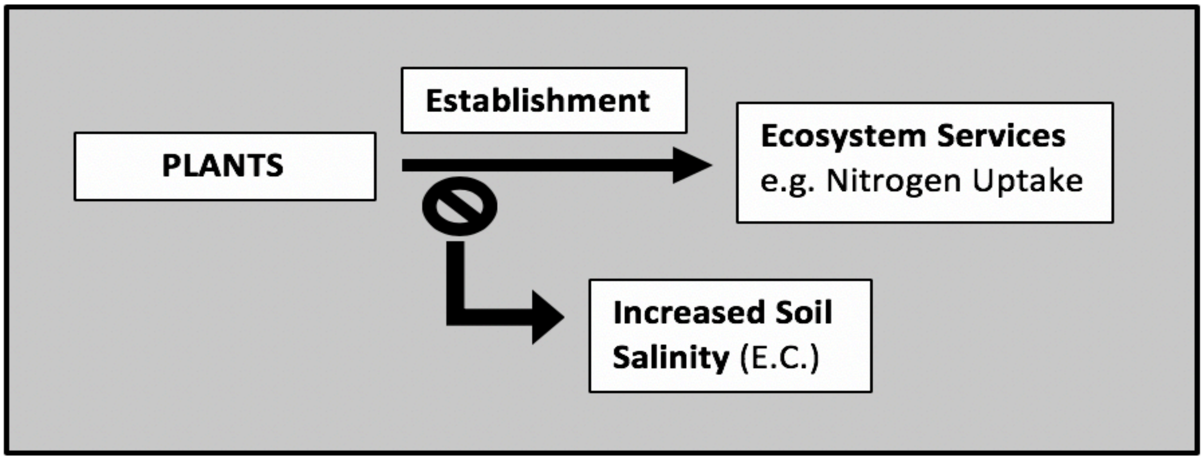

Figure 1.1. A conceptual diagram showing the relationship between riparian plants and the ecosystems services they provide when successful establishment occurs. Salinity affects the ability of plants to establish successfully. Thus, inhibiting or ceasing ecosystem services from the plants.

Salinity tolerance levels and responses of riparian species vary significantly within the northern Great Plains (Table 1.1). Generally, increased levels of salinity stunt plant growth because more energy is required by the plant to uptake water from the soil (Sigler et al. n.d.). Delayed germination and seedling development was observed which lead to evident water stress and temperature sensitivity. At high levels of salinity, physical damage and mortality are observed, significantly reducing plant population density and causing a major reduction in yields of their selected thirty-one native and culturally significant riparian species within the northern Cheyenne Reservation (Sigler et al. n.d.). Tober et al. (2007) suggested that graminoids are, in general, more salt tolerant (EC 5-25 dS m^{-1}) than forbs (EC 2-6 dS m^{-1}), legume (EC 2-6 dS m^{-1}), trees (EC 4-15 dS m^{-1}), and shrub species (EC 4-15 dS m^{-1}).

Tober et al. (2007) categorized moderately sensitive native forbs and legumes as being able to withstand electrical conductivity (EC) levels between 2-6 dS m^{-1}. Moderately sensitive herbaceous forage plants in their study were able to withstand EC levels between 5-10 dS m^{-1}, while Sigler et al. (n.d.) categorized "moderately sensitive" as 2-4 dS m^{-1} for all species in their study. Similar species categorized as moderately sensitive in both studies include: switchgrass

(*Panicum virgatum*), box elder (*Acer negundo*), and wild plum (*Prunus americana*) (Sigler et al. n.d.; Tober et al. 2007). Although similar species were assessed in both studies, little research exists evaluating the salinity tolerance of riparian species.

One of the greatest limitations to the establishment of riparian species in areas with salinity is the ability for the species to germinate. Schmer et al. (2012) tested both switchgrass and prairie cordgrass (*Spartina pectinata*) and found that switchgrass germinates successfully up to 8 dS m^{-1}; whereas, Kim et al. (2011) did not see successful results from switchgrass but did see promising results from prairie cordgrass. These two different experiments tested their grasses with the same salt; NaCl. These variable results may be to blame for the research gap in best establishment methods of riparian graminoid species. It is crucial for land managers to understand the type of salt present within their soil to be able to select species to plant that will establish successfully.

Table 1.1. Known salinity tolerance and EC range of riparian plant species native to the northern Great Plains Ecoregion.

Plant Type	Species	Salinity Tolerance	EC_e Range (dS m^{-1})	Reference(s)
Graminoids	*Spartina pectinata* (Prairie cordgrass)	Moderately Tolerant	10-15	Sigler et al. n.d.; Tober et al. 2007; Schmer et al. 2012; Kim et al. 2011.
	Elymus trachycaulus (Slender wheatgrass)	Tolerant	15-25	Tober et al. 2007
	Panicum virgatum (Switchgrass)	Moderately Sensitive	5-10	Schmer et al. 2012; Tober et al. 2007.
	Eleocharis palustris (Spikerush)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Typha latifolia (Broadleaf cattail)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Carex stipata (Saw beak sedge)	Moderately Sensitive	2-4	Maricle & Maricle 2018; Sigler et al. n.d.
	Scirpus nevadensis (Bulrush)	Moderately Tolerant/Tolerant	4-6/>6	Sigler et al. n.d.
	Equisetum arvense (Horsetail)	Moderately Sensitive	2-4	Sigler et al. n.d.
Shrubs/Trees	*Acer negundo* (Boxelder)	Moderately Sensitive	4-8	Sigler et al. n.d.; Tober et al. 2007.
	Salix exigua (Sandbar willow)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Prunus virginana (Chokecherry)	Sensitive	<2	Sigler et al. n.d.; Tober et al. 2007.
	(Amelanchier) Serviceberry	Sensitive	<2	Sigler et al. n.d.
	Cornus stolonifera (Red Osier dogwood)	Sensitive	<2	Sigler et al. n.d.
	Populus deltoides (Cottonwood)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Fraxinus pennsylvania (Green ash)	Moderately Tolerant	8-15	Sigler et al. n.d.; Tober et al. 2007.
	Populus tremuloides (Quaking aspen)	Sensitive	<2	Sigler et al. n.d.
	Prunus americana (Wild plum)	Sensitive/Moderately Sensitive	4-8	Sigler et al. n.d.; Tober et al. 2007.
	Symphoricarpos occidentalis (Snowberry)	Moderately Sensitive	2-4	Sigler et al. n.d.
Native Forbs	*Helianthus maximiliani* (Maximilian sunflower)	Moderately Sensitive	2-4	Tober et al. 2007.
	Mentha arvensis (Field mint)	Sensitive/Moderately Sensitive	<2/2-4	Sigler et al. n.d.
	Glycyrrhiza lepidota (Wild licorice)	Moderately Tolerant	4-6	Sigler et al. n.d.
	Nasturium officinale (Water Cress)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Aster foliatus (Leafy Aster)	Sensitive	<2	Sigler et al. n.d.
	Asclepias speciosa (Showy milkweed)	Moderately Sensitive	2-4	Sigler et al. n.d.
	Monarda fistulosa (Wild bergamont)	Moderately Sensitive	2-4	Sigler et al. n.d.

Several studies conducted within the northern Great Plains ecoregion evaluated the salinity tolerance of prairie cordgrass. All concluded that prairie cordgrass can be categorized as a moderately tolerant (EC 10-15 dS m^{-1}) grass species when established with an understanding of the soils conditions (i.e. broadcast seeding or rhizomes) (Table 1.1) (Sigler et al. n.d.; Tober et al. 2007; Kim et al. 2011; Schmer et al. 2012). In addition to prairie cordgrass, EC ranges used in this review revealed that many of the studies tested switchgrass at ranges between 0 and 20 dS m^{-1}. This is most likely because naturally occurring EC levels within soils typically fall within that range (Schmer et al. 2012). Switchgrass establishment in riparian ecosystems has a link to the amount or type of salinity in the soil system. This grass species has exhibited successful germination in previous research when exposed to high levels of salinity. Schmer et al. (2012) showed that switchgrass was able to germinate at EC levels ranging from 0 to 16 dS m^{-1}. This was revealed by germination percentages above 80% across the EC range (Schmer et al. 2012). This study tested four different grass species, including switchgrass, at EC levels ranging from 0-20 dS m^{-1}. Based on the current literature, we can conclude that EC ranges above 20 dS m^{-1} need to be researched more with a major focus on how switchgrass and other native graminoids respond to increased salinity levels.

In addition to the successful establishment switchgrass, slender wheatgrass (*Elymus trachycaulus*) also has potential for land managers focusing on riparian buffers. Tober et al. (2007) characterized slender wheatgrass as a tolerant grass, able to withstand EC levels from 15-25 dS m^{-1} (Table 1.1). These two native species: slender wheatgrass, switchgrass, will provide long-term ground cover and soil stability from their deep root systems (Hagerty et al. 1981; Henderson 1986; Medina 1995; Wynn et al. 2004; Brevik et al. 2015). As a result, they provide a viable option for riparian buffers in riparian ecosystems with saline soils within the northern Great Plains (Dodds et al. 2004; Lichvar et al. 2012; Brooks et al. 2013).

Effects of Excess Nitrogen on Riparian Ecosystems

In addition to increased riparian soil salinity, N is known as a primary stressor to aquatic ecosystems. When N enters water systems in abundance, it is classified as a pollutant that can eutrophication and contaminates groundwater (Fisher & Acreman 2004). Nitrogen enters riparian ecosystems in the form of organic N and inorganic N. Organic N is composed of leaf litter, fertilizers and animal waste. Anthropogenic structures such as roads and leaking sewer lines also contribute to overloading of N in aquatic systems. Inorganic inputs of N may include nitrate (NO_3^-), urea (NH^{+4}) and ammonia (NH_3) (Mayer et al. 2007; Obire et al. 2008; Islam et al. 2010). In these above-mentioned forms, N is classified as nonpoint source (NPS) pollution. The U.S. Environmental Protection Agency (U.S. EPA) defines NPS pollution as “pollution that is caused when rainfall or snowmelt, moving over and through the ground, picks up and carries natural and human-made pollutants, depositing them into lakes, rivers, wetlands, coastal waters and ground waters.” (U.S. Environmental Protection Agency 2018).

All organisms need N, but when it enters aquatic systems in large quantities, negative environmental impacts are observed (Carpenter et al. 1998). One potential negative environmental impact from N abundance is the process of eutrophication (Leakovic et al. 2000; Beutel et al. 2009), which results when oxygen is depleted in aquatic systems. Oxygen is depleted when algal blooms occur, and as a result, fish kills often rise (Carpenter et al. 1998; Carmichael and Boyer 2016). Impacts of these algal blooms can be seen in the Great Lakes and the Gulf of Mexico. Excess nutrient loading from the Mississippi leads to increases in phytoplankton growth (Campbell et al. 2019). The biomass of phytoplankton is then respired by aerobic microorganisms and the combination of respiration and the lack of ventilation to bottom waters leads to hypoxic zones (Dagg et al. 2007; Dagg et al. 2008). The effects of hypoxia in the northern Gulf of Mexico are most severe in the summer months because of the increased nutrient loading (N and P) from the Mississippi watershed (Chen et al. 2001; Rabalais et al. 2002; Rabalais et al. 2007).

Loss of biodiversity is a long-term symptom from excess nutrient loading (Carpenter et al. 1998). In addition to biodiversity loss, groundwater quality can be threatened when N enters water systems in abundance. Groundwater is extremely important to riparian ecosystems in periods of drought or low precipitation because it temporarily mitigates water stress in vegetation by supplying the roots with water until a rain event occurs (Chaves et al. 2002; Poff et al. 2011; Vivian et al. 2014). With NPS pollution as an environmental concern and groundwater quality at risk, riparian buffer strips have been created as an effective and sustainable way to protect aquatic ecosystems. These aquatic plants uptake and store N, mitigating the effects of N abundance within water resources (Mayer et al. 2007).

There is limited research on how excess N directly affects the ability of riparian vegetation to perform their respective ecosystem services (Wigington et al. 2003). However, it is known that the retention of NH_3-N occurs by plant uptake, soil microbial processes, and runoff infiltration (Vought et al.1995; Bunch and Bernot 2012). Wigington et al. (2003) assessed the nitrate removal effectiveness of riparian buffers along a small agricultural stream in western Oregon. They concluded that managing water quality effectively for nitrates must stem from sound agricultural practices and suggest the best way to combat excessive N in water systems is to apply fertilizers at the appropriate times and rates (Wigington et al. 2003). Lei et al. (2019) assessed the soil adsorption rate with increased levels of NH_3-N (ammonia). They found that soil adsorption rate and capacity increased with increased levels of NH_3-N. Soil that was waterlogged with NH_3-N exhibited characteristics of deceased adsorption rates over time. Lei et al. (2019) also found that the higher the NH_3-N levels, the more the adsorption rate decreases.

The ability of vegetation to influence water quality has been demonstrated by multiple researchers (Lowrance et al. 1984; Peterjohn and Correll 1984; Gilliam 1994; Correll 2000). A main contributor to the success of vegetation in reducing nitrates is understanding the hydrologic flowpath of water across the landscape. This is a critical component to consider when determining the effectiveness of these riparian systems and their ability to process nutrients and chemicals (Phillips et al. 1993; Lowrance et al. 1997). Water quality is more likely to be improved when it passes through the active root zone of riparian plants than water that exists as deep groundwater or surface runoff (Wigington et al. 2003).

Considerations for Riparian Buffer Establishment

Riparian species are impacted by ecosystem conditions (i.e. establishment methods, saline tolerance, and N uptake ability). Varying implications and conditions are evident depending on the nature of each previously mentioned ecosystem condition and the response each species has when exposed. Other confounding factors included: site environment, (e.g., greenhouse, germination chamber or natural setting) geographic location, and climate in which the study was conducted. These factors created an inability to draw definite conclusions as to how species actually respond when exposed to each of the riparian ecosystem factors researched in this review.

Further analysis revealed interesting trends in riparian plant species focused research. Kentucky bluegrass, willow, and alder are the top three most researched species when focusing on establishment within riparian buffers, tolerance to soil salinity and ability to uptake N. Definite reasons for the trends are unknown, but some general conclusions can be drawn.

Kentucky bluegrass is researched heavily because it is an invasive species that inhibits various hydrologic properties within riparian systems (Wynn et al. 2004; Eviner et al. 2012). Both willow and alder are used within riparian buffers to stabilize the soil with their moderate to deep rooting systems. These shrub species also establish quickly from cuttings when utilized in buffer strips (USDA Natural Resources Conservation Service 2011). The three most researched species analyzed in this review reveal definite needs for further research on native graminoids because they provide long-lasting soil stabilization (Hagerty et al. 1981; Henderson 1986; Medina 1995; Wynn et al. 2004; Brevik et al. 2015).

Prairie cordgrass, woolly sedge and green needlegrass are the three least likely species to be researched with respect to the riparian ecosystem factors included in this review. It is documented that prairie cordgrass and woolly sedge are difficult species to establish from seed but establish successfully in riparian buffers via plugs or rhizomes (Kim et al. 2011; Steed & DeWald 2003). The low number of studies focusing on green needlegrass may be explained because this species has a germination percentage close to 50% (AOSA 2010). The trends within these three species support the evidence that some species are difficult to study in lab settings. In these cases, vegetative establishment within natural settings may exhibit more successful results (AOSA 2010; Kim et al. 2011; Steed & DeWald 2003).

According to the literature, N uptake is researched the most regarding riparian species and buffer strips. This finding is not surprising as NPS pollution, specifically fertilizer runoff from crop fields, has become a major concern for water resource professionals (U.S. Environmental Protection Agency 2018). Best establishment methods of riparian species and tolerance to soil salinity are both researched around 20%. This is an important consideration when creating a direction for future research regarding riparian buffer establishment in locations that have compromised soil and environmental conditions.

It is crucial for land managers to understand the type of establishment the species needs in order to achieve successful long term ground cover (Kadlec & Wentz 1979; Steed & DeWald 2003; Tober et al. 2007; Kim et al. 2011). For example, it is documented that woolly sedge establishes better via rhizomes rather than seeding in compromised soil conditions (Kadlec & Wentz 1979; Steed & DeWald 2003). Furthermore, Kim et al. (2011) found that prairie cordgrass establishment is much more successful when planted via rhizomes or plugs instead of seeding. Results from these studies indicate that proper seed selection, proper establishment methods based on previous research, and having a thorough understanding of the type of salt and EC level associated with the site in which land managers are working, will aid in successful establishment of riparian buffers.

Conclusions

There is a limited body of research addressing the salinity tolerance of riparian plant species within the northern Great Plains. Future research is necessary to draw conclusions as to which plant species should be seeded or vegetatively established in riparian buffers to combat soil salinity impacts as well as mitigate the harmful effects of excess N within water systems. There has been a strong emphasis on width and type of riparian buffers that may prove to be successful, however, there is limited research on species selection. Moreover, when researching salinity tolerance of each species, differing salinity tolerance categories were evident.

Riparian buffers provide extremely important ecosystems services. They increase water infiltration rates and slow overland flow in major flooding events. Another extremely important ecosystem service these plants provide is N uptake. Threats to the effectiveness of riparian buffers include: soil salinity and N from NPS pollution. With increased soil salinity threatening plant establishment and N from NPS pollution as an increasing problem worldwide, it is crucial to understand the ecological impacts these two factors will have on riparian ecosystems.

This review reveals that it is critical to better understand the role that these environmental concerns have on the effectiveness of riparian buffers. Increased understanding of this process will increase the establishment of riparian plant species and improve ecosystem services in riparian ecosystems. Further research examining establishment methods of native graminoids exposed to increased soil salinity and increased levels of N, is crucial in identifying the best plant species for riparian buffers exposed to compromised environmental conditions.

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CHAPTER 2. GERMINATION THRESHOLDS OF RIPARIAN GRAMINOID SPECIES IN RESPONSE TO $MgSO_4$ INDUCED SALINITY

Materials and Methods

This study evaluated the final germination of seven species: *Andropogon gerardii* (big bluestem), *Spartina pectinata* (prairie cordgrass), *Panicum virgatum* (switchgrass), *Poa pratensis* (Kentucky bluegrass), *Elymus trachycaulus* (slender wheatgrass), *Nassella viridula* (green needlegrass), *Carex pellita* (woolly sedge), and *Hordeum vulgare* (barley). We analyzed these species to determine saline tolerance during the fragile stage of germination. This study was conducted at the North Dakota State Seed Lab in Fargo, North Dakota.

Saline tolerant species in this study were prairie cordgrass (up to 15 dS m^{-1} - NRCS) and barley (up to 25 dS m^{-1} - NRCS) (Table 1.1). These saline tolerant species served as reference when compared to others evaluated in the research. We chose riparian graminoid species that naturally thrive in riparian or wet meadow areas within the northern Great Plains. In addition, they tend to be acclimated to locations that are moderately to highly saline. Seeds were obtained from Agassiz Seed Supply, West Fargo, ND.

All species were evaluated at six salinity levels with EC values of 0, 4, 8, 16, 24, and 32 dS m^{-1}. Salt solutions at each EC level consisted of varying amounts of crystallized $MgSO_4$ and distilled water. Solutions were made by dissolving $MgSO_4$ in distilled water until the desired EC level was reached. Once dissolved, each EC level was verified with a Sension 378 conductivity probe. (Hach Co., Loveland, CO, USA). Distilled water (0 dS m^{-1}) was used as the control in the experiment. High salinity was expressed as 16-32 dS m^{-1}. Each species was represented in three replications exposed at the six EC levels. A total of 144 units were observed in this experiment (3 reps x 8 species x 6 EC_1 levels x 1 solution: $MgSO_4$).

Germination testing was conducted at the North Dakota State Seed Lab in Fargo, North Dakota. Each species was planted in a separate petri dish with a base layer of blotter paper and germination paper on top (Schmer et al. 2012). The blotter paper was then saturated with approximately 10 mL of salt solution. This provided the seed with adequate moisture for one full week of germination.

Following the saturation of the blotter paper, seeds of switchgrass, Kentucky bluegrass, slender wheatgrass, and barley were prepared as stated in Table 2.1 to break seed dormancy as specified in Association of Official Seed Analysts (AOSA) guidelines (AOSA 2010). Following the initial preparation, petri dishes were wrapped in plastic to prevent desiccation and then arranged on trays in a completely randomized design (CRD). This ensured all seeds were exposed to similar conditions throughout the germination period.

Table 2.1. Seed characteristics and germination testing procedures of plant species based on the Association of Official Seed Analysts (AOSA) rules for testing seeds.

Species	Number of Seeds Planted per Species	Chill Period	Count Interval (days)	Pure Seed (%)	Germination (%)
Red River prairie cordgrass	38	none	7_14_21	66.1	19
Revenue slender wheatgrass	22	5 days	14	98.5	96
Big bluestem	29	none	7_14	95.5	60
Arrowhead Kentucky bluegrass	100	5 days	14_21_28	used AOSA standard	
Woolly sedge	21	none	7_14_21	97.2	72.9
Dacotah switchgrass	23	14 days	21_28	99.3	86
Lodorm green needlegrass	32	none	7_14	99.8	4
Hays forage barley	23	3 days	7	99.8	94

Germination took place in a Hoffman seed germinator (model no. SG30SC, Hoffman Mfg. Inc. Jefferson, OR U.S.A.) for a varying number of days. Seeds were placed in their respective growth chamber based on guidelines for seed testing set up by the AOSA. The germination chambers utilized in the experiment were programmed to expose the species to alternating photoperiods throughout the day. The germinator that housed switchgrass, green needlegrass, and Kentucky bluegrass was programmed to run with 8 hours of light at 25 ˚C and 16 hours without light at 15 ˚C. A separate germination chamber housed big bluestem, prairie cordgrass, woolly sedge, and slender wheatgrass. This particular germinator ran with 8 hours of light at 30 ˚C and 16 hours without lights at 20 ˚C. Lastly, barley was kept in a germinator that held a consistent temperature while light availability varied. This germinator had 8 hours of light at 20 ˚C and 16 hours of no light also at 20 ˚C. Once in the growth chamber, we monitored seeds on a weekly basis for the duration of germination (Zuk et al. 2012).

Seeds were determined as germinated when the presence of both the radicle and hypocotyl were evident (AOSA 2010). After a seed germinated, it was recorded and removed from the petri dish. Germination percentage was based on seeds that germinated within the respective number of growth days set for that species (Table 2.1). Some seeds were abnormal in that they started, but did not successfully complete germination. Abnormal seeds were categorized as not germinated, and germination percentage was calculated as the number of seeds that successfully completed germination over the total number of seeds tested (x 100). Final germination of species at each EC level was calculated and analyzed to determine germination thresholds of riparian graminoid species.

Final germination (FG) was calculated by dividing the number of germinated seeds by the total number of seeds planted in each petri dish (i.e. Pure Live Seed) and multiplied by 100.

To determine if EC level had a significant effect on the final germination percentage of the species, a general linear model analysis of variance was used in SAS® (version 9.4, SAS Institute, Inc.) with a Tukey's adjustment significance were determined at the 0.05 level of confidence for graminoid species.

Results

Germination responses differed by both EC levels and species. Significant differences ($p \leq 0.05$) were observed for the main effects of species and EC levels, as well as species x EC level interactions ($p \leq 0.05$) (Table 2.2 and Table 2.3). Germination percentage for individual species significantly decreased as EC levels increased (Table 2.3). The exception would be prairie cordgrass and woolly sedge, which had low germination rates regardless of EC level. The EC level where FG significantly decreased varied depending on species.

Table 2.2. Analysis of variance for the germination of riparian graminoid species in $MgSO_4$ saturated medium as affected by species and electrical conductivity (EC) level.

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr>F
Plant	7	107188.0642	13398.508	430.42	<.0001
EC level	5	61727.8495	12345.5699	396.60	<.0001
Plant*EC level	35	64559.5122	1613.9878	51.85	<.0001

At the EC level of 4 dS m^{-1}, Kentucky bluegrass experienced a significant decline in FG from the control. Whereas, the FG of slender wheatgrass increased at 4 dS m^{-1} and had FG similar to the control at 8 and 16 dS m^{-1}. At 16 dS m^{-1}, big bluestem (14.9%) and green needlegrass (0.0%) FG decreased compared to the control, however, FG of slender wheatgrass (68.1%), barley (92.8%) and switchgrass (86.9%) did not differ from the control. At an EC of 24 dS m^{-1}, switchgrass (44.9%), slender wheatgrass (9.1%), and barley (2.9%) all experienced a significant decrease in FG from the control, but FG rates were still higher than that of prairie cordgrass (0.0%), big bluestem (0.0%), green needlegrass (0.0%), woolly sedge (0.0%), and

Kentucky bluegrass (0.0%). At 32 dS m^{-1}, switchgrass experienced a significant decrease in FG from 44.9% to 0.0%. Big bluestem was the only species that germinated at 32 dS m^{-1} with a FG of 1.1%. Of the graminoid species tested in this study, barley, switchgrass, and slender wheatgrass all exhibited a significantly higher FG than the other graminoid species tested (Table 2.3). Therefore, we can conclude that barley, switchgrass, and slender wheatgrass would be expected to perform best in environments with $MgSO_4$ induced salinity.

Table 2.3. Mean values (with standard deviation) for the germination percentage as affected by electrical conductivity (EC; dS m^{-1}) and graminoid species.

EC (dS m^{-1})	Barley	Big bluestem	Green needlegrass	Kentucky bluegrass	Prairie cordgrass	Slender wheatgrass	Switchgrass	Woolly sedge†
0	92.9±2.7 Aa	71.3±8.7 Ab	52.1±0.1 Ac	89.5±3.0 Aa	0±0 Ad	78.8±7.0 Ab	88.4±10.9 Aa	1.6±2.8 d
4	91.5±7.7 Aa	65.5±6.0 Ab	56.3±3.2 Ab	1.9±1.6 Bc	0±0 Ac	95.5±4.6 Ba	94.2±10.1 Aa	4.8±4.8 c
8	96±0 Aa	59.8±14.4 Ab	33.4±11.0 Ac	0±0 Bd	0±0 Ad	72.7±13.7 Aa	89.9±6.7 Aa	0±0 d
16	92.9±2.7 Aa	14.9±7.2 Bc	0±0 Bd	0.6±0.5 Bd	0±0 Ad	68.2±16.4 Ab	87.0±7.5 Aa	0±0 d
24	2.9±2.5 Bb	0±0 Bb	0±0 Bb	0±0 Bb	0±0 Ab	9.1±7.9 Cb	44.9±6.7 Ba	0±0 b
32*	0±0 B	1.1±2.0 B	0±0 B	0±0 B	0±0 A	0±0 C	0±0 C	0±0

Upper case letters separate germination means across EC levels for each individual plant and lower case species separate germination (%) means across plant species by EC level. *Denotes no differences observed across species and † denotes no difference observed across EC level.

When exposed to 24 dS m^{-1} and higher, barley germinated at less than 3%. At lower EC levels, 0-16 dS m^{-1}, barley germinated >92%. Switchgrass was by far the most saline tolerant perennial grass species. At levels ranging from 0-16 dS m^{-1}, germination percentages were FG>85%. Switchgrass had the highest final germination percentage of 44.9% at 24 dS m^{-1}. This result was much greater than the crop species, barley, which germinated at less than 3% at EC 24 and 32 dS m^{-1}.

The highest final germination percentage recorded for prairie cordgrass had was less than 2% at the EC level 0. Similar results were exhibited for woolly sedge. The highest final germination of woolly sedge was at 4 dS m^{-1} (4.8%). Kentucky bluegrass revealed a very interesting response to $MgSO_4$ induced salinity. At the control level, EC 0 dS m^{-1}, Kentucky bluegrass germinated at 89.5%. However, when exposed to germination levels greater than 4 dS m^{-1}, Kentucky bluegrass germination was less than 2%. Slender wheatgrass had final germination rates greater than 70% at EC levels from 0-8 dS m^{-1}. Development of green needlegrass decreased significantly when exposed to EC levels greater than 4 dS m^{-1}. At all EC levels higher than 4 dS m^{-1}, green needlegrass germinated under 33.3%. At 0 dS m^{-1}, big bluestem germinated at 71.3%.

Discussion

Species with the highest salinity thresholds included slender wheatgrass, switchgrass, and barley. Based on our results, these three species may be able to germinate successfully on $MgSO_4$ saline soils with EC levels up to 16 dS m^{-1}. The results slender wheatgrass exhibited in our study are consistent with the results of Tober et al. (2007). They found that slender wheatgrass is tolerant of salinity levels ranging from 15-25 dS m^{-1}. Switchgrass was also able to germinate at levels from 0-16 dS m^{-1}, which is not consistent with results of Tober et al. (2007) and Schmer et al. (2012), which both categorized switchgrass as moderately sensitive and only able to with stand EC ranges between 5-10 dS m^{-1}. The results barley exhibited in our study are consistent with many studies assessing the salinity tolerance of barley; it is well established from previous research that barley is a saline tolerant crop species (Tober et al 2007; Akbarimoghaddam et al. 2011; Wu et al 2013; Fu et al 2019).

We found green needlegrass can successfully germinate under $MgSO_4$ induced saline soils with EC levels up to 8 dS m^{-1}. In this study, green needlegrass was able to germinate in $MgSO_4$ induced salinity at EC levels up to 8 dS m^{-1}. Information on the salinity tolerance of green needlegrass is limited. Therefore, there is a need for more research assessing the salinity tolerance of green needlegrass and its ability to germinate in $MgSO_4$ induced saline soils. Although there is limited research on the ability of green needlegrass to germinate in saline soils, cool season native grasses alike have been tested in previous research with regards to saline tolerance. Cool-season grasses may be tolerant of soil salinity in their adult stages but soil salinity threatens the initial stages of seedling growth and emergence (Gazanchian et al 2006, 2007; Masoudi et al 2010). Masoudi et al (2010) tested two cool-season grasses, bulbous barley (*Hordeum bulbosum* L.) and tall wheat grass (*Agropyron elongatum* Host.), for saline tolerance during initial stages of emergence and seedling growth. These two species were tested in order to determine if seed priming could improve root and shoot growth at early stages of seedling emergence (Masoudi et al 2010). Masoudi et al (2010) found that priming did increase the seeds ability to withstand the accumulation of Na, Na:K ratio in the shoot and root when compared to non-primed seeds (Masoudi et al 2010).

Results from this study suggest that big bluestem may not be suitable for seed establishment in soils with EC levels of 8 dS m^{-1} or higher of $MgSO_4$ salts. These findings are similar to that of the two cultivars of big bluestem tested by Schmer et al. (2012). Both of their cultivars were able to germinate above 50% at 8 dS m^{-1} of NaCl induced salinity. Big bluestem is a very dominant species in lowland sites within the tall-grass prairie; it is adapted to high soil moisture and moderately saline subsoils (Schmer et al. 2012).

Switchgrass had the highest final germination percentage while woolly sedge and prairie cordgrass had the lowest germination percentages under both saline and non-saline conditions. These findings differ greatly from those of Kim et al. (2011) in which prairie cordgrass had a final germination percentage of 40% and exhibited much higher tolerance than switchgrass when exposed to NaCl induced salinity (Kim et al. 2011). In comparison, both Schmer et al. (2012)

and this study reported low germination rates for prairie cordgrass and salinity thresholds ≤ 4.5 dS m^{-1}. We documented similar germination rates as Kim et al. (2011) for switchgrass (44.9% at 24 dS m^{-1}). These results compare to the unsuccessful germination rates Kim et al. (2011) reported for switchgrass at increased levels of NaCl induced salinity. Whereas, both Kim et al. (2011) and Schmer et al. (2012) reported switchgrass had germination rates above 40% at EC 8 dS m^{-1}. The findings show the variation in FG when prairie cordgrass and switchgrass are exposed to differing salts like NaCl and $MgSO_4$. This is an important consideration for water resource professionals when planning stream bank reestablishment projects. An understanding of the type of salt present within the soil will aid in successfully selecting species to plant (Kim et al. 2011).

Kentucky bluegrass, woolly sedge, and prairie cordgrass seeds did not exhibit successful germination in the experiment. Kentucky bluegrass showed that EC level has a greater impact on seedling growth than germination. We found that at salinity levels greater than EC 0 dS m^{-1} many of the seeds were classified as abnormal, indicating the seeds started to germinate but were unable to successfully complete germination. These results are consistent with results from Zuk et al. (2012)'s finding that as salinity increased, Kentucky bluegrass growth and development decreased significantly. Unsuccessful germination may have occurred because the specific cultivar used is not tolerant to salinity. There is a need for the development of cultivars that are more tolerant of salinity (Zuk et al. 2012).

Information on germination and revegetation strategies of woolly sedge is limited (Steed & DeWald 2003). Research on this species was imperative in understanding how this abundant riparian graminoid thrives in wet and moderately saline soils. Woolly sedge thrives in wet conditions (Steed & DeWald 2003) and did not exhibit successful germination under optimum germination conditions. It is documented that woolly sedge requires abundant moisture and sunlight for successful germination (Steed & DeWald 2003). This may account for the low FG rates we observed for woolly sedge, regardless of EC level. In addition, site characteristics such as physical stress (erosion or salinity) influence seeding success (Allen 1978; Kadlec & Wentz 1979). Transplanting in the summer will most likely exhibit the best establishment, especially when using rhizomes from large wildling transplants (Steed & DeWald 2003). Steed and DeWald (2003) found that the survival of their sedges tested was significantly greater for summer transplants (55.1%) than that of fall transplants (24.1%). Sedge production is generally more successful when rhizomes are planted to appropriate groundwater depths, especially on unstable grounds (Allen 1978; Kadlec & Wentz 1979; Steed & DeWald 2003).

The poor germination rate exhibited of prairie cordgrass was unexpected because this perennial grass species is typically found in abundance in riparian systems and possesses a cellular tolerance to salinity much like halophytes; salt tolerant plant species (Warren et al. 1985; Kim et al. 2011). Schmer et al. (2012) experienced similar results when testing prairie cordgrass germination. They reported that prairie cordgrass had the lowest germination tolerance to increased salinity levels of the other riparian species tested. Results of our study and Schmer et al. (2012)'s suggest that prairie cordgrass likely didn't germinate because initial germination may take longer than the duration of germination specified for prairie cordgrass in the AOSA guidelines (Shipley & Parent 1991). In addition to a long duration of germination, prairie cordgrass is a protogynous plant which means female reproductive parts mature before male parts (Prasifka et al. 2011). This can restrict the fertilization and flowering process (Prasifka et al. 2011). Although further research on prairie cordgrass in regards to saline and non-saline conditions is warranted, successful establishment may require planting plugs or rhizomes (Kim et al. 2011).

Conclusions

Currently, there is limited to no research on the ability of riparian plants to germinate in $MgSO_4$ induced salinity within riparian areas. The purpose of this study was to determine salinity thresholds of seven graminoid species commonly found in riparian ecosystems across increasing EC levels of $MgSO_4$ induced salinity. This study determined the final germination of seven riparian graminoid species found in riparian areas throughout the northern Great Plains. We tested each species and their ability to germinate across increasing EC levels of $MgSO_4$ induced salinity. Our threshold results can be used to select species that can germinate successfully in saline soils for riparian restoration and buffer establishment. Threshold results of each species can help researchers draw conclusions as to what species may be best for reestablishment from seed (Figure 2.1).

Results from this study indicate that switchgrass, big bluestem, slender wheatgrass, and barley are able to successfully germinate at EC levels ranging from 0-16 dS m^{-1} in soils with $MgSO_4$ induced salinity. When establishing riparian vegetation from seed in an area with $MgSO_4$ induced salinity, switchgrass, big bluestem, and slender wheatgrass should be considered. This study exhibits that selection of proper species is critical for the successful establishment of riparian vegetation. Furthermore, proper seed selection along with a thorough understanding of the salt type and EC level, will ensure water resource managers can have successful establishment results.

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CHAPTER 3. DETERMINATION OF BEST VEGETATION FOR RIPARIAN BUFFER SITES EXPOSED TO EXCESS NITROGEN FROM FIELD RUNOFF

Materials and Methods

This study was conducted for a 12 week period (November 15th, 2018 – February 7th, 2019) in a greenhouse located on the campus of North Dakota State University (Fargo, North Dakota). Mean air temperature and relative humidity within the greenhouse were 26.5 ± 3.2 °C and 18.6 ± 0.07%, respectively. The experiment was set up as a randomized complete block design (RCBD). This study had 3 replications. Each rep had 28 (7 spp x 4 treatments) pots, which were arranged randomly by table in the greenhouse. Each of the reps were rotated on a weekly basis to compensate for variability of conditions within the greenhouse throughout the growing period. The pots within the reps were also rotated on a weekly basis. The total number of pots (units) in the experiment was 84 (7 spp x 4 trt x 3 reps).

The seven species evaluated were big bluestem (*Andropogon gerardii*), smooth brome (*Bromus inermis*), switchgrass (*Panicum virgatum*), Kentucky bluegrass (*Poa Pratensis*), slender wheatgrass (*Elymus trachycaulus*), green needlegrass (*Nassella viridula*), and Alsen wheat which served as our crop species comparison. These species were chosen based on their abundance within riparian ecosystems in the northern Great Plains.

Seed was obtained from Agassiz Seed and Supply (West Fargo, North Dakota). Seeding rate (kg/ha of pure live seed (PLS)) for the study was determined based on the recommended seeding rate as per NRCS standards (USDA-NRCS – North Dakota 2018). This rate was converted to grams of seed per pot, given a seeding area of 325 cm^2 for each pot (Table 3.1). The seeds were broadcasted in the pots and covered with a thin layer of soil to ensure adequate seed to soil contact and improve seedling establishment. Wheat seeds were sown in rows about 5 cm in the soil. Seeds in this study were not exposed to any form of specialized coating or chemical treatments.

Table 3.1. Study seeding rate per species broadcast in each pot as per NRCS recommended seeding rates.

Species	Study Seeding Rate (grams of seed/pot factoring in PLS)	Recommended Seeding Rate (kg/hectare)	Pure Seed %
Smooth brome	5.0	9.0	83.5
Green needlegrass	8.8	8.4	52.0
Slender wheatgrass	6.7	6.2	94.6
Big bluestem	7.9	8.4	57.3
Switchgrass	9.0	5.0	85.4
Kentucky bluegrass	12.6	3.4	88.9
Alsen wheat	13 seeds per pot	67.3	---

Seeds were planted into sandy loam (77.5% sand, 13.6% silt, 8.9% clay) textured soil that was obtained from S&S Landscaping located in Fargo, North Dakota. No prior treatment was done to the soil before seeding. Species were grown in individual pots (one broadcasted species per pot). The pots had a total volume of 325.16 cm^3. Each pot was weighed on a daily basis and brought up to field capacity (5.8 kg) of the soil in order to ensure the plants had adequate water.

To determine application rates of N to be used in the pots, calculations were based on 300 mg of N per pot/12 wheat plants, the recommended base rate of N application for Alsen wheat in North Dakota (Goos, J., Personal interview 2018). Each pot in the study held 5 kg of soil. Plastic bags lined the pots to prevent leaching of N and water.

Solutions were prepared using N in the form of an NH_4NO_3 (ammonium nitrate) powdered solution dissolved in distilled water. 0.7g of NH_4NO_3 was dissolved into 10 mL of distilled water to create a solution with 50 ppm of N. The solution was applied to each treatment at three week intervals until the desired rate of N application was reached. The number of individual applications varied depending on the treatment with the 0 ppm treatment receiving zero, 50 ppm receiving one, 100 ppm receiving two, and 150 ppm receiving three.

Data Collection

After termination, the above ground biomass was clipped 1 cm above the soil surface. After clipping, biomass was dried in a Grieve Oven for one week at 60 °C (Model SB-350. The Grieve Corporation. Round Lake, IL 60073) and weighed. Once dried, the biomass for each sample weighing over 0.5 grams (dry weight) was ground to 2 mm in a Thomas-Wiley Laboratory Grinder. (Model 4. Thomas Scientific, USA, Swedesboro, NJ 08085). Samples were then analyzed to determine total N using the block digestion method using copper catalysts and steam distillation into boric acid (AOAC Official Method) in the Animal Sciences nutrition laboratory at North Dakota State University, Fargo, ND.

All species but switchgrass yielded enough biomass to run total N. Of our 84 samples, all but nineteen samples yielded more than 0.5 grams of dry biomass. Thus, the total number of samples analyzed in the nutrition lab was 65. Nitrogen use efficiency (NUE) or nitrogen recovery was calculated using the equation below (Raun and Johnson 1999):

$$\mathbf{NUE} = (NF) - (NC) / R$$

NF = total N uptake in species from N fertilized pots

NC = total N uptake in species from unfertilized pots

R = rate of fertilizer N applied

Statistical Analysis

To determine if species selection or the level of N influenced total biomass production, percent N uptake by the biomass, and how our N levels influenced our species' NUE, an

ANOVA model was created using the basic ‘stats’ package in R 3.5.1 and a post-hoc Tukey test was used to determine differences within each factor.

Results

In table 3.2, means and standard deviations of N content of plants, biomass production and NUE are shown for the seven species and four treatment levels. The main effects of species and N level as well as the interactions of species*N level were all found to be statistically significant (Table 3.2). Species selection significantly impacted the N content of plants ($p \leq 0.05$), biomass production ($p \leq 0.001$) and NUE ($p \leq 0.001$).

Table 3.2. Mean values with standard deviation of N content in plants (N%), biomass production (g) and N use efficiency as affected by species and N level (treatment).

Effect	Level	N%	Biomass	NUE
Species	Big bluestem	1.6±1.0 b	1.8±1.8 c	23.8±46.6 a
	Smooth brome	2.5±0.8 ab	8.0±2.8 a	15.1±25.8 ab
	Slender wheatgrass	2.2±0.9 ab	9.0±3.2 a	23.8±40.6 a
	Green needlegrass	2.2±1.4 ab	1.7±1.4 c	7.2±18.6 bc
	Switchgrass	0±0 c	0.0±0.0 d	0±0 d
	Kentucky bluegrass	3.0±1.0 a	2.6±1.4 c	23.4±43.6 a
	Alsen Wheat	2.5±0.4 ab	4.3±1.4 b	19.2±34.0 ab
	p	*	***	***
Treatment	150 ppm	2.3±1.5 a	4.4±4.5 ab	0.5±0.9 b
	100 ppm	2.2±1.3 ab	4.7±4.5 a	0.5±1.2 b
	50 ppm	1.9±1.2 ab	3.3±3.2 c	0.3±1.7 b
	0 ppm	1.6±0.9 b	3.4±2.2 bc	62.9±32.0 a
	p	***	**	***
Species x Treatment	*p*	**	***	***

Significant levels are denoted as $^*p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$. Lower case letters separate means between plants species and treatment levels.

Biomass Production

When we look across species and treatments it is evident that N level significantly influenced biomass production, but there is no clear trend due to the differing responses of individual species (Table 3.2). Our top two performing species, slender wheatgrass and smooth brome were not significantly different from each other, but were significantly different from the performance of their next closest competitor, Alsen wheat. Green needlegrass, big bluestem, and Kentucky bluegrass were all significantly similar to each other but were not similar to the performance of wheat, smooth brome, slender wheatgrass, or switchgrass. Overall, the increasing levels of N had significant impacts on the production of our riparian species.

However, a closer look at the species*treatment interactions shows that only big bluestem and smooth brome biomass production were influenced by the treatment levels (Table 3.3). As N level increased, production of smooth brome also increased from 4.0g (control) to 10.1g (150 ppm). Increasing N levels had a significant negative impact on the production of big bluestem, reducing biomass production from 4.7g (control) to 0g (150 ppm).

Table 3.3. Mean values with standard deviation of biomass (g) for individual species as affected by N level (treatment).

Rate	Big bluestem	Smooth brome	Slender wheatgrass	Green needlegrass	Kentucky bluegrass	Wheat
150 ppm	0.5±0.5 b	10.1±1.1 a	11.5±0.4	2.1±0.4	2.8±1.5	3.8±2.6
50 ppm	1.4±2.4 b	7.6±0.7 a	6.9±3.7	1.6±1.9	1.2±1.1	4.1±0.7
100 ppm	0.6±0.5 b	10.2±0.4 a	11.5±2.1	1.9±1.8	3.0±1.5	5.4±0.2
0 ppm	4.7±0.6 a	4.0±2.1 b	6.2±0.1	1.2±1.5	3.4±0.5	4.0±0.6
p	***	***	ns	ns	ns	ns

Significant levels are denoted as *p≤0.05, **p≤0.01, ***p≤0.001, and ns. Lower case letters separate means between plants species and treatment levels.

Nitrogen Content

When we look across treatments and species we observed an increase in N content as ppm N increases (Table 3.2). The two species that exhibited the highest percentage of N in their aboveground biomass are Kentucky bluegrass (3.3%) and green needlegrass (3.0%). Wheat, smooth brome, and slender wheatgrass all had a N content of 2.4%-2.5%. Big bluestem had an aboveground biomass concentration of 2.2% N.

The increased application of N only significantly influenced N uptake of three species, wheat, big bluestem and smooth brome (Table 3.4). Whereas, N uptake of slender wheatgrass, green needlegrass and Kentucky bluegrass was not influenced by N application. Wheat and smooth brome increased N uptake by 0.9% and 2.0% with increased levels of N, respectively. Conversely, N uptake of big bluestem decreased from 2.1% (control) to 0.0% (150 ppm). Switchgrass did not produce enough biomass to run N analysis in this study.

Table 3.4. Mean values with standard deviation of N content (%) for individual species as affected by N level (treatment).

Rate	Big bluestem	Smooth brome	Slender wheatgrass	Green needlegrass	Kentucky bluegrass	Wheat
150 ppm	0.0±0.0 b	3.4±0.02 a	3.1±0.1	3.3±0.1	3.6±0.04	2.8±0.2 a
50 ppm	2.0±0.4 a	2.4±0.1 b	2.4±0.1	1.9±1.7	2.2±1.9	2.5±0.1 a
100 ppm	2.4±0.1 a	2.7±0.2 b	1.9±1.63	2.1±1.8	3.6±0.2	2.7±0.3 a
0 ppm	2.1±0.4 a	1.4±0.1 c	1.5±0.1	1.6±1.4	2.8±0.2	1.9±0.1 b
p	***	***	ns	ns	ns	*

Significant levels are denoted as *p≤0.05, **p≤0.01, ***p≤0.001, and ns. Lower case letters separate means between plants species and treatment levels.

Nitrogen use efficiency

Overall, NUE decreased with increasing N levels across all species tested (Table 3.2). This response was exhibited by all species with the exception of green needlegrass, which had no significant change in NUE across treatments (Table 3.5). Slender wheatgrass (1.8) and smooth brome (1.9) had the highest NUE at the 150 ppm treatment. Slender wheatgrass had NUE concentrations between 1.4 at 50 ppm and 1.8 at 150 ppm. Smooth brome had concentrations of 2.5 at 50 ppm and 1.9 at 150 ppm. Wheat had NUE concentration levels of 0.6 at 50 ppm and decreased to 0.2 (150 ppm), which was significantly lower than that of smooth brome and slender wheatgrass. Kentucky bluegrass and big bluestem exhibited the greatest decline in NUE. Kentucky bluegrass had a NUE of 94.7 (control) but ≤0.10 with N application; similarly, big bluestem had a NUE of 98.0 (control) but ≤-0.65 when N was applied. However, none of the species tested had a significant change in NUE between the three treatment levels (Table 3.5).

Table 3.5. Mean values with standard deviation of NUE for individual species as affected by N level (treatment).

Rate	Big bluestem	Smooth brome	Slender wheatgrass	Green needlegrass	Kentucky bluegrass	Wheat
150 ppm	-0.7±0.5 b	1.9±0.6 b	1.8±0.1 b	0.3±0.3	0.0±0.3 b	0.2±0.5 b
50 ppm	-1.4±0.5 b	2.5±0.6 b	1.4±1.6 b	0.4±1.6	-1.4±1.1 b	0.6±0.5 b
100 ppm	-0.8±0.4 b	2.2±0.3 b	1.0±1.7 b	0.3±0.3	0.1±0.6 b	0.7±0.2 b
0 ppm	98.0±29.5 a	53.7±26.0 a	90.7±8.2 a	27.9±32.2	94.7±16.6 a	75.3±6.9 a
p	***	**	***	ns	***	***

Significant levels are denoted as *p≤0.05, **p≤0.01, ***p≤0.001, and ns. Lower case letters separate means between plants species and treatment levels.

Discussion

The rate of N fertilizer application has varying effects on the production of cool and warm season grasses. Bartholomew (2015a) reported that the yield of their cool-season species, annual ryegrass (*Lolium multiflorum* Lam.), rye (*Secale cereale* L.) and oats (*Avena sativa* L.), increased as N application rates increased. Similarly, we observed a positive response in biomass production and N uptake for smooth brome. These results are consistent with the results Bartholomew (2015a) who showed that cool-season grass growth increased in response to N fertilizer application, reporting a strong liner relation between yields and N rates. However, the native cool-season species did not have a significant response to N application. In contrast, Martin and Chambers (2001) found 20 native forb and graminoid riparian species in central Nevada increased levels of N resulted in greater biomass production. Furthermore, they reported that N addition plus clipping had a significant effect on the species during all 3 years within their sites. Martin and Chambers (2001) reported that the greater response of their clipped plots was likely the result of better contact with the fertilizer and warmer temperatures that likely occurred as a result of clipping. Overall, development of the plants assessed were accelerated when exposed to N (Martin and Chambers 2001).

In contrast to the cool-season grasses, warm season grasses exhibited a negative response to N application. In our study we found switchgrass had no response in biomass production, which may have been because of low germination. Whereas, big bluestem exhibited a decrease in both biomass production and N content as application rates increased. This is not consistent with Bartholomew (2015b) reports that neither their N level or timing of application significantly affected their warm-season grass harvest regrowth year after year. However, they did report an increase in biomass production of warm season grasses for N application rates up to 75.1 kg/ha. Both our study and Bartholomew (2015a) found big bluestem had a more positive response to N than switchgrass. In contrast, Friesen and Cattani (2017) reported that switchgrass produced 78.5% more biomass at their high N rate whereas their other species, big bluestem, produced only 58.1%.

The cool season grasses in the study had an increased capacity to utilize the N in the environment, which is reflected in the higher N content and NUEs than the warm season species. These findings are consistent with those of Zhong et al (2019). Zhong et al. (2019) tested warm and cool-season grass species, false wheatgrass (*Leymus chinensis*), feather windmillgrass (*Chloris virgata*), and limpo grass (*Hemarthria altissima*), and concluded that N application resulted in a higher N content in the leaves of both their cool and warm-season species. This led to faster recovery during drought conditions but did not have positive effects of plant NUE for the cool-season grass, false wheatgrass (Zhong et al. 2019). While all species NUE responded negatively to increased levels, slender wheatgrass and smooth brome had the highest NUEs at increase N levels; whereas, big bluestem had the lowest. However, prior to N application big bluestem had the highest, this higher NUE rate for big bluestem is consistent with the research of Friesen and Cattani (2017). Isbell et al. (2013) tested nine native perennial warm-season grasses in a natural grassland setting and found that increased levels of N initially increased plant productivity, but then productivity declined over time. At high rates of N, a shift in plant communities was observed. High diversity native-dominated sites shifted to a low-diversity state where non-native species eventually dominated (Isbell et al. 2013).

In our controlled greenhouse setting, smooth brome performed best when compared to the other species tested. Smooth brome performed best in both biomass and NUE analysis. Therefore, this species may be recommended for riparian buffer strip establishment. However, there may be some limitations associated with smooth brome, and further research is warranted. For example, results from our study suggest smooth brome is the best suited species for mitigating negative impacts of excess N in riparian ecosystems. Smooth brome responds positively to nitrogen application rates as high as 308.2 kg/ha. It can provide quick forage and help prevent soil erosion in riparian settings when planted with native grasses (Roberts and Kallenbach 2000; Bahm et al. 2011). One potential negative to establishing smooth brome in riparian ecosystems is that it is an aggressive grass that can become weedy and invade native grasslands throughout the Great Plains (Bush 2006; Bahm et al. 2011). Another potential negative to establishing smooth brome in riparian ecosystems is that the root system is fibrous (Brown et al. 2010). Compared to native graminoid species, such as big bluestem, its root system does not anchor as far into the soil. When planted as a monoculture, smooth brome would not provide adequate streambank stability (Bush et al. 2006; Brown et al. 2010). Therefore, it may not be a desired species to plant in buffers.

Conclusions

Riparian buffers provide important ecosystem services imperative to the health of humans and the natural environment that surrounds them. This study aimed to identify species to be planted in riparian buffers with the greatest potential to mitigate the impacts of excess N. The species that exhibited successful results in our greenhouse study was smooth brome.

The production and N uptake responses of our native grasses to increasing levels of N were analyzed separately to reflect differences between cool and warm-season grasses, N uptake, NUE, and their ability to produce adequate biomass. Land managers with a focus on riparian buffers, have a goal to remediate the soil from a variety of environmental conditions as well as provide sufficient ground cover to mitigate erosion within these fragile ecosystems. Based on our results, we suggest that the cool-season grass, smooth brome, may be best to plant in locations with increased levels of soil N. While one of our warm-season grasses, switchgrass, did not perform best in this study, further research is warranted on how warm-season grass species may respond over a longer period of time once proper establishment has occurred.

The northern Great Plains is not only known for its vast rangelands but also for its large agricultural influence within the United States. This large agricultural influence has, unintentionally, created water quality issues throughout much of this region from nonpoint source pollution such as fertilizer runoff. Due to the severe effects of excess N within the water resources of this region, it is crucial to implement riparian restoration efforts to restore these ecosystems back to where their ecosystem services can be maximized to their full potential. In this study, smooth brome exhibited positive responses to increased levels of N within the soil. Smooth brome can be used in riparian reestablishment plans to provide adequate NUE ability as well as produce enough biomass to stabilize fragile soils within riparian ecosystems. Issues that may arise from establishing smooth brome in riparian settings are twofold: first, its root system is fibrous and shallow and may not provide adequate streambank stability, and second, it can become weedy or invasive and dominate over native riparian plant species. To refine our recommendations, further research is warranted on how warm and cool-season grasses will respond to increased levels of N when they have been established in a more natural setting and observed for longer periods of time.

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GENERAL CONCLUSIONS

The general goal of this research was to determine the best riparian graminoid species to plant in riparian buffers to aid in mitigating the harmful effects of excess N and salinity in riparian settings. We found that the best species to plant to combat both increased soil salinity and excess N is smooth brome. This species may be used in conjunction with the species found in the MN Buffer Seed Mix which has already been tested throughout the northern Great Plains region. Although smooth brome was not tested in the germination study, it is found in abundance in locations with known increased soil salinity throughout the northern Great Plains. Slender wheatgrass was able to germinate within EC levels up to 16 dS m^{-1}. Furthermore, slender wheatgrass, a native species, exhibited both an increase in biomass production and increased NUE as N levels increased in the form of our NH_4NO_3 fertilizer application. Overall, smooth brome and slender wheatgrass showed promising results in controlled settings but should be tested further in natural settings to determine if they can be incorporated into riparian buffer seeding plans in the future.