

BRINE IMPACTED SOILS IN SEMI-ARID RANGELANDS: GREENHOUSE ELECTRICAL  
CONDUCTIVITY THRESHOLDS AND EX SITU/IN SITU REMEDIATION  
COMPARISONS

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**Title**

Brine Impacted Soils in western North Dakota: Greenhouse Electrical  
Conductivity Thresholds and Ex-Situ and In-Situ Remediation Comparisons

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## ABSTRACT

Brine is a by-product of oil and gas extraction that can have profound impacts on soil chemistry and vegetation assemblages when discharged onto the soil surface. Brine spill remediation aims to remove or minimize the saturated paste electrical conductivity ( $EC_e$ ) to levels suitable for plant growth. My research focused on evaluating  $EC_e$  and plant parameters on topsoil excavation and chemical amendment sites, and examining brine thresholds of several native grasses. Halophytic grasses had a higher brine threshold between 18.6 and 34.6  $dS\ m^{-1}$  whereas glycophytic grasses exhibited a lower threshold (9.70 and 18.6  $dS\ m^{-1}$ ). Halophytes examined in this study may aid in revegetating salt-affected sites. Soil  $EC_e$  was not different ( $p \geq 0.05$ ) between remediation techniques to the 60 cm depth. Functional plant groups were also not different ( $p \geq 0.05$ ) between remediation techniques. Both techniques were successful at reducing soil  $EC_e$  to facilitate natural and assisted vegetation recovery.

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## PREFACE

The first chapter is a general literature review detailing concepts and justification for chapters two and three, and follows the style and format for *Rangeland Ecology and Management Journal*. Chapters two and three are independent manuscripts prepared for submission to peer-reviewed journals, both chapters follow the style and format for the *Rangeland Ecology and Management Journal*.

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## GENERAL INTRODUCTION

Oil and gas production can increase the presence of anthropogenic surface salinity through accidental or deliberate discharge of oil-produced water (i.e. brine) onto the soil surface. Brine is a by-product of oil and gas extraction process and contains high concentrations of sodium chloride (NaCl) salts that overwhelm plants' ability to process large quantities of salt ions and maintain a favorable cell-water gradient in the presence of osmotic stress. High concentrations of NaCl lead to ion toxicity, cell dehydration and plasmolysis that ultimately result in plant death. Brine negatively influences vegetation as it lowers the osmotic potential in soil water, which makes it difficult for plant roots to extract water and essential nutrients from the soil matrix. Excess Na<sup>+</sup> ions impede vegetation establishment by weakening aggregate stability and reducing hydraulic conductivity. Brine spill remediation through topsoil excavation (*ex situ*) or chemical amendment (*in situ*) techniques aim to remove or minimize the abiotic stressor to levels suitable for plant growth. The establishment of halophytes post remediation may provide additional protection from the rise of salt-laden water and continuous vegetation cover on remediated brine spill sites.

Halophytes represent a unique group of plant species that can tolerate high salt concentrations ( $\geq 15 \text{ dS m}^{-1}$ ; Keiffer and Ungar 2001), — concentrations that would otherwise kill 99% of neighboring species (Flowers and Colmer 2008). Salt-rich environments exert high selection pressures and enable halophytes to develop morphological and anatomical characteristics to cope with salt stress (Flowers et al. 2010). However, abrupt increases in soil salinity, such as brine spills, overwhelm both glycophytes' and halophytes' ability to cope with large amounts of salt at one discrete time. These brine salts kill plants shortly after coming into contact with live plant parts (Murphy et al. 1988; Aschenbach 2006; Aschenbach and Kindscher

2006). Plants die because they are unable to maintain favorable cell-water relations (i.e. turgor) and transport salt ions at a fast enough pace into plant cell vacuoles at high salt concentrations (Flowers et al. 1977; Shavrukov 2013). Brine spill remediation aims to remove or minimize the amount of brine salts to facilitate establishment of plant species. Assisted reintroduction of halophytic species through reseeded treatments on remediated brine spill sites may stabilize soil and provide continuous vegetation cover in the presence of recalcitrant brine salts.

Remediation is essential for improving the physico-chemical properties of contaminated soil to enhance growing conditions for plants (Wong 2003), and it should occur as soon as possible to prevent contaminants from permeating through soil layers (Harris et al. 2005). Topsoil excavation is an *ex situ* method that permanently reduces the amount of contaminated soil onsite by moving it to a new location, such as a landfill. Alternatively, environmental consultants and oil and gas personnel use chemical amendments, such as calcium-based salts, with water to displace sodium ( $\text{Na}^+$ ) and allow for its leaching to lower soil depths. However, this method, without a supplemental water source, is often intensive and ineffective in semi-arid to arid climates (Jury and Weeks 1978; Keiffer and Ungar 2001; Ammari et al. 2013), where limited precipitation prevents ions from leaching below the plant root zone (Keiffer and Ungar 2002). Unfortunately, the majority of high oil-producing regions are located in semi-arid and arid regions in the Great Plains of North America (Keiffer and Ungar 2001), such as western North Dakota. My research aims to evaluate brine thresholds of northern mixed-grass prairie species and evaluate efficacy of *ex situ* and *in situ* remediation techniques.

## **CHAPTER 1: LITERATURE REVIEW**

### **Natural Abiotic Stressors and Biotic Disturbances**

Natural abiotic and biotic factors exerted strong selective pressure on grassland flora that contributed to spatial and temporal heterogeneity in the northern mixed-grass prairie (NMGP) (White 1979; Sousa 1984; Pickett and White 1985). The most influential abiotic factors affecting vegetation composition and productivity in grassland biomes are temperature and precipitation gradients (Mitchell and Csillag 2001; Fredlund and Tieszen 1994). Additionally, small-scale factors such as soil texture, hydrology, nutrients, and natural salt deposits are important for the separation of distinctive plant groups that occupy a variety of microhabitat niches (Keith 1958; Barnes and Harrison 1982; Tilman 1994). The historic presence of biotic disturbances such as fire and grazing, altered vegetation structure to maintain a grassland dominated ecosystem (Collins and Barber 1986; Fuhlendorf and Engle 2004; Davies et al. 2009). The long-term influence of both abiotic and biotic environmental factors resulted in the natural selection of different plant strategies to persist in these habitats (White 1979; Fuhlendorf et al. 2009; Bui 2013).

The vegetation within the NMGP evolved under a system of frequent fires and herbivore grazing (Axelrod 1985; Collins and Barber 1986; Fahnestock and Detling 2002; Janis et al. 2002). Biotic disturbances, such as fire and grazing, resulted in subtle or abrupt changes in ecosystem structure and community. These historic disturbances were beneficial to grassland ecosystems because they removed stagnant biomass, thus stimulating forage regrowth (Johnson and Matchett 2001). Higher forage quality after a burn attracted bison and other herbivores to these areas (Bonham and Lerwick 1976; Coppedge and Shaw 1998). The patchiness of fire followed by grazing created a shifting mosaic of vegetation patches, which provided habitat to a

variety of prairie fauna (Vinton et al. 1993; Fuhlendorf and Engle 2004; Fuhlendorf et al. 2009). These keystone processes were largely removed from grassland ecosystems following the European settlement (Knapp et al. 1999; Fahnestock and Detling 2002). Native plants did not evolve at a fast enough pace to effectively compete or survive in these human-altered ecosystems. This human-plant relationship is particularly apparent when examining plants' response to anthropogenic soil salinity.

### **Saline Seep Formation**

The European settlers converted productive grasslands into agriculture farmland by the late 1800's. The change in historical land cover increased the evapotranspiration rate near the soil surface, which consequently increased the development of saline seeps throughout the northern Great Plains (Miller et al. 1981; van Schilfgaarde 1981; Timpson and Richardson 1986). The natural salt deposits in saline seeps are common abiotic stress in semi-arid climates where evaporation exceeds precipitation (Brown 1971; Miller et al. 1981). Saline seeps develop when water carrying dissolved salts from weathered geologic materials, predominantly mixed Na-Mg-Ca-(SO<sub>4</sub>) minerals, percolate through the soil profile until it reaches impermeable soil layers (rock or limestone; Miller et al. 1981; van Schilfgaarde 1981; Timpson and Richardson 1986; Timpson et al. 1986). The layers restrict downward water movement, allowing soluble salts to only travel laterally with subsurface water and accumulate in depositional areas (Miller et al. 1981). The soluble salts gradually seep towards the surface through capillary action, especially during dry summer months when soil water travels upward to meet atmospheric evaporative demands (Miller et al. 1989). Furthermore, natural precipitation is limited in semi-arid and arid regions (< 25 cm yr.<sup>-1</sup>) (Keiffer and Ungar 2001; Keiffer and Ungar 2002). This prevents soluble salts from leaching to depths below the root zone. Wet and dry cycles increase

salt accumulation near the soil surface, which accelerate saline seep formation and intensify soil salinity. Plants negatively respond to high concentration of salts in the center of saline seeps by decreasing plant growth and future plant recruitment, which lead to increases in bare soil spots.

### **Salt Stress**

Germination is the most critical growth stage in a plants life cycle (Macke and Ungar 1971). Reducing salt concentrations in the top 15 cm of soil is a prerequisite for successful plant germination on salt-affected soils (Chapman 1942; Pujol et al. 2000). Surface salts retard germination of many native plant seeds, especially during periods of water scarcity when salts accumulate on the soil surface. Salts influence plant germination by decreasing the osmotic pressure of the soil, which make it increasingly difficult for seeds to extract water from the soil matrix (Ayers 1952; Ungar 1978). The lack of available water leads to permanent damage of seed embryos or osmotic induced dormancy (Ayers 1952; Ungar 1978; Ungar 1995; Keiffer and Ungar 1997). Surges in soil moisture are a dormancy-release factor (Badger and Ungar 1989), creating suitable places for seedlings to emerge on salt-affected sites, such as saline seeps. Therefore, germination typically occurs in early spring or late fall when water is more available to dilute salt concentrations on the soil surface (Chapman 1942, Ungar 1978; McMahon and Ungar 1978). This strategy enables plants to complete critical growth stages during times of reduced soil salinity prior to periods of increased salt stress (Singh et al. 1983; Schwarz and Redmann 1990; Keiffer and Ungar 2002).

Excessive soluble salts in the soil medium restrict plant growth at the cellular and whole level (Hasegawa et al. 2000). Salts within the root zone induce osmotic stress, disrupt metabolic homeostasis and ion distribution (Serrano et al. 1999; Zhu 2001), and damage important plant tissue through cell plasmolysis (Shavrukov 2013), which can all decrease plant productivity or



induce plant death. Salt affected soils impact vegetation by altering the osmotic gradient between soil and plants. Lower osmotic potential in soil water increases the hydraulic gradient towards salt-rich areas and away from plant roots (Bernstein and Hayward 1958; Shavrukov 2013). Additionally, the decline in osmotic potential alters the uptake and transport of water and essential plant nutrients ( $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ) through the cell membrane of plant roots, leading to nutrient deficiencies and ion imbalances (Munns and Termaat 1986; Hu and Schmidhalter 2005). Plants that exhibit salt stress may attempt to regulate water loss by closing stomata cells to limit gas diffusion (carbon dioxide ( $\text{CO}_2$ )) through plant leaves, preventing photosynthesis from occurring (Longstreth and Nobel 1979; Chaves et al. 2009). Prolonged salt stress prevents plants from re-opening stomata cells and taking in  $\text{CO}_2$ , which is necessary to produce photosynthetic energy to sustain metabolic activity, leading to plant death. However, some plant species are less sensitive to increases in soil salinity (Bernstein and Hayward 1958; Ungar 1995; Pujol et al. 2000).

### **Halophytes**

Scholars have recognized salt tolerance as a plant trait for more than 200 years (Flowers et al. 1986). Flowers et al. (2010) estimates there are over 350 salt tolerant plant species (i.e. halophytes) that can complete their lifecycle in at least  $20 \text{ dS m}^{-1}$  (Flowers et al. 2010). Halophytic species evolved in salt-rich environments (e.g., saline seeps, coastal wetlands, and salt plains) where they developed morphological and anatomical characteristics to cope with salt stress (Flowers et al. 2010). Halophytes are able to exclude, regulate, or transport salt ions to maintain homeostasis and ion distribution in the presence of salt stress (Flowers et al. 1977; Salt et al. 1998; Zhu 2003). Plants can survive salt stress by remobilizing water and nutrients to younger leaves and by storing salt ions in older, less productive leaves to complete their life

cycle (Munns 2002; Munné-Bosch and Alegre 2004; Munns 2005). Older plant leaves are also more susceptible to salt accumulation in leaf vacuoles because they have been transpiring longer, thus bringing salt ions into leaf vacuoles, and reaching storage capacity sooner than younger plant leaves (Munns 2002). Exceeding the storage capacity in plant cell vacuoles can cause these vacuoles to rupture and leak salt ions into the plant cytoplasm where they become toxic to plant cell enzymes, and/or build up in the surrounding plant cell wall, leading to cellular dehydration (Munns and Passioura 1984; Yeo and Flowers 1986; Munns 2002). Compared to younger plants, older plant species have more plant tissues to transport and store salt ions in leaf vacuoles, which is generally why salt tolerance generally increases with age of plant. However, salt sensitivity can also increase right before reproductive stages when plant resources are allocated to support reproductive structures (Läuchli and Grattan 2007; Oliveira et al. 2013). Some plant species may attempt to accelerate phenological development or switch between sexual and asexual reproduction as a last effort to pass on genetic material (Adams 1990; van Zandt et al. 2003). This survival tactic may produce sterile offspring, reducing the long-term sustainability of their population in salt-affected areas.

Halophytes stabilize and desalinate soils and may act as ecological proxies to replace salt sensitive species (i.e. glycophytes). Native halophytes are preferable over exotic halophytes because native halophytes are usually restricted to native habitats and are adapted to colonizing and completing their cycle in hypersaline environments, such as saline seeps (Flowers et al. 2008; Flowers et al. 2010). Halophytes can remove and contain salt ions and other industrial solutes (e.g., boron (B), cadmium ( $\text{Cd}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and copper ( $\text{Cu}^+$  &  $\text{Cu}^{2+}$ ); Rozema et al. 1992; Manousaki and Kalogerakis 2011) by metabolizing them in plant tissues (Salt et al. 1998). These plants can accumulate contaminants in plant roots and shoots, but

removal usually only occurs for the above ground portion (Keiffer and Ungar 2002). Harvesting halophytes above ground biomass at the end of the growing season can permanently reduce soil contaminants to create a conducive environment and favor the establishment of less salt tolerant plant species (i.e. glycophytes) (Salt et al 1995; Salt et al. 1998; Manousaki and Kalogerakis 2011). Most importantly, halophytes stabilize soil in salt-rich environments to prevent further degradation. Belowground roots play an important role in increasing hydraulic conductivity and providing a surface area for microbial communities (Anderson et al. 1993; White et al. 2003).

Halophytes are preferential for revegetating salt-affected sites because they provide additional protection from the gradual rise of salt-laden water during periods of dryness (Barrett-Lennard, 2002; Rabhi et al. 2009; Lokhande and Suprasanna 2012). Although halophytes are beneficial for restoring the biotic processes in salt-affected areas, they are less adaptive to sudden increases in soil salinity (Shavrukov 2013). Plants are not likely to recover even if removal of salts occurred shortly after introduction into the growing medium (Shavrukov 2013). Oil-produced water (i.e., brine) spills from oil and gas development occur suddenly and result in unprecedented amounts of anthropogenic surface salts throughout the Great Plains (Aschenbach and Kindscher 2006).

### **Anthropogenic Salinity: Brine**

Improvements in oil and gas technology have increased access to shale reservoirs and consequently these improvements have increased the amount of brine produced in North America (Rahm 2011; Mason 2012; Brantley et al. 2014). Hydraulic fracturing— also known as hydrofracturing — is a process in which water, sand, and additive chemicals travel at high pressure through an underground wellbore, creating micro fractures in the permeable rock, releasing trapped oil and gas (Brantley et al. 2014). Sand particles prop-open crevices in the

shale, allowing water and solvents to travel through the permeable shale rock. The shale formation contributes large amounts of dissolved salts (Meissner 1978; Whittig et al. 1982). These salts mix with water to create brine that travels back to the soil surface during oil and gas production (Harkness et al. 2015). Brine is a regulated waste by-product of the oil and gas extraction process. Brine is usually stored in tank battery containers where it is recycled, transported, or permanently re-injected into underground storage facilities (Brittingham et al. 2014).

Brine spills are arguably the most environmentally destructive and controversial aspect of the oil and gas extraction process (Jager et al. 2005; Aschenbach and Kindscher 2006). Sodium chloride (NaCl) comprises 90% of brine salts and can have a saturated paste electrical conductivity ( $EC_e$ ) magnitude ( $\leq 200 \text{ dS m}^{-1}$ ) higher than saline seep concentrations ( $\geq 15 \text{ dS m}^{-1}$ ) (Merrill et al. 1990; Jong 1982; Keiffer and Ungar 2002; Aschenbach and Kindscher 2006). Brine contamination occurs when there is a tank battery overflow, pipeline leak, or migration of salts from a reserve pit (Keiffer and Ungar 2002; Sublette et al. 2007). These types of uncontrollable releases leave permanent marks on the landscape (Jager et al. 2005), which can persist for decades in semi-arid to arid regions in the absence of remediation techniques.

Brine spills are a severe abiotic site modification that can have long lasting impacts on soil and vegetation parameters. Salts from brine spills are considered especially problematic because they are both saline and sodic ( $EC_e \geq 4 \text{ dS m}^{-1}$  and  $SAR > 13$ ) (USDA 1954). Excessive  $\text{Na}^+$  ions can lead to dispersion of clay particles on soil aggregates, thus weakening soil structure and resulting in reduced hydraulic conductivity. Additionally, salts near the soil surface sterilize the soil seed bank and prevent successful plant migration from nearby propagules (Ungar 1978; Keiffer and Ungar, 1977; Ungar 1995; Ungar 2001; Keiffer and Ungar 2002). The high

concentrations of salts in brine require an active approach to speed-up natural processes towards a predefined state. Brine spill remediation aims to remove or minimize the abiotic stressor to increase the success rate of both natural and assisted plant reintroduction. The goal of remediation is to reinstate some ecological processes to assist in the long-term sustainability of the site. Reduction of salt concentrations through *ex situ* and *in situ* remediation techniques is the first step towards creating a more conducive environment for soil and plant organisms.

### **Ex-Situ and In-Situ Brine Spill Remediation**

Topsoil excavation (*ex situ*) permanently reduces the volume, toxicity, and mobility of contaminants (Steele and Pichtel 1998). The physical removal of contaminants to an approved disposal location decreases off-target effects and future liability (Steele and Pichtel 1998). Although this technique is effective in reducing the inhibitory effect of brine salts and other industrial effluents, the removal of soil contaminants and introduction of replacement soil destroys thousands of years of soil development (Bradshaw 1997). Replacement soil also poses its own ecological implications because it may unintentionally contain exotic seeds in the soil seed bank that may germinate and spread into the surrounding area. Furthermore, new topsoil may differ in soil chemical and physical properties (e.g. soil texture, nutrients, organic matter) and may yield a different plant community than what previously existed before the spill. The permanent loss of the original soil source and lack of adequate soil structure associated with the topsoil excavation technique increases soil erosion and decreases soil porosity when replacement soil is mechanically compressed (Stylinski and Allen 1996; Angers and Caron 1998), which can negatively influence plant root growth (Milchunas et al. 1999; Stylinski and Allen 1996; Pagliai et al. 2004). Roots unable to grow and develop lead to declines in microbial activity, nutrient reserves, and moisture availability (Bronick and Lal 2005). The lack of plant inputs reduces

overall soil fertility. The loss of original soil structure is defensible only when contamination poses a significant threat to human and/or environmental health (Wilson and Jones 1993; Efraymson et al. 2004).

Alternatively, chemical amendments (*in situ*) are a common practice to remediate salt-affected soils and minimally disrupt soil integrity. The minimal disruption of the original soil has a greater public acceptance among soil and ecologists because it does not transport contaminated soil from one place to another (Khan et al. 2000). Chemical amendments are typically calcium ( $\text{Ca}^{2+}$ ) based salts, such as gypsum ( $\text{CaSO}_4^{2-} \cdot 2\text{H}_2\text{O}$ ) and calcium chloride ( $\text{CaCl}_2$ ), to replace sodium ( $\text{Na}^+$ ) ions from soil exchange sites and move them from upper to lower soil depths (Gharaibeh et al. 2009; Mahmoodabadi et al. 2013; Ammari et al. 2013). Calcium based salts differ from  $\text{Na}^+$  based salts because they flocculate clay particles (Peterson 1948), as opposed to  $\text{Na}^+$  ions, which disperse clay particles. Divalent cations ( $\text{Ca}^{2+}$ ) replace monovalent cations like  $\text{Na}^+$  ions because they have a higher affinity to bond with negatively charged sites on soil particles (Alva et al. 1991; Gharaibeh et al. 2009). This technique requires water to facilitate  $\text{Na}^+$  exchange because it favors the adsorption of  $\text{Ca}^{2+}$  ions to displace and leach  $\text{Na}^+$  ions (Reeve and Bower 1960). Preliminary studies estimate a 100:1 dilution ratio of fresh water to brine salts to permit plant growth on brine-affected soils (Munns and Stewart 1989). Jury and Weeks (1978) estimate that the leaching process for chemical amendments can take upwards of 3,000 years depending upon soil texture and exchangeable sodium percentage (Jury and Weeks 1978; Atalay et al. 1999; Harris et al. 2005). The process in which chemical amendments displace lower valence cations ( $\text{Na}^+$ ) with higher valence cations ( $\text{Ca}^{2+}$ ) can lead to a reduction in soil EC and sodium adsorption ratio (SAR) (Reeve and Bower 1960; Hamza and Anderson 2003; Hanay et

al. 2004). The rate of  $\text{Na}^+$  displacement depends upon soil structure, activity of  $\text{Ca}^{2+}$  in the soil matrix, and electrolyte concentration of water (Reeve and Bower 1960; Gharaibeh et al. 2009).

Although chemical amendments are effective at displacing unwanted salt ions, they may also indiscriminately leach macro and micronutrients to lower soil depths where they are inaccessible to plant roots (Yazdanpanah et al. 2013). This remediation technique is not suitable in the presence of a shallow water table, where displaced salt ions and additive brine chemicals can inadvertently contaminate groundwater. Furthermore, chemical amendments such as gypsum are only effective at the depth of application, requiring some surface disruption to incorporate the amendment to lower soil depths (Jong 1982; Robbins 1986).

Organic materials serve a dual purpose by providing protection from erosional processes and improving the soil conditions for revegetation of salt-affected soils (Wong 2003; Tejada et al. 2006; Tejada et al. 2009). Organic materials are organic waste products from living organisms such as plants and/or animals. Coupling organic amendments with remediation techniques reduces erosional hazards on newly disturbed soil and increases the efficacy of  $\text{Na}^+$  leaching. A study using a subsurface draining system and hay decreased brine salts ( $\text{NaCl}$ ) by an average 93 and 78% after a four year period, with hay playing an important role in limiting the rate of evaporation from the soil surface to enhance salt leaching (Harris et al. 2005). Several other studies observed similar results using organic materials to desalinate surface salts (Dorado et al. 2003; Wong 2003, Zhang et al. 2008). Organic materials increase percent organic matter that binds humic colloids together (Dorado et al. 2003), promoting microbial activity and soil structure formation (Roldán et al. 1996; Bulluck et al. 2002; Liang et al. 2003; García-Orenes et al. 2005; Tejada et al. 2006; Tejada et al. 2009; Diacono and Montemurro 2010). Additionally, organic amendments replenish nutrients lost by leaching to improve soil fertility and facilitate

revegetation of saline-sodic soils (Liang et al. 2003; Tejada et al. 2009; Yazdanpanah et al. 2013). Organic additions, such as wood chips, hay, mulch, straw, wattles, and biodegradable netting may accelerate plant succession by ameliorating edaphic conditions to stimulate succession of human-disturbed sites.

Brine is an abiotic stress (i.e. soil salinity) that exerts a continuous negative impact on soil and plant parameters when spilt on the soil surface during the growing season. Remediation via chemical amendments and topsoil excavation are two common remediation techniques to lower soil salinity. Remediation attempts to lower soil salinity to facilitate revegetation efforts, however, physical disturbances surrounding oil and gas activities further weaken intact plant communities and can delay plant recovery on post-remediated spill sites. Anthropogenic disturbances associated with oil and gas development include roads, vehicle movement, tilling, and trampling (Larson 2003; Efrogmson et al. 2004). These disturbances occur daily in the oil fields and can have negative effects (Rapport and Whitford 1999; Borics et al. 2013) on nearby remediated brine spill sites by preventing the plant community from returning to pre-spill conditions. Anthropogenic disturbances disrupt the soil surface to expose available plant resources (e.g., water, light, nutrients, and space resources) that inhibit the establishment of later successional species and promote the continual dominance of early successional species (Grime 1977; Grubb 1977; Daehler 2003).

### **Plant Succession**

Early successional species, such as ruderals and exotics, tend to have small seeds, high dispersal capabilities, and rapid growth (Huston and Smith 1987; Eriksson and Eriksson 1997). All of these strategies enable early successional species to move and quickly establish on disturbed sites. Common early successional species in the NMGP include foxtail barley



(*Hordeum jubatum* L.), curlycup gumweed (*Grindelia squarrosa* (Pursh) Dunal), field brome (*Bromus arvensis* L.), eastern daisy fleabane (*Erigeron annuus* (L.) Pers.), and annual sunflower (*Helianthus annuus* L.). These early successional species are likely to dominate disturbed sites until later competitive, successional species become established (Mcintyre et al. 1995).

Succession is the change in vegetation overtime (Huston and Smith 1987). However, the rate of replacement and facilitation of vegetation, such as early successional species by later successional species, may occur more slowly in the presence of soil salinity or when topsoil has been removed (Keiffer and Ungar 2002; Prach and Pyšek 2001).

Short-lived ruderal and exotic species may be benign and even beneficial in the early stages of the reclamation process. Early successional species provide plant nutrients through high species turnover and stabilize loose soil to rebuild soil structure, which facilitate the replacement of later successional species (Tansley 1935; D'Antonio and Meyerson 2002; Ewel and Putz 2004). Later successional species do not readily establish on disturbed sites because they are slower growing and primarily reproduce by vegetative means (Eriksson and Jakobsson 1998). Later succession species also tend to have heavier seeds (Leishman 1999) that are less likely to reach disturbed sites as compared to lighter, smaller seeds of early successional species (Primack 1987; Reader 1993). Differences in plant life histories may create distinct contrasts in plant community assemblages on the bordering edge of native prairie and disturbed sites.

Successional trends on remediated brine spill sites are largely unknown, and perpetual disturbances surrounding these areas can lead to permanent shifts in vegetation cover. Exotics are detrimental to disturbed sites if they are long-lived colonizers that persist in plant communities, ultimately becoming invasive (D'Antonio and Meyerson 2002). Invasive plant species are difficult to eradicate because they compete for the same resources as native plant

species, but aggressively take over and create monotypic stands of only a few species (Lesica and DeLuca 1996; Henderson and Naeth 2005; Murphy and Grant 2005; DeKeyser et al. 2015). Common invasive species in the NMGP include Kentucky bluegrass (*Poa pratensis* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), Canada thistle (*Cirsium arvense* (L.) Scop.), and leafy spurge (*Euphorbia esula* L.). The long-term occupancy of these invasive species irreversibly change plant community assemblages and make it difficult to reach reclamation objectives (Brandt and Rickard 1994; Stylinski and Allen 1996). Residual brine salts also alter successional sequences by exerting a strong abiotic gradient (i.e. soil salinity) that inhibits salt sensitive species from successfully establishing on these sites. Assisted revegetation using native halophytic species (e.g., *Pascopyrum smithii* (Rydb.) Á. Löve, *Puccinellia nuttalliana* (Schult.) Hitchc., *Sporobolus airoides*, *Distichlis spicata*, and *Hordeum jubatum*) are desirable because they grow naturally in the NMGP and are commercially available (Dodd and Coupland 1966; Braidek et al. 1984). These grass species are also more aesthetically pleasing than early successional species, and the establishment of native halophytic grasses on remediated brine spill sites may help land managers achieve reclamation goals.

Characterizing plant community assemblages and quantifying plant productivity on remediated brine spill sites is useful for determining if remediation was successful at restoring the plant community to pre-spill conditions (Halvorson and Lang 1989). Our research aims to investigate plant community responses to different levels of salinity through a greenhouse study and to quantify residual brine salts and plant establishment on remediated sites in a field study. The objectives of our greenhouse study were to determine brine/plant survival thresholds of nine plant species at five brine-derived soil saturated paste extract ( $EC_e$ ) levels, and evaluate plant sensitivity at seedling and late vegetative — pre-boot growth stages. Our research will help

establish ECE benchmarks as well as identify suitable species for reclamation of remediated brine spill sites for oil and gas personnel and environmental consultants. The objective of the field study was to compare efficacy of the two soil amendment techniques by evaluating remaining brine salts and quantifying plant establishment on remediated spill sites to determine which method is more appropriate for the environmental conditions in semi-arid rangelands.

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## CHAPTER 2: OIL-PRODUCED WATER THRESHOLDS ON RANGELAND PLANT'S SURVIVAL UNDER GREENHOUSE CONDITIONS

### Abstract

Oil and gas production activities in western North Dakota increased the presence of anthropogenic surface salinity in the Northern Mixed Grass Prairie (NMGP). Oil-produced water (i.e. brine) is a regulated waste product of oil and gas extraction that can kill actively growing plants shortly after coming into contact with plant leaves and roots when discharged onto the soil surface. We conducted a non-leaching greenhouse experiment on nine plant species at five brine-derived saturated paste electrical conductivities ( $EC_e$ ; 2, 4, 8, 16 and  $32 \text{ dS m}^{-1}$ ) at seedling (SDLG) and late vegetative — pre-boot (LV) growth stages. Concentrated brine ( $210 \text{ dS m}^{-1}$ ) was diluted with distilled to create five  $EC_e$  concentrations, with no added brine as the control, and applied to five pots per  $EC_e$  treatment in a loam soil. We then collected survival and biomass data on greenhouse plants and analyzed with regression. Halophytic and glycophytic plants exhibited increased mortality at  $EC_e$  greater than  $9.7 \text{ dS m}^{-1}$ . Above and belowground biomass declined with each additional  $EC_e$  level for all species at both growth stages. Decreases in aboveground biomass ranged from 4 to 60% whereas belowground biomass declined by 4 to 50% when compared to the control treatment for each species across all  $EC_e$  levels. Halophytic species exhibited a brine threshold between  $18.6$  and  $34.2 \text{ dS m}^{-1}$ , whereas glycophytic species exhibited a lower  $EC_e$  survival threshold ( $9.70$  and  $18.6 \text{ dS m}^{-1}$ ). We did not identify brine thresholds ( $\geq 34.2 \text{ dS m}^{-1}$ ) for *Hordeum jubatum* and *Puccinellia nuttalliana*. The halophytic species examined in this paper have different vacuole capacities and transpiration rates as well as different salt tolerance mechanisms to revegetate salt-affected areas. The halophytic species

examined in this greenhouse experiment are suitable candidates to diversify oil and gas reclamation seed mixes.

## **Introduction**

Soil salinity is a major limiting factor to plant productivity, affecting approximately 7% of the earth's land surface (Ghassemi et al. 1995). Both natural and human-induced processes cause soil salinity. Oil and gas production activities, such as the oil production in the Northern Plains, increased the presence of anthropogenic salinity in the Northern Mixed Grass Prairie (NMGP) region with over 78 million liters of oil-produced water (i.e. brine) spilled in North Dakota since 2001 (ND DOH 2015). Accidental or deliberate discharges of brine adversely affect soil and vegetation parameters, and in the absence of remediation can create areas that are unable retain soil resources to support vegetation (Jager et al. 1995). Salt tolerant plant species (i.e. halophytes) aid in the amelioration of salt-affected sites, but their value as possible candidates to revegetate remediated brine spill sites on western rangelands has not been evaluated in a greenhouse experiment (Aschenbach and Kindscher 2006). This study examines the ability of halophytic and glycophytic cool and warm-season grasses to grow and survive under non-leaching greenhouse conditions at two growth stages.

High salt concentrations impede plant recovery in semi-arid to arid climates where limited precipitation restricts downward leaching of salt ions below the plant root zone. Leached salt-laden water travels upward to the soil surface via capillary action during hot, dry months (Thimm 1990), preventing plant seeds from germinating, and killing seedlings (Ungar 1978; Keiffer and Ungar 1997). This salt migration and subsequent reduction in plant establishment will result in the need for additional soil treatments and reseeding applications. Salt tolerant plant species are better equipped to handle gradual changes in soil salinity. Halophytes have evolved

under natural saline environments such as saline seep formations, allowing them to develop morphological and anatomical characteristics to deal with salt stress (Flower et al. 2010). Halophytes maintain plant performance in salt-rich environments ( $\geq 15 \text{ dS m}^{-1}$ ; Keiffer and Ungar 2001) by closing stomata cells and accumulating salt ions in leaf vacuoles. Leaf vacuoles will accumulate salt ions until they reach capacity causing them to rupture and leak salt ions into the cell cytoplasm and cell wall, thus leading to cellular dehydration (Munns 2002; Munné-Bosch and Alegre 2004; Munns 2005). Salt tolerance generally increases with age of plant even though older plants have been transpiring for longer and have brought in more salt ions through plant roots (Munns 2002; Munné-Bosch and Alegre 2004; Munns 2005). Mature plants can survive in the presence of salt stress through senescence of older plant leaves first and remobilization of water and nutrients to younger photosynthetically active leaves (Munns 2002; Munné-Bosch and Alegre 2004; Munns 2005). However, salt sensitivity can increase right before maturity when plants allocate resources to support reproductive structures (Läuchli and Grattan 2007; Oliveira et al. 2013). Although halophyte species exhibit higher salt tolerances than less salt tolerant plant species (glycophytes), halophytes are less equipped to handle large doses of salt at one discrete time, leading to cell plasmolysis (Shavrukov 2013).

Cell plasmolysis is the process in salt concentrations build-up to toxic levels in the cytoplasm and leak into the plant cell wall. Cell plasmolysis occurs when there is an abrupt increase in salinity in the growing medium that leaves plants unable to mobilize available resources and initiate survival mechanisms fast enough to combat changing soil conditions (Shavrukov 2013). Plant survival mechanisms include accumulation of ions, solutes, and carbohydrates to aid in osmotic adjustment as well as salt ion exclusion and compartmentalization to maintain ion homeostasis (Munns 2002; Shavrukov 2013). Fortunately,

plants rarely experience sudden increases in soil salinity in field conditions (Shavrukov 2013), with the exception of low quality irrigation water and brine spills. Brine (90% NaCl) is a regulated waste product of oil and gas extraction process that contains salt concentration magnitudes higher than natural saline environments (McMillion 1965). Accidental and deliberate discharges of brine kill actively growing plants shortly after coming into contact with plant parts (Murphy et al. 1988; Aschenbach and Kindscher 2006). The loss of stabilizing root structures and protective plant cover leave soil susceptible to erosional hazards. Salts from brine spills also have long-term impacts on soil and vegetation parameters because they alter the osmotic potential energy of soil water making it difficult for plants to colonize brine-affected sites without some type of soil amelioration technique.

Brine spill remediation techniques can remove or minimize the abiotic stressor (salt) to levels suitable for plant growth. Although brine spill remediation can reduce the overall concentration of brine salts, recalcitrant salts can resurface during dry months and result in plant death (Thimm 1990). Halophytes provide short-term protection from the rise of salt-laden water by up-taking salt ions to create a more conducive environment that facilitates the establishment of less salt tolerant plant species (Barrett-Lennard 2002; Manousaki and Kalogerakis 2011). Harvesting of aboveground parts of halophytes at the end of the growing season can also permanently decrease salt concentrations. Additionally, some halophytes can also uptake ions that exceed plant nutrient requirements from industrial solutes containing boron (B), cadmium ( $\text{Cd}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), and copper ( $\text{Cu}^+$  &  $\text{Cu}^{2+}$ ; Rozema et al. 1992; Manousaki and Kalogerakis 2011). Halophytes are desirable for reclaiming salt-affected sites because they are naturally present in the environment and commercially available (Aschenbach and Kindscher 2006; Manousaki and Kalogerakis 2011).

Evaluation of salt tolerance includes examining plant survival and growth at different saturated paste electrical conductivity ( $EC_e$ ) levels. Identifying plant survival thresholds of salt tolerant and sensitive NMGP species at seedling and pre-mature growth stages along an  $EC_e$  gradient may provide insight into acceptable  $EC_e$  ranges and the type of species that would succeed on newly remediated brine spills in western rangelands. Objectives of this study were to 1) determine plant survival thresholds of nine plant species treated with five brine-derived soil  $EC_e$  levels and 2) evaluate plant sensitivity at seedling (SDLG) and late vegetative—boot stage (LV) stages. We hypothesized that plants will exhibit salt sensitivity at concentrations greater than  $2 \text{ dS m}^{-1}$ , as suggested by preliminary greenhouse trials. We expected plant mortality to increase with each additional brine concentration for SDLG and LV growth stages. Halophytic plant species (*Pascopyrum smithii* (Rydb.) Á. Löve, *Puccinellia nuttalliana* (Schult.) Hitchc. *Sporobolus airoides*, *Distichlis spicata* (L.) Greene, and *Hordeum jubatum* L.) are likely to display higher EC thresholds than less salt tolerant species in this experiment due to salt ion accumulation and avoidance mechanisms.

### **Materials and Methods**

We conducted a greenhouse experiment at the North Dakota State University Agricultural Experiment Station Research Greenhouse Complex in Fargo, North Dakota, USA to evaluate brine thresholds of nine NMGP species. Greenhouse conditions were with a 14:10 hour day:night cycle and a constant temperature ( $25^\circ\text{C}$ ). The growth medium for plants in the experiment was topsoil from a Williams Soil Series (34% clay, 22% silt, 44% sand, and  $0.38 \text{ dS m}^{-1} EC_e$ ). The Williams Series is a moderately, well-drained soil that is the most extensive soil series in western North Dakota and is a suitable soil to study brine and plant relationships. The brine used to derive the soil EC's was collected from a wellhead near the Wiley field in

Maxbass, North Dakota. This brine had a 210 dS m<sup>-1</sup> EC, and sodium (74,930 parts per million (ppm)), chloride (149,908 ppm), potassium (2,809 ppm), calcium (2,140 ppm), sulphate (1,540 ppm), magnesium (602 ppm), and boron (81.7 ppm) levels (Midwest Laboratories, Inc., Omaha, Nebraska). The plants used to test brine thresholds in this experiment are all native to the NMGP with the exception of Kentucky bluegrass (*Poa pratensis* L.) (Table 1).



**Table 1:** Reported survival and/or biomass electrical conductivities of saturated paste extract (EC<sub>e</sub>) thresholds (dS m<sup>-1</sup>) of nine Northern Mixed-Grass Prairie species at germination, seedling, and mature growth stages.

	EC Threshold (dS m <sup>-1</sup> )	Reference
<b>Cool Season (C<sub>3</sub>) Grasses</b>		
<i>Pascopyrum smithii</i> †	10 <sup>G</sup> & 58 <sup>S</sup>	(Moxley et al. 1978; Aschenbach 2006)
<i>Poa pratensis</i>	22 <sup>S</sup> , 3 <sup>S</sup> & 5-6 <sup>M</sup> , 23 <sup>S</sup> , & 8 <sup>S</sup>	(Kenkel et al. 1991; Carrow and Duncan 1998; Alshammmary et al. 2004; Gilbert and Fraser 2013; Hu et al. 2015)
<i>Hordeum jubatum</i> †	26 <sup>S</sup> & 22 <sup>S</sup>	(Badger and Ungar 1990; Kenkel et al. 1991)
<i>Nassella viridula</i>	9-10 <sup>G</sup>	(Ries and Hofmann 1983)
<i>Puccinellia nuttalliana</i> †	22 <sup>S</sup> , 19 <sup>G</sup> , 23 <sup>S</sup>	(Kenkel et al. 1991; Tarasoff et al., 2007b; Gilbert and Fraser, 2013)
<b>Warm Season (C<sub>4</sub>) Grasses</b>		
<i>Distichilis spicata</i> †	42 <sup>M</sup> , 66 <sup>M</sup> , 23 <sup>M</sup> , & 35-41 <sup>M</sup>	(Ungar 1966; Ungar 1969; Hansen et al. 1976; Alshammmary et al. 2004)
<i>Bouteloua gracilis</i>	31 <sup>G</sup> & 16 <sup>G</sup>	(Zhang et al. 2012; Dudley et al. 2014)
<i>Schizachyrium scoparium</i> †	9-10 <sup>G</sup> & 3-16 <sup>G</sup>	(Ries and Hofmann 1983; Dudley et al. 2014)
<i>Sporobolus airoides</i> †	11-25 <sup>G</sup> & 9-10 <sup>G</sup>	(Hyder and Shamsa 1972; Ries and Hofmann 1983)

† = Halophytic species

G = Germination, S = Seedling, M = Mature

We air-dried field soil and sieved it through a 16 mm mesh to remove rocks and debris, then mechanically ground the soil before transferring into 15.2 x 10.8 cm pots (1,450 g per pot) lined with plastic to prevent leaching. Seed germination occurred in vermiculite, and then transplantation of seedling to pots occurred between 7 and 14 days post-germination. Each pot contained two seedlings with five pots per EC treatment for all nine species. Target brine-derived soil  $EC_e$  treatments were 2, 4, 8, 16, and 32  $dS\ m^{-1}$ . The control treatment for each species was no brine (0.38  $EC_e$ ). We determined the initial brine-derived soil  $EC_e$  by estimating field capacity of the soil using particle size analysis, bulk density, and a pedotransfer function to determine the proportion of concentrated brine (210  $dS\ m^{-1}$ ) to add to each deionized water solution (Saxton and Rawls 2006). The differences in target versus actual soil  $EC_e$  (3.3, 5.8, 9.7, 18.6, and 34.6  $dS\ m^{-1}$ ) are attributed to the fact that brine is not a uniform solution but a mixture of petroleum hydrocarbons, industrial effluents, and other soluble salts. Additionally, the  $EC_e$  values from the soil do not include ions exchanged onto the mineral surface complexes.

Application of brine-derived soil  $EC_e$  concentrations occurred at two different growth stages for each plant species and included 1) seeding stage (SDLG) and 2) late vegetative—pre-boot (LV) growth stage. Assessment of plant survival occurred 30 days after brine application. Survival assessment of individual plants included visually examining green plant parts and creasing plant leaves to observe water transport via xylem to aerial plant parts. Shortly after visual assessment, aboveground and belowground biomass were harvested, separated and oven dried (49 °C) to a constant weight. Data analysis included linear regression models for brine survival thresholds done in SAS<sup>®</sup> (version 9.3, SAS Institute, Inc., Cary, NC). We then compared first-order regression models (linear, exponential decay, power, growth, sigmoidal, and logistic) using a small-size corrected version of Akaike information criterion (AIC) to select

the best model for above and belowground biomass weights in SAS<sup>®</sup> (version 9.3, SAS Institute, Inc., Cary, NC; Hurvich and Tsai 1989). Significance for all regression models were based on an alpha  $p < 0.05$ .

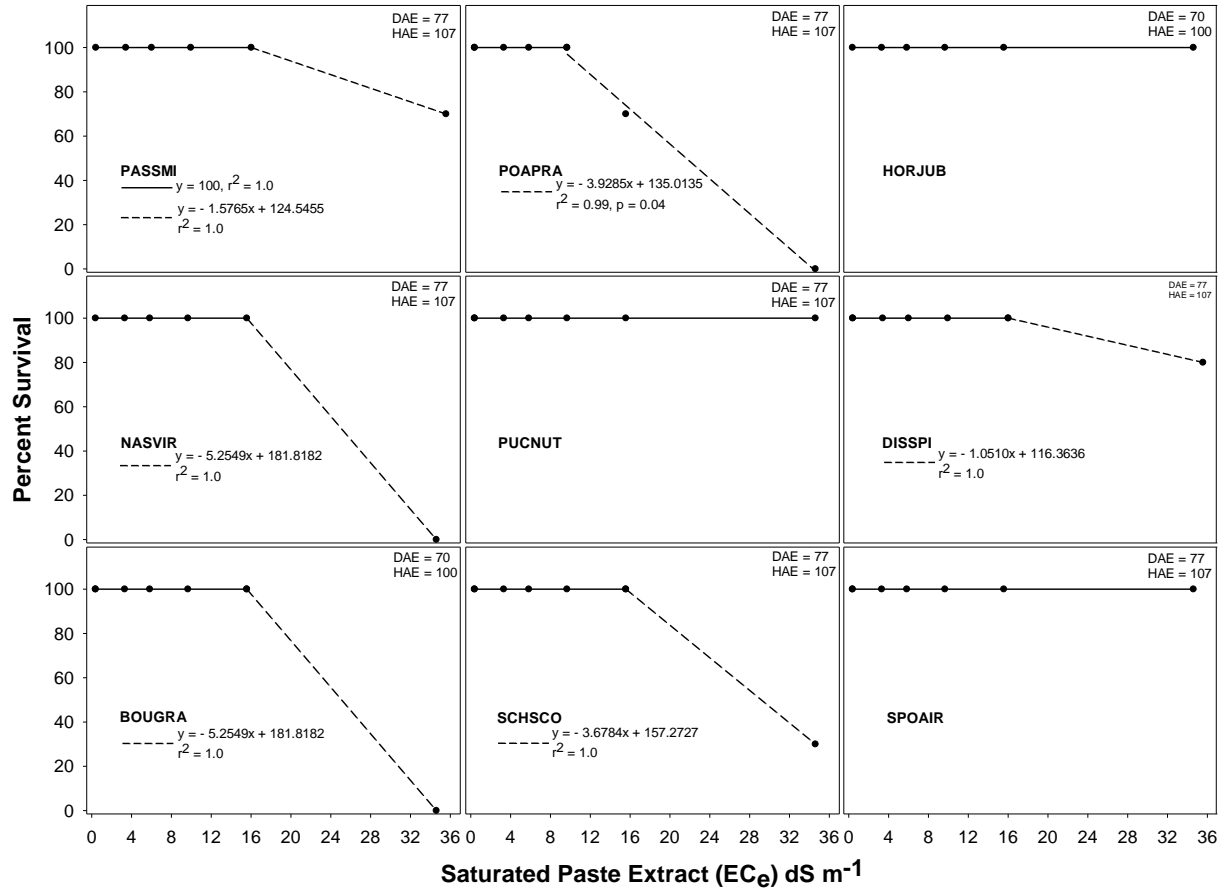
## Results

Halophytic and glycophytic plant species exhibited salt sensitivity at soil EC's greater than  $8 \text{ dS m}^{-1}$  under greenhouse conditions (Figure 1 & 2). The two growth stages of halophytic plant species played a minimal role in plant survival at the respective brine-derived soil EC's ( $p \geq 0.05$ ). The most resilient halophytic species in this experiment were *Hordeum jubatum*, *Distichlis spicata*, *Puccinellia nuttalliana* and *Sporobolus airoides* with no tolerance thresholds up to  $34.6 \text{ dS m}^{-1}$  detected at one or both growth stage(s) (Figure 1 & 2). *Pascopyrum smithii* had approximately 50% and 75% survival at SDLG and LV growth stages at  $34.6 \text{ dS m}^{-1}$  and less salt tolerant plant species, such as *Poa pratensis*, *Nassella viridula* (Trin.) Barkworth, *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, and *Schizachyrium scoparium* (Michx.) Nash exhibited no plant mortality at soil EC<sub>e</sub> less than  $9.70 \text{ dS m}^{-1}$ .

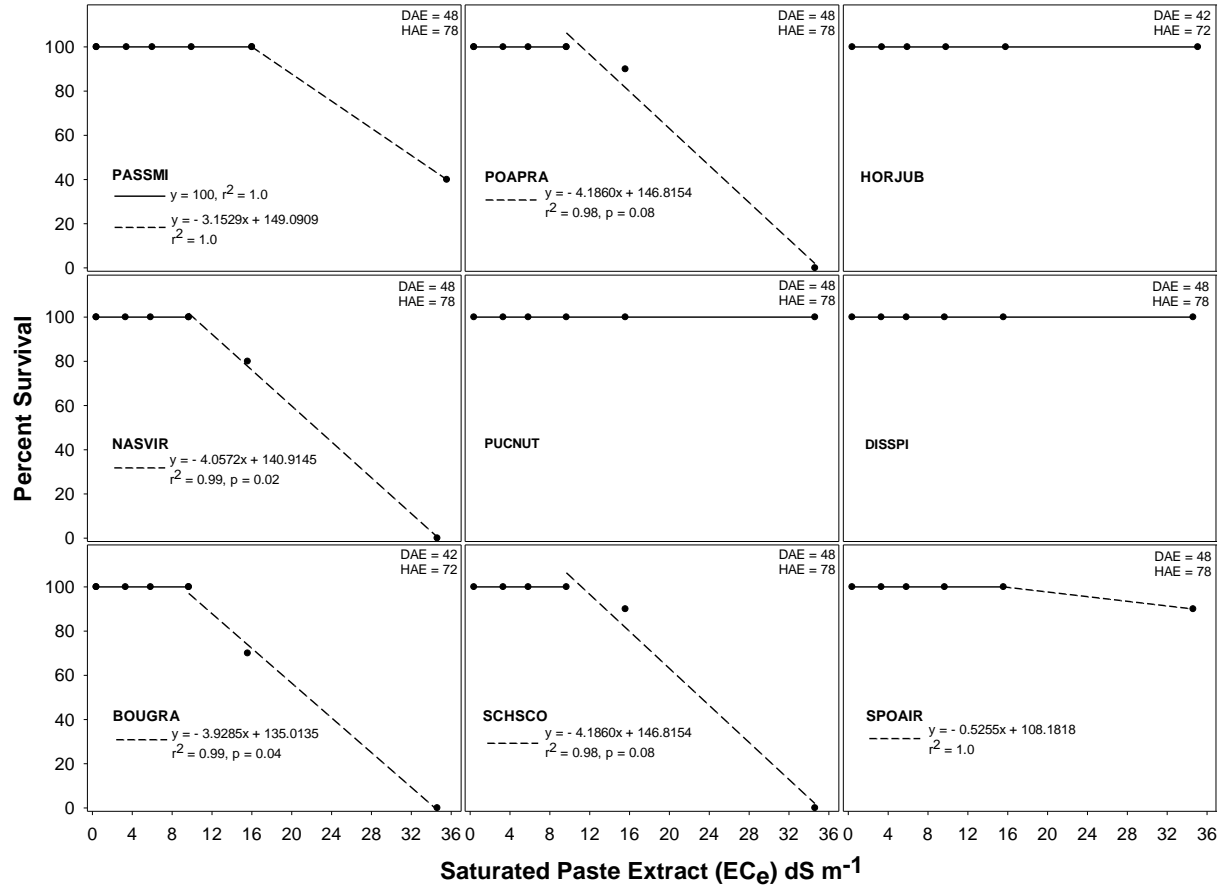
Aboveground biomass for SDLG and LV growth stage treatments declined by 4 to 60%, with each treatment increase in soil EC for all plant species when comparing differences among treatments as opposed to differences between treatments. Glycophytic species (*Poa pratensis*, *Nassella viridula*, *Bouteloua gracilis*, and *Schizachyrium scoparium*) experienced a 45 to 100% reduction in overall biomass between the  $18.6$  and  $34.6 \text{ dS m}^{-1}$  treatments for both growth stages when compared to the control treatment (Figures 3 & 4). Halophytic species also showed a gradual decline in aboveground biomass, but much less than glycophytic species. Overall biomass reduction was higher for *Pascopyrum smithii* at the SDLG growth (84%) than at the LV growth stage (72%) between  $34.2 \text{ dS m}^{-1}$  as compared to the control treatment. This trend was

also similar for overall biomass production of *Hordeum jubatum* (70% (SDLG) & 40% (LV)), *Puccinellia nuttalliana* (77% & 30%), *Distichilis spicata* (49 & 42%), and *Sporobolus airoides* (74%; SDLG only stage) with the largest reduction occurring between 18.6 and 34.6 dS m<sup>-1</sup> treatments. However, there was no relationship ( $p \geq 0.05$ ) between increasing soil EC and biomass reduction of *Sporobolus airoides* at the LV growth stage.

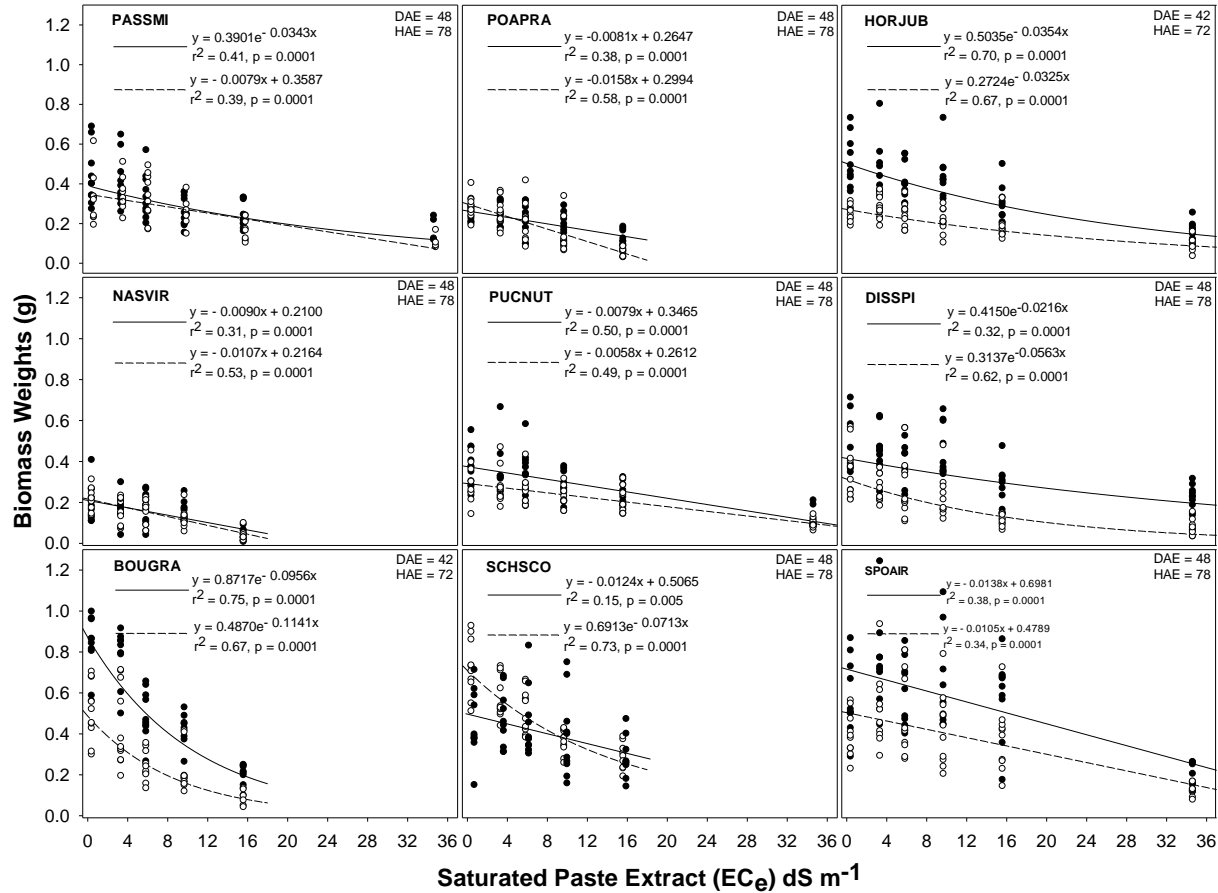
Belowground biomass for SDLG and LV plants were slightly less than aboveground biomass, declining by 4 to 50%, when comparing differences among treatments as opposed to differences between treatments. Roots exhibited less of a decline with each increase in soil EC<sub>e</sub> at both the SDLG and LV growth stages when compared to the aboveground portion (i.e. leaves and stems). Reductions in overall belowground biomass for halophytes and glycophytes mirrored that of aboveground biomass with the lowest difference in reduction occurring between the control (0.38 dS m<sup>-1</sup>) and 3.30 dS m<sup>-1</sup> treatment and the largest difference between EC<sub>e</sub> 18.6 and 34.6 dS m<sup>-1</sup> treatments, respectively. SDLG survival and biomass weights was lower than LV plants. The reduction in biomass between the control treatment (0.38 dS m<sup>-1</sup>) and 34.6 dS m<sup>-1</sup> was 100% for SDLG and 97% for LV glycophytes. Halophytes exhibited lower biomass reduction between 18.6 and 34.6 dS m<sup>-1</sup> for SDLG (75%) and LV (50%) plants. The results of this greenhouse experiment support our initial hypotheses.



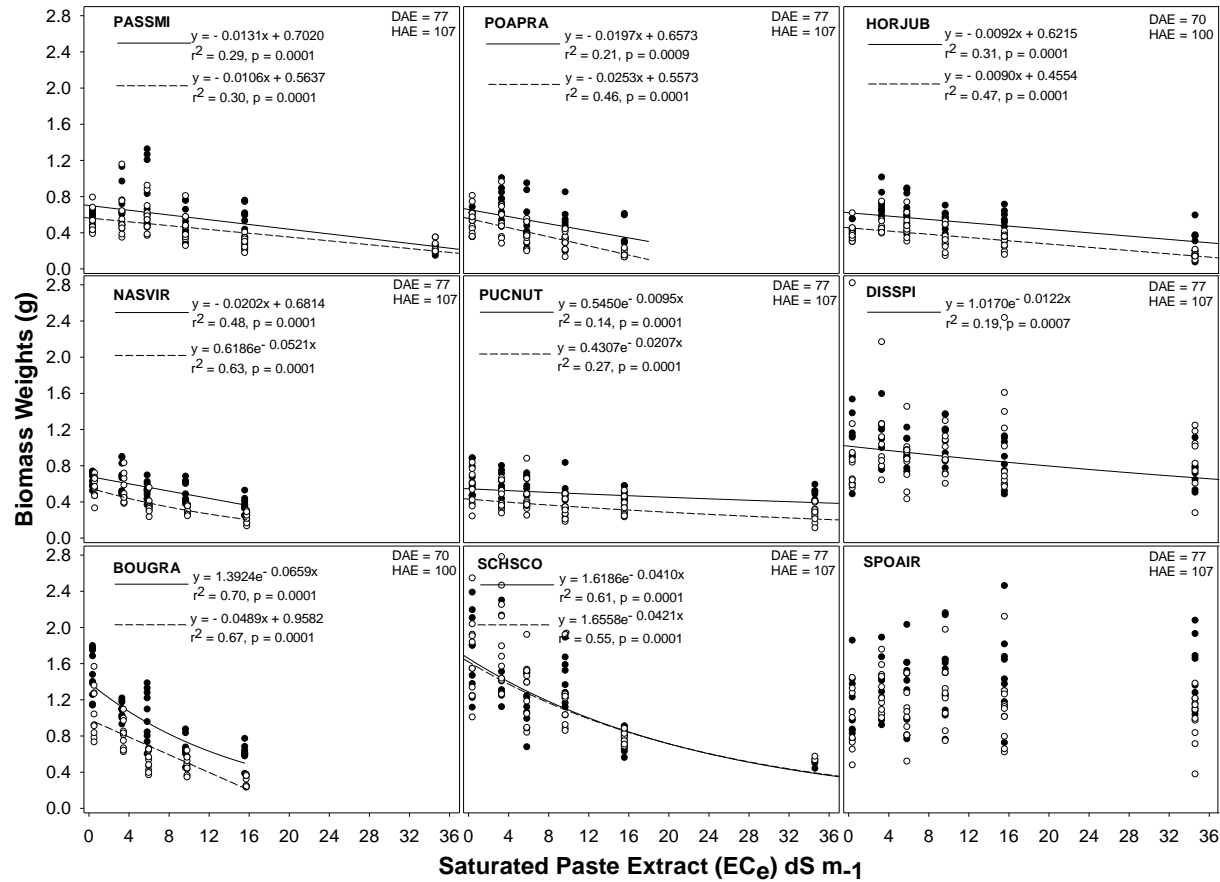
**Figure 1:** Percent survival of seedlings on an  $EC_e$  gradient with piecewise-linear regression models,  $r^2$ , pvalue, brine application days after emergence (DAE), and days harvested after emergence (HAE); plants starting from the upper left-hand corner are *Pascopyrum smithii* (PASSMI), *Poa pratensis* (POAPRA), *Hordeum jubatum* (HORJUB), *Nassella viridula* (NASVIR), *Puccinellia nuttalliana* (PUCNUT), *Distichlis spicata* (DISSPI), *Bouteloua gracilis* (BOUGRA), *Schizachyrium scoparium* (SCHSCO), and *Sporobolus airoides* (SPOAIR).



**Figure 2:** Percent survival of late vegetative — pre-boot plants on an  $EC_e$  gradient with piecewise-linear regression models,  $r^2$ , pvalue, brine application days after emergence (DAE), and days harvested after emergence (HAE); plants starting from the upper left-hand corner are PASSMI, POAPRA, HORJUB, NASVIR, PUCNUT, DISSPI, BOUGRA, SCHSCO, and SPOAIR.



**Figure 3:** Above (closed circles & solid line) and belowground (open circles & dashed line) biomass weights of seedlings on an  $EC_e$  gradient with regression model equations,  $r^2$ , pvalue, brine application days after emergence (DAE), and days harvested after emergence (HAE); plants starting from upper left-hand corner are PASSMI, POAPRA, HORJUB, NASVIR, PUCNUT, DISSPI, BOUGRA, SCHSCO, and SPOAIR.



**Figure 4:** Above (closed circles) and belowground (open circles) biomass weights of late vegetative — pre-boot plants on an  $EC_e$  gradient with linear and exponential decay regression equations,  $r^2$ , pvalue, brine application days after emergence (DAE), and days harvested after emergence (HAE); plants starting from upper left-hand corner are PASSMI, POAPRA, HORJUB, NASVIR, PUCNUT, DISSPI, BOUGRA, SCHSCO, and SPOAIR.



## Discussion

The salt ions in the brine limit plants' ability to function properly, requiring immediate human action to reduce ion toxicity and osmotic stress in the soil root zone. This greenhouse study identified potential survival threshold windows (between 9.70 and 18.6 dS m<sup>-1</sup>) for rangeland plants to determine abiotic levels for revegetation. The five halophytic species (*Pascopyrum smithii*, *Hordeum jubatum*, *Distichlis spicata*, *Puccinellia nuttalliana*, and *Sporobolus airoides*) in this study exhibited higher salt tolerance above 18.6 dS m<sup>-1</sup> when compared to survival threshold of glycophytes, with all but two halophytes displaying differences in percent survival at the two growth stages along the soil EC<sub>e</sub> gradient. Cool-season grasses *Hordeum jubatum* and *Puccinellia nuttalliana* exhibited 100% survival at the SDLG and LV growth stage along the soil EC<sub>e</sub> gradient. The brine thresholds of less salt tolerant plant species (*Poa pratensis*, *Nassella viridula*, *Bouteloua gracilis*, and *Schizachyrium scoparium*) suggest the unlikelihood of natural or artificial reintroduction of these species on salt-affected sites with EC<sub>e</sub> above 9.70 dS m<sup>-1</sup>.

Percent survival of the less salt tolerant plant species, *Bouteloua gracilis*, *Nassella viridula*, and *Schizachyrium scoparium*, increased at the LV growth stage. The observed differences between percent survival and biomass reduction of NMGP species at the two growth stages are likely a result of increased ion transport and storage of salt ions in leaf vacuoles (Munns and Tester 2008). The results of this experiment were similar to other studies that found seedlings were sensitive to increases in soil salinity than at older growth stages (Chartzoulakis and Klapaki 2000; Heo et al. 2007). Mature plants are generally better at regulating water intake and loss, as well as partitioning Na<sup>+</sup> ions to prevent salt accumulation in the cytoplasm and cell wall (Munns 2002; Munné-Bosch and Alegre 2004; Munns 2005). The majority of species

observed in this experiment with the exception of *Poa pratensis*, exhibited increased salt tolerance at the older growth stage. *Poa pratensis* responded the same to soil salinity levels regardless of growth stage, which may indicate similar physiological restrictions occur at both stages. Along with physiological traits, salt tolerance is also associated with grass morphology as it relates to water uptake and plant establishment processes (De Jong 1978; Schwarz and Redmann 1990; Flowers et al. 2010).

Cool-season ( $C_3$ ) grasses tend to dominate the plant community during the early spring and late fall portion of the growing season in the NMGP when lower temperatures and evaporation rates occur (Barnes et al. 1983). The survival of these halophytes in salt-rich environments may be due to their ability to cease tiller development in late-May before salts rise to the soil surface to meet the evaporative demand of the soil surface, which can inhibit plant growth (Singh et al. 1983; Schwarz and Redmann 1980; Tarasoff et al 2007b). The initial development of roots and access to valuable plant resources can potentially make them more competitive and less sensitive to salinity than warm season grasses (Mahall and Park 1976; Tiku 1976). Cool-season halophytic grasses in this experiment were *Hordeum jubatum*, *Puccinellia nuttalliana*, and *Pascopyrum smithii*. *Pascopyrum smithii* was the least salt tolerant of the three with a survival threshold between 18.6 and 34.6 dS  $m^{-1}$ .

*Pascopyrum smithii* is the most widely used native halophyte in seed mixes for reclamation in western rangelands (Richards et al. 1998). The species' survival and biomass production were more affected at the SDLG growth stage than at the LV growth stage in this experiment, which may pose long-term problems for plant establishment and recruitment if salinity levels remain high ( $> 9.70 \text{ dsm}^{-1}$ ). Differences between SDLG and LV percent survival and productivity may depend upon osmotic adjustment of cell-sap molarities to maintain turgor

and cell volume during salt stress (Bernstein 1961; Munns 1988), and selective uptake and transport of potassium ( $K^+$ ) ions over sodium ( $Na^+$ ) ions (Apse and Blumwald 2007; Guo et al. 2015) at different growth stages. Additionally, *Pascopyrum smithii* may lose the ability to selectively uptake  $K^+$  over  $Na^+$  ions and transport them into plant tissues at high salinity levels (Munns and Tester 2008; Guo et al. 2015), such as concentrations ( $EC_e$  18.6 and 34.6  $dS\ m^{-1}$ ) tested in this experiment.

Short-lived halophytic grasses circumvent osmotic stress to some extent by fast tracking their life cycle to avoid costly plant inputs. *Hordeum jubatum*, ruderal halophyte, exhibited 100% survival at both growth stages up to  $EC_e$  of 34.6  $dS\ m^{-1}$ . *Hordeum jubatum*, is successful in saline environments because it can selectively uptake potassium ( $K^+$ ) and calcium ( $Ca^{2+}$ ) ions to young plant shoots, while reducing  $Na^+$  and  $Cl^-$  uptake and storing salt (NaCl) ions into above and belowground vacuoles (Badger and Ungar 1990; Keiffer and Ungar 2001; Keiffer and Ungar 2002). This mechanism is beneficial for temporarily reducing soil salinity in the growth medium to facilitate the reintroduction of less salt tolerant plant species as soil salinity decreases (Keiffer and Ungar 2001; Keiffer and Ungar 2002). However, harvesting aboveground biomass is necessary to permanently remove salt ions stored in leaf vacuoles and prevent long-term salt ion cycling in the soil matrix. Biomass production of seedlings were more sensitive to soil salinity than at the older growth stage and is comparable to observed field conditions (Cords 1960; Badger and Ungar 1989). *Hordeum jubatum* compensates for this failure by maturing early, which allow brittle heads to break away from the rachis and disperse seeds across the landscape (Cords 1960; Best et al. 1978). These seeds then germinate shortly after dispersal or during periods of reduced soil salinity to yield monotypic stands (90 to 100%) of *Hordeum jubatum* on salt-rich areas (Badger and Ungar 1989; Badger and Ungar 1994). Although *Hordeum jubatum*

can readily inhabit these types of habitats, it is a poor competitor with perennial, deep-rooted plants at lower salinities (Cords 1960; Wilson 1967; Best et al. 1978). The results of this greenhouse experiment display the potential use of *Hordeum jubatum* as a field indicator species to identify problematic salt-affected areas on remediated brine spill sites.

*Puccinellia nuttalliana* is a perennial bunchgrass that provides ground cover, forage resources, and wildlife habitat once established (Liu and Coulman 2015). Similar to *Hordeum jubatum*, *Puccinellia nuttalliana* was unaffected at the higher EC treatments, making it a more desirable and competitive species as compared to shallow rooted, short-lived *Hordeum jubatum*. However, the specific salt tolerant mechanism of *Puccinellia nuttalliana* is unknown (Tarasoff et al. 2007a; Tarasoff et al. 2010), and reportedly does not store salt ions in above or belowground plant parts (Tarasoff et al. 2007a; Tarasoff et al. 2010). *Puccinellia nuttalliana* may use salt-avoidance strategies, such as delaying germination at high salinities, excluding salt ion uptake at the root interface, and ceasing tiller development before salt-laden water rises when the soil becomes dry and moves towards the soil surface (Schwarz and Redmann 1990; Tarasoff et al. 2007b). The high salt tolerance of *Puccinellia nuttalliana* at early growth stages, as indicated by this greenhouse experiment, may enable this plant to establish in salt-rich areas and take advantage of plant resources (e.g., water, nutrients, light, and space) that glycophytes are unable to access (Macke and Ungar 1971; Tarasoff et al. 2007; Gilbert and Fraser 2013). Although we did not assess the competitive ability of *Puccinellia nuttalliana* in this experiment, this plant is a deep-rooted, perennial halophyte that can stabilize soil to minimize wind and water erosion on salt-affected areas (Kenkel et al. 1991; Gilbert and Fraser 2013).

Warm-season (C<sub>4</sub>) grasses, to some extent, are salt tolerant in order to survive in semi-arid to arid climates where drought and salinity often coincide (Bromham 2015). These two

abiotic stressors behave similarly by activating and regulating different stress response mechanisms to cope with a reduction in total soil water potential energy in the growing medium (Wang et al. 2003; Hu and Schmidhalter 2005). Warm-season grasses differ from cool-season grasses in that they can better regulate water intake and loss during periods of drought and salt stress (Barnes et al. 1983; Schwarz and Redmann 1990; De Jong 1978). Warm-season halophytic grasses in this experiment were *Distichlis spicata* and *Sporobolus airoides*, each with a detected brine threshold between 18.6 and 34.6 dS m<sup>-1</sup> at the LV and SDLG, respectively.

*Distichlis spicata* and *Sporobolus airoides* both belong to the Chloridoideae subfamily and are salt accumulating halophytes (Marcum 1999). Salt accumulating halophytes compartmentalize salt ions in leaf vacuoles, utilizing specialized glands to excrete salt (Na<sup>+</sup> & Cl<sup>-</sup>) ions to outside the plant (Hansen et al. 1976; Marcum 1999). Salt accumulating halophytes reportedly recover faster from high concentrations of salts in growing medium than salt-excluding halophytes that tend to grow better at lower soil EC<sub>e</sub> levels (Greenway 1968). *Distichlis spicata* was slightly more salt tolerant at the LV growth stage than at the seedling stage, and was the only species to display this relationship in our study. Increased mortality at the LV growth stage is likely associated with the divergence of resource allocation to support reproductive structures and lower salt-accumulation at high salinity levels (Marcum 1999). *Sporobolus airoides* differed from *Distichlis spicata* in having a lower brine threshold at the SDLG growth stage (between 18.6 and 34.6 dS m<sup>-1</sup>) and no response at the LV stage (> 34.6 dS m<sup>-1</sup>) and vice versa. *Sporobolus airoides* and *Distichlis spicata* can accumulate and compartmentalize brine salts (Na<sup>+</sup> & Cl<sup>-</sup>) in plant tissues (Hansen et al. 1976; Marcum 1999), allowing for the harvesting of aboveground plant growth and permanently reducing salt ions in the plant root zone.

## Management Strategies

The native halophytes examined in this project are viable options for reclaiming salt-affected rangelands, while providing food and cover to wildlife species and forage for livestock. *Distichlis spicata* and *Pascopyrum smithii* provide high crude protein (CP) content (CP; 15.0 and 18.5%, respectively) during early spring when high quality forage is necessary for maintenance of lactating mammals (Hart et al. 1963; Hansen et al. 1976). In addition to forage value, *Sporobolus airoides* grows tall and dense, providing shelter and camouflage for wildlife species (Hickey and Springfield 1996). These native halophytes are preferable over non-native halophytes because they facilitate the reintroduction of less salt tolerant grasses and forbs to increase biodiversity and are less likely to create monotypic communities. Dominance of non-native halophytes, such as *Agropyron cristatum* (L.) Gaertn., and *Bassia scoparia* (L.) A.J. Scott, on salt-affected soils prevent ecosystem recovery and delay the bond release process for remediated brine spill sites. Reclaiming recently remediated brine spill sites with native halophytes may increase the success rate of reseeding applications and possibly reduce exotic halophyte establishment.

## Conclusions

Evaluation of native halophytic plant species through greenhouse and field studies creates new science for improving remediation techniques on land affected by natural and anthropogenic surface salinity. The halophytes in this greenhouse experiment exhibited a brine threshold between 18.6 and 34.6 dS m<sup>-1</sup> EC<sub>e</sub>, which was higher than the brine threshold of glycophytes (9.70 and 18.6 dS m<sup>-1</sup> EC<sub>e</sub>). *Hordeum jubatum* and *Puccinellia nuttalliana* exhibited 100% survival up to 34.6 dS m<sup>-1</sup> at both SDLG and LV growth stages, indicating a higher brine threshold. SDLG percent survival and biomass production was lower than the LV growth stage,

with increasing soil salinity. Since plant mortality and biomass production declined with plant age, it may be beneficial to transplant seedlings to promote plant establishment and growth during periods of increased soil moisture sooner, before the soil dried out, to circumvent scorching of newly established seedlings when salt-laden water rises from deeper to upper soil horizons. Unfortunately, many of the native halophytic species discussed in this paper are not widely used in oil and gas reclamation seed mixes. *Pascopyrum smithii* is the most popular halophyte species for revegetating western rangelands due to its availability and moderate price (Richards et al. 1998). The results of this paper provide an opportunity for oil and gas personnel to diversify their native seed selection by selecting native halophytes (e.g., *Distichlis spicata*, *Puccinellia nuttalliana* and *Sporobolus airoides*) to increase plant establishment and future recruitment on salt-affected sites. *Hordeum jubatum* is a ruderal halophyte that is likely to revegetate salt-affected sites without artificial assistance and replaced by perennial, deep-rooted species when  $EC_e$  levels decline to acceptable levels for glycophytes. Selection of halophytes with different salt tolerant mechanisms and life histories are important for ensuring permanent vegetation cover on salt-affected sites.

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## CHAPTER 3: EVALUATION OF SOIL TREATMENT TECHNIQUES ON REMEDiated BRINE WATER SPILL SITES IN SEMI-ARID RANGELANDS

### Abstract

Oil-produced water (i.e. brine) is a waste product of oil and gas extraction that can cause severe changes to soil chemistry and plant communities when discharged onto the soil surface. Brine spill remediation through topsoil excavation (*ex situ*) or the addition of chemical amendments (*in situ*) aims to remove or minimize the abiotic stressor to levels suitable for plant growth. We quantified residual brine salts and plant establishment on 10 chemical amendment and 11 topsoil excavation sites in semi-arid rangelands of western North Dakota. Paired reference and remediated sites had similar soil texture and landscape position. We hypothesized no difference in soil and plant parameters between remediation techniques. Results concluded electrical conductivity ( $EC_e$ ) was not different ( $p > 0.05$ ) between remediation treatments for all three depths. Overall plant productivity was different ( $p \leq 0.05$ ) between remediated and reference sites, but not between remediation techniques ( $p > 0.05$ ). Remediation techniques successfully lowered  $EC_e$  to facilitate natural and assisted revegetation. Species composition and diversity on remediated sites were different ( $p \leq 0.05$ ) between remediated and reference sites, indicating that remediated sites are undergoing succession and not yet similar to neighboring plant communities. Based on our findings, chemical amendments are the preferred remediation technique on small brine spill sites because they are less invasive and a more sustainable option than topsoil excavation.

## Introduction

Global oil and gas exploration and extraction contributes to environmental degradation (Asif and Muneer 2007), loss of ecosystem services (Allred et al. 2015), and the development of novel rangeland ecosystems when abiotic and biotic properties are altered (Hobbs et al. 2006; Hobbs et al. 2009). Oil and gas development increase human access to once remote areas (Finer et al. 2008), and weaken the structure and function of ecosystems by introducing a suite of new species (i.e. exotics; Hobbs and Huenneke 1992). While surface disturbances from oil and gas development disrupt the biotic component of ecosystems, contaminants from these human-induced activities change the abiotic properties of soil, making it increasingly difficult for plants to survive in these areas (Murphy et al. 1988; Keiffer and Ungar 2001). Discharges of oil-produced water (i.e. brine) and hydrocarbons can contain salt concentrations that kill vegetation and change plant community assemblages. Brine contaminated land, if left untreated, can create novel ecosystems that put additional pressure on intact remnant communities, requiring human-intervention to lessen the negative impacts caused by surface contamination and disturbance.

Oil-produced water (i.e. brine) spills are arguably the most environmentally destructive contaminant and controversial aspect of the oil and gas extraction process (Jager et al. 2005; Aschenbach and Kindscher 2006). Brine can contain up to 90% NaCl salts (McMillion 1965; Merrill et al. 1990) and can induce soil electrical conductivities (EC) much higher ( $\leq 200 \text{ dS m}^{-1}$ ) than natural salinity (Jong 1982; Keiffer & Ungar 2002). Spills may occur when there is a tank battery overflow, pipeline leak, or migration of salts from a reserve pit (Murphy et al. 1988; Sublette et al. 2007). Accidental or deliberate releases of brine can have long lasting impacts on soil and vegetation parameters.



Sodium from spills weaken soil aggregate stability by dispersing clay particles and plugging soil pores and thus reducing hydraulic conductivity (Jager et al. 2005; Leskiw et al. 2012). Releases of brine onto the soil surface overwhelm a plants' ability to cope with large quantities of salt at one discrete time, leading to ion toxicity (Munns and Termaat 1986; Halvorson and Lang 1989; Munns 2002). Brine salts also impede future plant establishment and recruitment by decreasing the soil water osmotic potential (Harris et al. 2005; Jager et al. 2005; Leskiw et al. 2012), requiring plants to expend more energy to extract water and nutrients from the soil matrix.

The severity of brine spills depends on the environment in which they occur. Brine in semi-arid to arid climates ( $\leq 25 \text{ cm year}^{-1}$ ) tend to have long-term impacts due to low moisture and soil leaching (Keiffer and Ungar 2001; Keiffer and Ungar 2002). Increases in soil moisture during months with ample precipitation lead to vertical and horizontal expansion of brine salts (brine plumes; Murphy et al. 1988), which can resurface during dry months when leached soil water rises to the soil surface via capillary action to meet atmospheric demand (Thimm 1990). Natural processes do not lessen the negative effects of brine salts over a reasonable management timescale. A legacy non-remediated brine spill sites ( $> 60$  year-old) in western North Dakota had a saturated paste electrical conductivity ( $EC_e$ ) value of  $126 \text{ dS m}^{-1}$  (Klaustermeier et al. 2016), which is 30 times higher than what the USDA classifies as saline-sodic soils ( $EC_e$  of  $4 \text{ dSm}^{-1}$ ; USDA 1954). Brine spill remediation through soil treatment techniques such as topsoil excavation (*ex situ*) and leaching with calcium amendments (*in situ*) are used to ameliorate site conditions and increase the success rate of both natural and assisted plant reintroduction towards a predefined state.

Traditional remediation techniques aim to reduce adverse effects on human health or ecological risks associated with brine contamination. Topsoil excavation (*ex situ*) removes the impacted soil to the depth of infiltration and introduces replacement soil. This method permanently reduces the quantity of brine salts but requires a source for the replacement soil. The most common *in situ* method involves divalent ( $\text{Ca}^{2+}$ ) based salts, primarily gypsum ( $\text{CaSO}_4^{2-}$ ) and calcium chloride ( $\text{CaCl}_2$ ), to displace monovalent sodium ( $\text{Na}^+$ ) ions from soil cation exchange sites and move them from upper to lower soil depths (Jong 1982; Halvorson and Lang 1989; Atalay et al. 1999). Differences in soil and vegetation parameters between remediation techniques may play an important role in determining plant community assemblages long after completion of remediation.

Brine spills can create novel ecosystems when remediation techniques do not return soil and plant parameters to pre-spill conditions. Chemical amendments are often preferred over topsoil excavation as they minimize the disruption of the soil structure when reducing soil  $\text{EC}_e$  levels, allowing plant roots to penetrate deeper into the soil profile to extract water and nutrients. However, the application of this technique indiscriminately discharges desirable plant nutrients below the plant root zone where they are inaccessible to plant roots, thus reducing soil fertility and reducing plants ability to propagate these sites. Topsoil excavation invokes a different strategy by rapidly reducing soil contaminants all at once. However, replacement soil has less soil structure due to the multiple disturbances needed to collect, haul, and replace soil. Soil with less structure will influence plant composition and abundance by restricting plant roots from entering soil pores that are smaller than their diameter (Wiersum 1957), resulting in slower root growth (Pardo et al. 2000; Bronick and Lal 2005). The loss of plant productivity can lead to patches of bare soil that are susceptible to erosional processes, reducing soil fertility and

preventing plant establishment and recruitment. Given the variability in performance between remediation techniques, an assessment is necessary to determine how each remediation technique affects soil and plant parameters under a semi-arid climate.

Current remediation practices aim towards reducing the amount of time, money, and energy spent at each contaminated site. However, “fast and effective” do not always complement each other, and sites may require additional follow-up treatments to finalize the remediation process. A formal assessment of these two traditional remediation techniques (leaching with chemical amendments and topsoil excavation) across a variety of ecological sites is necessary to determine which method is more appropriate for the environmental conditions in semi-arid rangelands. The objective of this field study was to compare the efficacy of the two soil remediation techniques (*in situ* & *ex situ*) by evaluating residual brine salts and quantifying plant establishment on remediated brine spill sites on semi-arid rangelands. We hypothesize no difference in soil and plant parameters between reference sites and respective remediation techniques.

### **Materials and Methods**

The study area was located on the Little Missouri National Grasslands (LMNG) in western North Dakota, USA. The LMNG is primarily managed for cattle grazing and wildlife habitat (Reeves et al. 2006), but is experiencing an increase in oil and gas development in the last decade (NDIC-OGD 2016). The dominant soil types in this area include well-drained loams, clay loams, and sandy loams (Setter and Lym 2013). Climate is continental and semi-arid, with mean temperatures ranging from -15 °C in winter to 23 °C during the growing season (April-September), and a mean annual precipitation and potential evaporation of 425 and 3.81 mm, respectively (NDAWN 2016), with 70% of precipitation occurring between May and September.

Historic natural vegetation includes western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths), needle and thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth subsp. *comata*), green needlegrass (*Nassella viridula* (Trin.) Barkworth), prairie junegrass (*Koeleria macrantha* (Ledeb.) Schult.), and threadleaf sedge (*Carex filifolia* Nutt.) (Reeves et al. 2006; Setter and Lym 2013). Plant nomenclature follows the USDA Plants Database (USDA 2016).

We sampled 10 chemical amendment and 11 topsoil excavation sites in the summer of 2015 across seven ecological sites. Sites varied by time since remediation (2007-2014), size ( $14 \times 14 \text{ m}^2$  to  $45 \times 45 \text{ m}^2$ ), and amount of brine split (3 to 690 bbls; Appendix A). We used a paired-plot design, pairing each remediated brine spill site with a nearby reference site similar in soil texture and landscape position. We also compared our reference sites to random sites located within the LMNG to determine if our reference sites represent the current state of the grasslands across sampled ecological sites. These random sites are a more accurate representation of the plant community and productivity of the LMNG because data collection occurred at a minimum distance of 200 m from anthropogenic structures, such as fences, roads, and water sources.

Since brine spills are irregular in size, the number of data frames collected from each site reflected the size of the remediated brine spill area ( $250 \text{ frames/ha}^{-1}$ ). Soil samples were collected at three depths (0-15, 15-30, and 30-60 cm) along two intersecting transects at each site. We analyzed subsamples of remediated and reference sites for topsoil excavation sites at the 0-15 cm depth for soil texture using the pipette method (Black 1965).

Preparation of soil samples followed guidelines for 1:1 soil-to-water suspensions ( $\text{EC}_{1:1}$ ) as described in Handbook 60 of the United States Department of Agriculture (USDA 1954). The  $\text{EC}_{1:1}$  were then converted to saturated paste extract ( $\text{EC}_e$ ) values as described by Klaustermeier

et al. (2016). We converted our  $EC_{1:1}$  to  $EC_e$  because  $EC_e$  naturally simulates saturated soil conditions to predict plants' response to soil salinity (Zhang et al. 2005). Statistical analysis for soil data included one-tailed t-tests at a specified value of zero to compare the mean differences in  $EC_e$  between reference and remediated sites and two-tailed t-tests between remediation techniques.

Visual estimation of plant cover occurred in 1×1 m frame using a modified cover class method (0-1%, 1-2%, 2-5%, 5-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-100%) (Daubenmire 1959) and using the midpoint values for each class for statistical analysis. We harvested and separated plants into native and non-native life form groups (forb, grass, and shrub) in the 1×1 m frame and oven dried (48 °C) them to a constant weight. Statistical analysis included two-tailed t-tests to compare the mean difference between reference and remediated sites, and between remediation techniques to analyze biomass and ground cover parameters. Ground cover parameters included visual estimation of basal and canopy cover of bare ground and litter (plant residue and man placed plant materials). Data analysis included subtracting the mean differences between reference and remediated sites in the same direction each time. Mean differences of values are either positive or negative to reflect changes in plant productivity among ecological sites. We compared plant productivity of paired reference sites with data collected from random sites in the summer of 2013 and 2014 on the LMNG using similar ecological sites. Sites were analyzed using Nonmetric Multidimensional Scaling (NMS) ordination in PC-ORD® vs. 6.0 multivariate statistical software program.

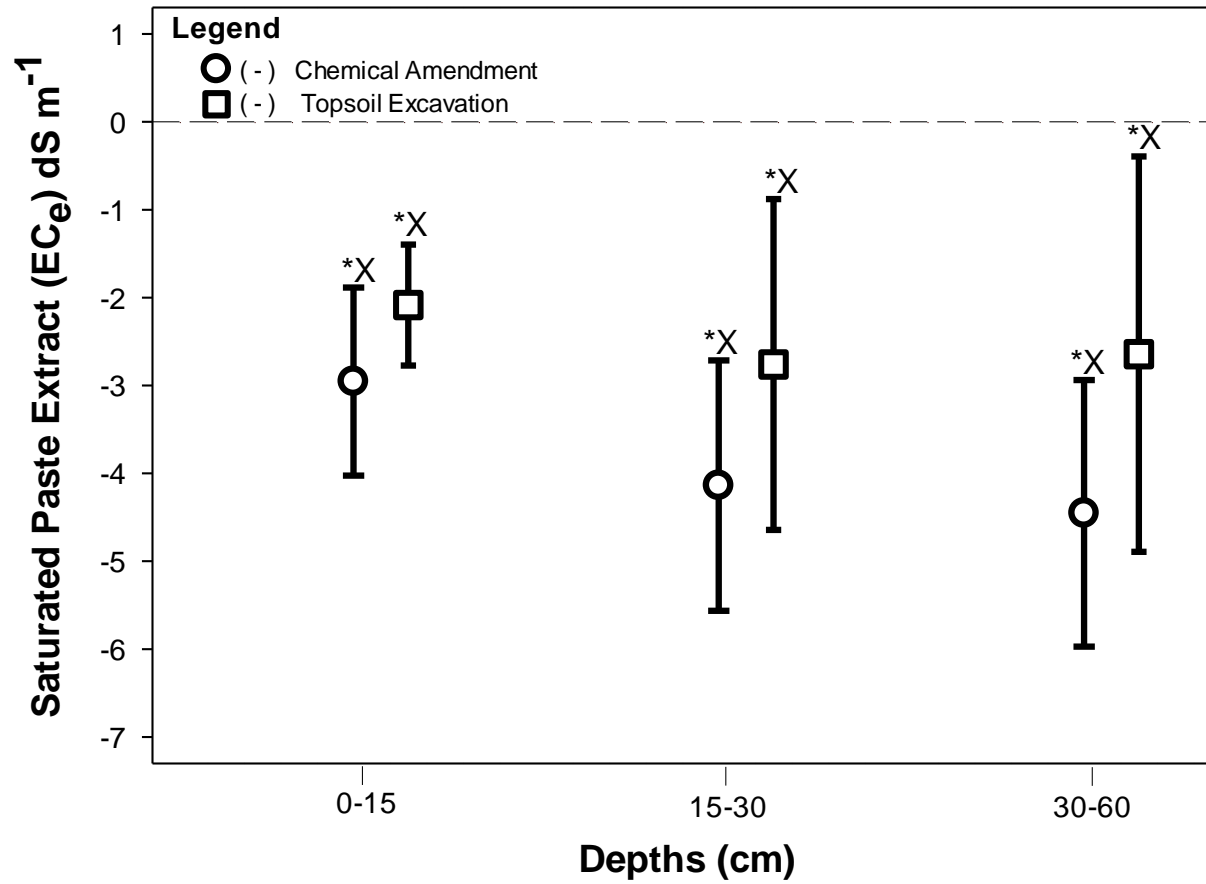
The Relative Sørensen Dissimilarity Index was used to determine contrast differences in species proportions between remediated reference sites (McCune and Grace 2002). We also used

PC-ORD to compare differences between reference and remediated sites using the PerMANOVA function and to compute diversity indices. Statistical analysis of diversity indices and Sørensen dissimilarity index include one-tailed t-tests at a specific value of zero and two-tailed t-tests.

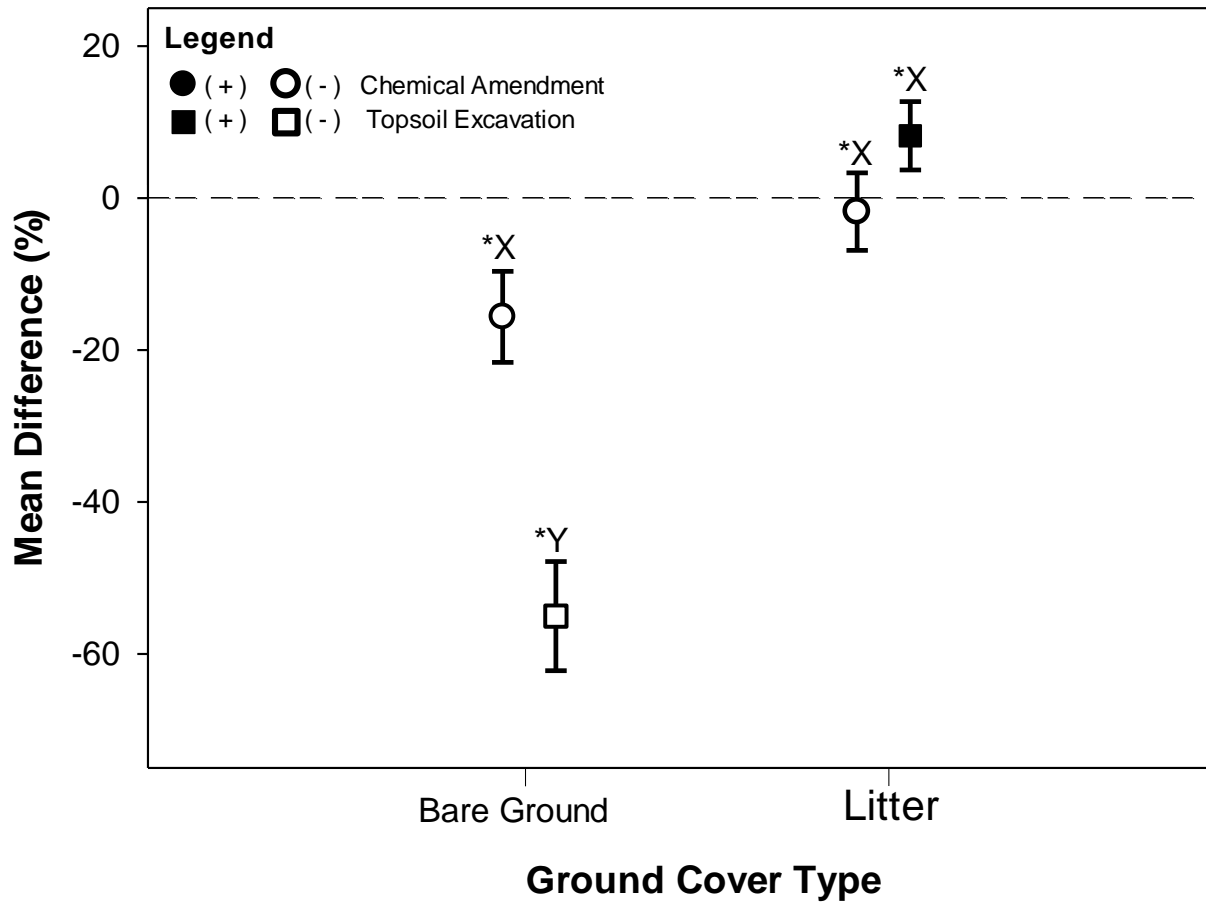
## Results

The mean soil  $EC_e$  difference was not different ( $P \geq 0.05$ ) between remediation techniques (Figure 5) for all three depths. Soil  $EC_e$  had the lowest values for both remediation techniques at the 0-15 cm depth, with chemical amendment sites ( $-2.96 \pm 1.07 \text{ dS m}^{-1}$ ) having a slightly higher  $EC_e$  than topsoil excavation sites ( $-2.08 \pm 0.69 \text{ dS m}^{-1}$ ). Electrical conductivity at the 15-30 cm depth for chemical amendments and topsoil excavation sites were  $-4.14 \pm 1.42$  and  $-2.76 \pm 1.88 \text{ dS m}^{-1}$ , respectively. The highest soil  $EC_e$  for both chemical amendments ( $-4.45 \pm 1.52 \text{ dS m}^{-1}$ ) and topsoil excavation sites ( $-2.64 \pm 2.25 \text{ dS m}^{-1}$ ) occurred at the 30-60 cm depth. The soil  $EC_e$  values were different ( $P < 0.05$ ) between remediated and reference sites for all three depths at the specified value of zero.

The mean percent difference for bare ground was different ( $P < 0.05$ ) between chemical amendment ( $-15.6 \pm 5.99\%$ ) and topsoil excavation sites ( $-55.0 \pm 7.18\%$ ), and between remediated and reference sites (Figure 6). Chemical amendments ( $18.3 \pm 6.19\%$ ) had less bare ground than topsoil excavation ( $58.7 \pm 6.67\%$ ) sites, and was more similar to reference sites ( $2.68 \pm 0.91$  and  $3.62 \pm 1.45\%$ , respectively). Litter was not different ( $P \geq 0.05$ ) between chemical amendment sites ( $-1.77 \pm 5.12\%$ ) and topsoil excavation sites ( $8.22 \pm 2.54\%$ ), but was different ( $p < 0.05$ ) between remediated and reference sites (Figure 6). Litter was higher on chemical amendment ( $13.0 \pm 3.12\%$ ) than on topsoil excavation sites ( $1.54 \pm 0.57\%$ ), and was more similar to reference sites ( $11.2 \pm 2.61$  and  $9.75 \pm 2.53\%$ ).



**Figure 5:** Significant mean difference of soil EC<sub>e</sub> between remediated and reference sites at a specified value of zero are indicated by an asterisk (\*), and between chemical amendment and topsoil excavation techniques (X's) at 0-15, 15-30, and 30-60 cm across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

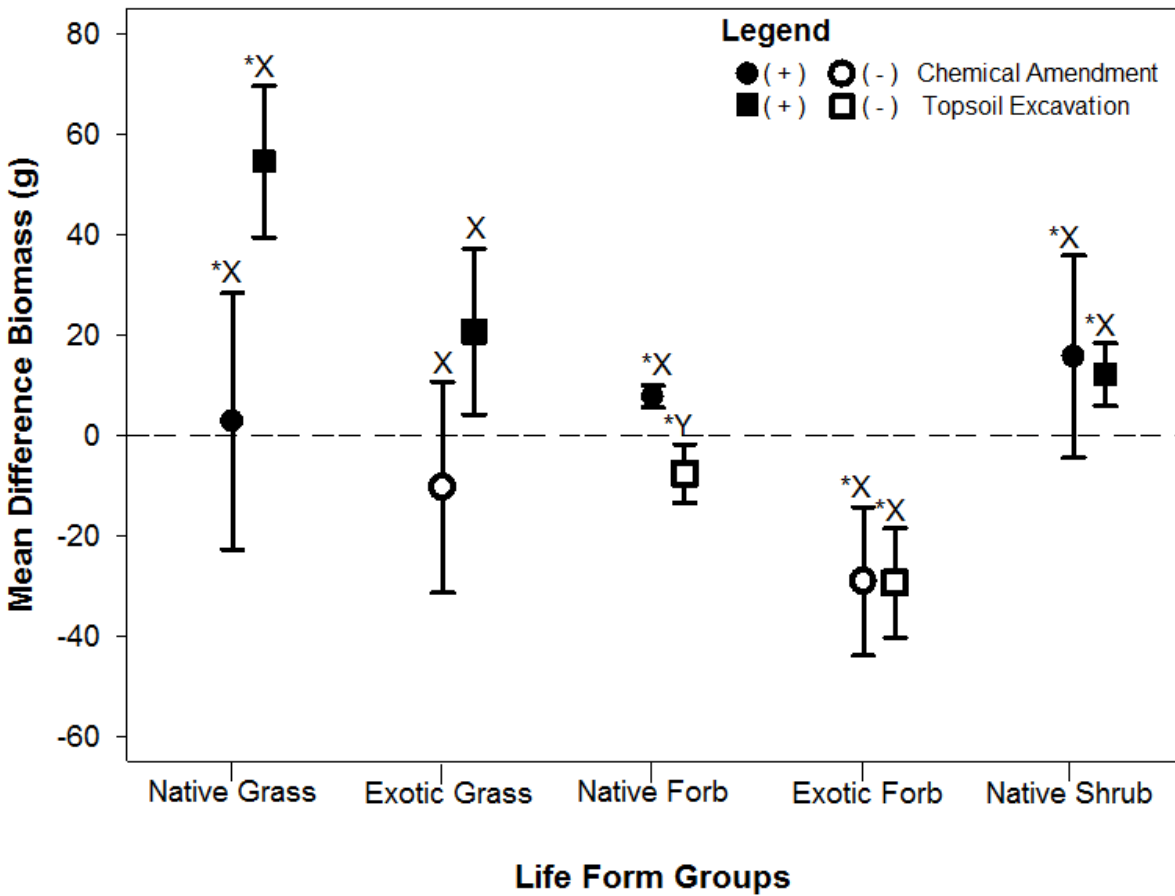


**Figure 6:** Percent mean difference in basal and canopy cover between remediated and reference sites. Significant differences between remediated and reference sites are indicated by an asterisk (\*) and between chemical amendment and topsoil excavation techniques (X's & Y's) for bare ground and litter across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.



Native grass biomass was not different ( $P \geq 0.05$ ) between chemical amendment ( $2.76 \pm 25.5$  g) and topsoil excavation sites ( $54.5 \pm 15.1$  g), but was different ( $P < 0.05$ ) between remediated and reference sites (Figure 7). Native grass biomass was considerably higher on chemical amendment ( $63.8 \pm 34.6$  g) than topsoil excavation ( $10.0 \pm 2.86$  g) sites, as compared to reference ( $60.6 \pm 17.0$  and  $64.5 \pm 16.0$  g) sites. Exotic grass biomass was not different ( $P \geq 0.05$ ) between chemical amendment ( $-10.4 \pm 21.1$  g) and topsoil excavation ( $20.6 \pm 16.5$  g) sites, or between remediated and reference sites (Figure 7). Exotic grass biomass on chemical amendment ( $32.1 \pm 14.4$  g) and topsoil excavation sites ( $14.7 \pm 6.70$  g) were similar to exotic grass biomass on reference sites ( $19.7 \pm 10.0$  and  $35.3 \pm 14.3$  g).

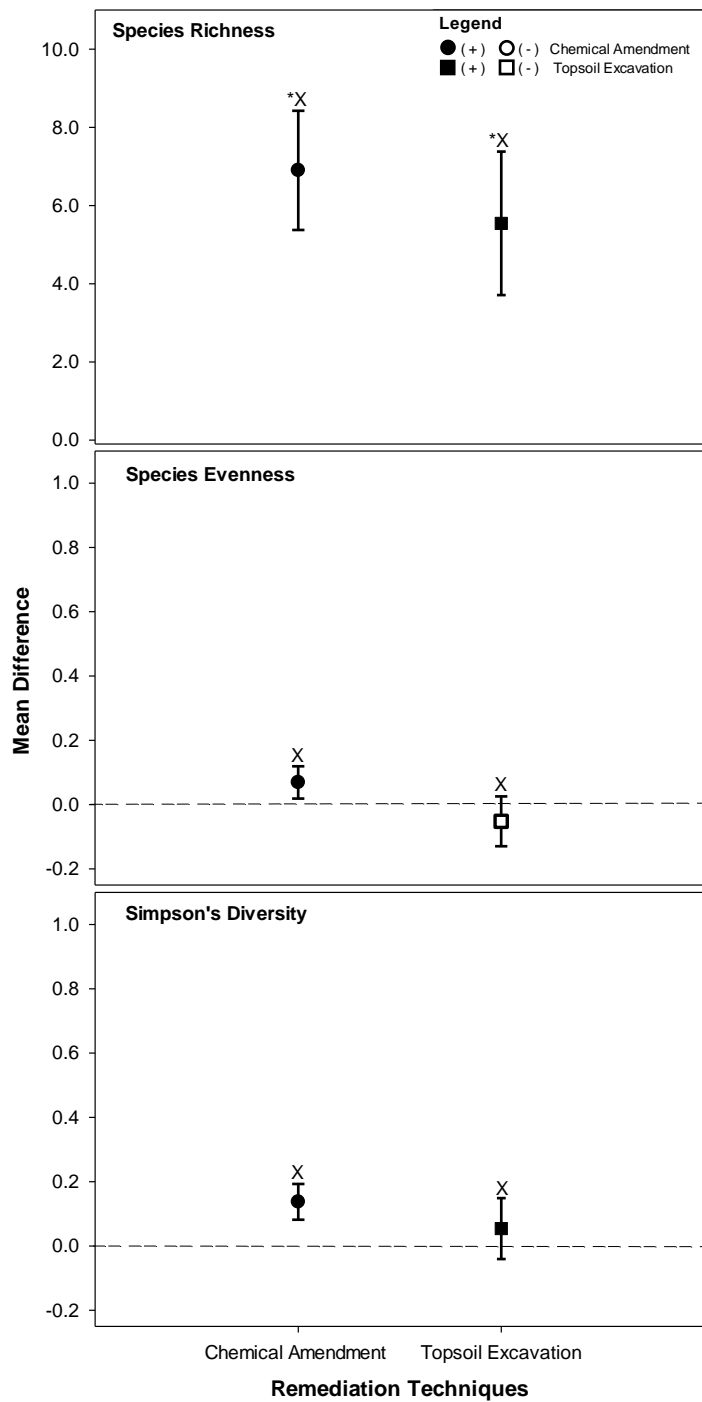
Native forb biomass was different ( $P < 0.05$ ) between chemical amendment ( $7.66 \pm 2.24$  g) and topsoil excavation sites ( $-7.65 \pm 5.85$  g), and between remediated and reference sites (Figure 7). Chemical amendments sites ( $2.29 \pm 0.89$  g) had a lower native forb biomass than topsoil excavation sites ( $13.0 \pm 6.12$  g), with topsoil excavation sites more closely resembling reference sites ( $9.08 \pm 2.17$  and  $5.39 \pm 1.20$  g). Exotic forb biomass was not different ( $P \geq 0.05$ ) between chemical amendment ( $-29.2 \pm 14.8$  g) and topsoil excavation sites ( $-29.5 \pm 10.9$  g), but was different ( $P < 0.05$ ) between remediated and reference sites (Figure 7). Chemical amendment ( $32.4 \pm 15.6$  g) and topsoil excavation sites ( $29.8 \pm 10.9$  g) had higher exotic forb biomass as compared to reference sites ( $3.01 \pm 1.57$  and  $0.31 \pm 0.19$  g).



**Figure 7:** Significant mean differences in biomass between remediated and reference sites are indicated by an asterisk (\*), and between chemical amendment and topsoil excavation techniques (X's & Y's) for native grass, exotic grass, native forb, exotic forb, and native shrub across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

Native shrub biomass was not different ( $P \geq 0.05$ ) between chemical amendment ( $15.6 \pm 20.1$  g) and topsoil excavation sites ( $12.1 \pm 6.25$  g), but was different ( $P < 0.05$ ) between remediated and reference sites (Figure 7). Chemical amendment sites ( $18.5 \pm 10.1$  g) had higher native shrub biomass than topsoil excavation sites ( $2.15 \pm 2.15$  g), with chemical amendment sites more closely resembling native shrub biomass of reference sites ( $31.1 \pm 16.8$  and  $14.2 \pm 5.98$  g).

We observed 140 species across remediated, reference, and random sites. Species richness did not differ ( $P \geq 0.05$ ) between remediation techniques ( $6.90 \pm 1.52$  and  $5.55 \pm 1.84$ ) (Figure 8). However, species richness was different ( $P < 0.05$ ) between remediated and reference sites at a specified value of zero. Species evenness were not different ( $P \geq 0.05$ ) between remediation techniques, or between remediated and reference sites (Figure 8).



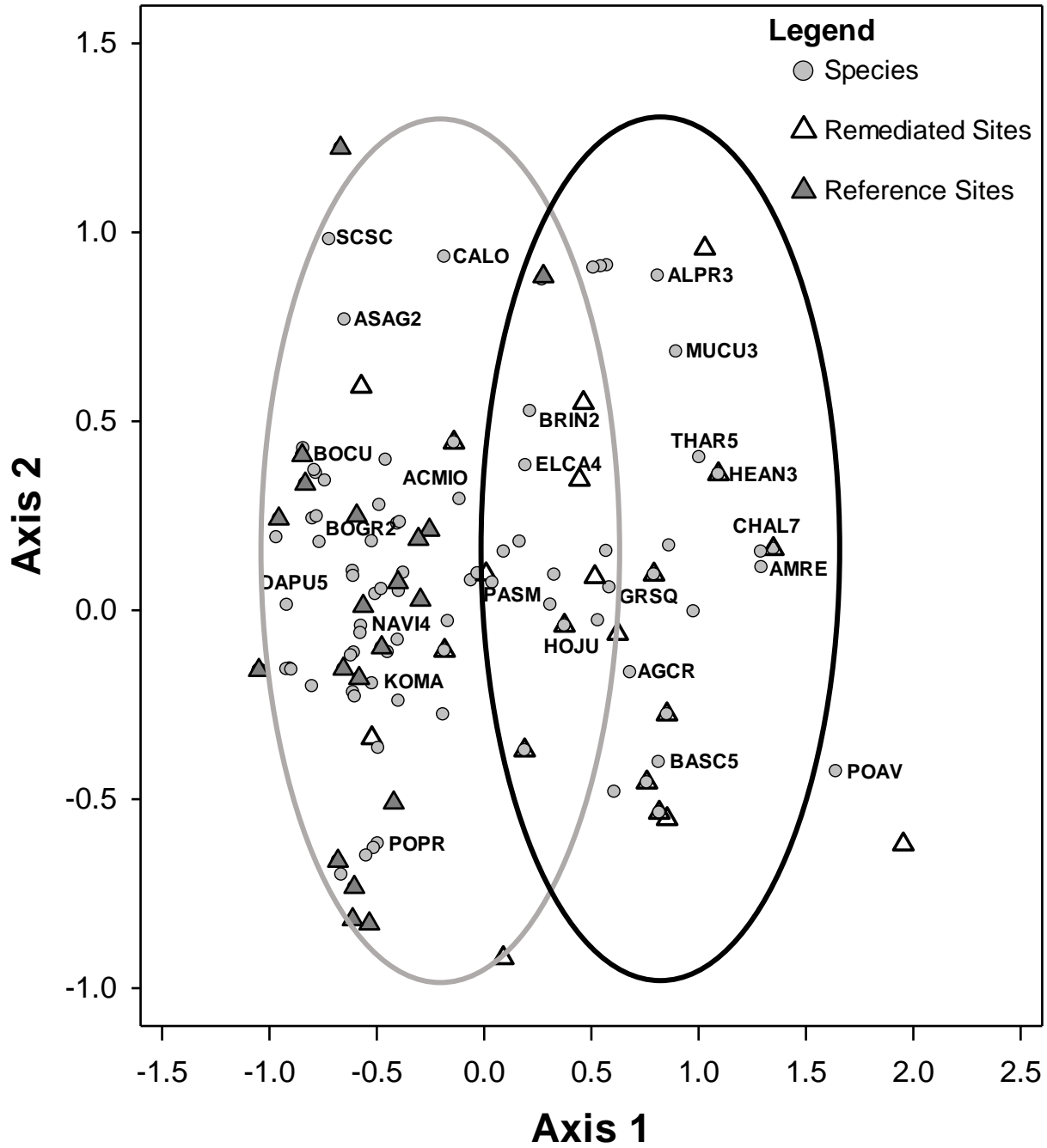
**Figure 8:** Significant mean differences in diversity indices between remediated and reference sites are indicated by an asterisk (\*), and between chemical amendment and topsoil excavation techniques (X's & Y's) for species richness and evenness, and Simpson's diversity across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

The remediated brine spill sites were different ( $P < 0.05$ ) from the reference sites as indicated by the PerMANOVA (Figure 9). The NMS ordination diagram separates Axis 1 and Axis 2, with Axis 1 corresponding to cover of early successional and exotic species across remediated and reference sites (Figure 9). Axis 1 (25%) represents the majority of variation in the dataset, whereas Axis 2 represents 15% and Axis 3 (not shown) represents 11% of the variation in the dataset. The three axes explain 51% of the total variation with a final stress of 14. Nineteen reference sites were located on the left side of the ordination axis, correlating with native grass species such as blue grama, green needlegrass, and prairie junegrass as well as the majority of native forb species. Nine remediated sites were located on the right side of the ordination axis, correlating with ruderal and exotic plant species, such as field pennycress (*Thlaspi arvense* L.), annual sunflower (*Helianthus annuus* L.), wormwood (*Artemisia absinthium* L.), and kochia (*Bassia scoparia* (L.) A.J. Scott). The middle of the ordination where reference (one site) and remediated (eight sites) sites overlap correlated with salt tolerant plant species (e.g. western wheatgrass, foxtail barley (*Hordeum jubatum* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn). Percent dissimilarity was not different ( $P \geq 0.05$ ) between chemical amendment ( $0.76 \pm 0.08$  %) and topsoil excavation sites ( $0.87 \pm 0.04$  %) (Figure 10).

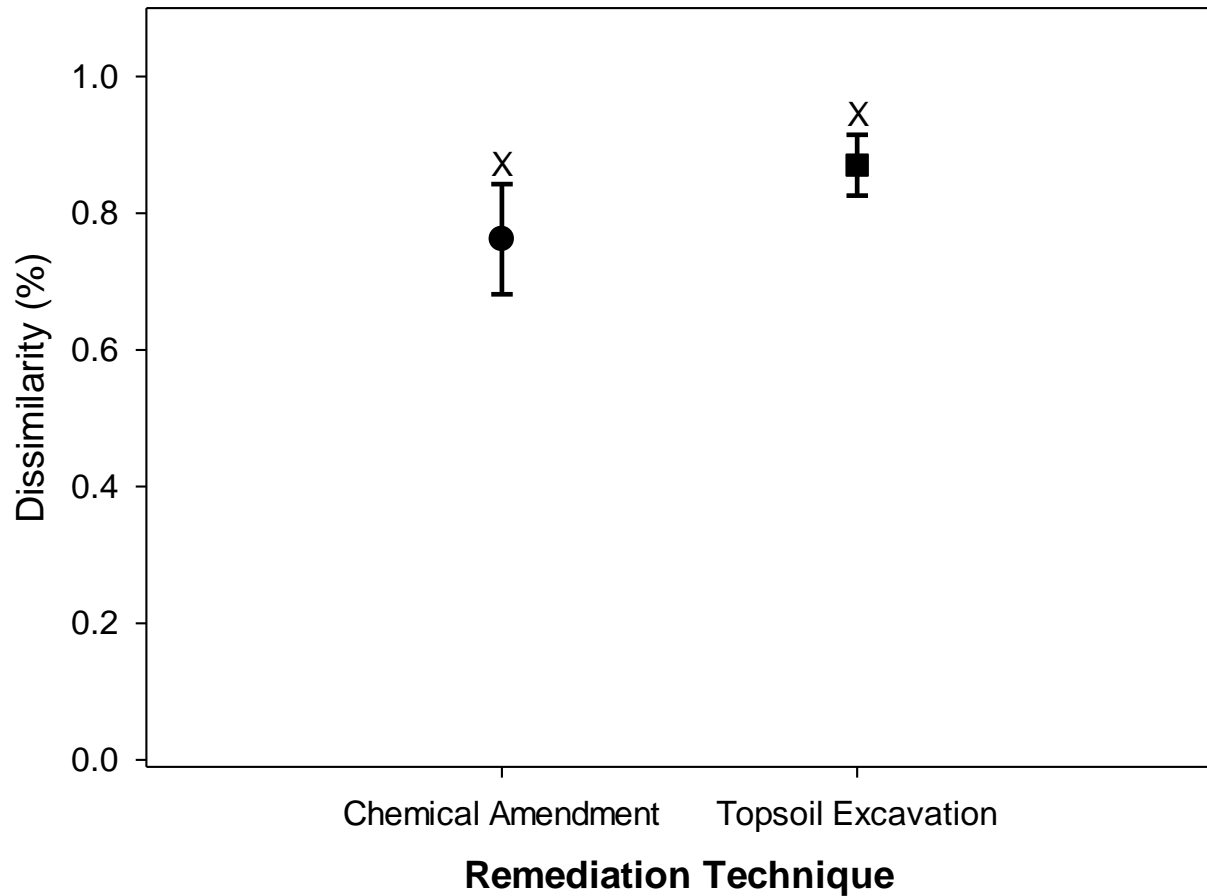
The reference sites were not different ( $P \geq 0.05$ ) from the random sites as indicated by the PerMANOVA (Figure 11). Axis 1 (29%) and Axis 2 (20%) explained almost 50% of the variation of the dataset, with early successional and exotic species corresponding to Axis 1 (Figure 11). The two dimensional solution comprise a final stress of 20 (Figure 11). Forty-two random sites concentrated around native grasses, such as sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.), little bluestem (*Schizachyrium scoparium* (Michx.) Nash), inland saltgrass

(*Distichlis spicata* (L.) Greene), and blue grama as well as the majority of native forb species (Figure 11).

# NMS Ordination



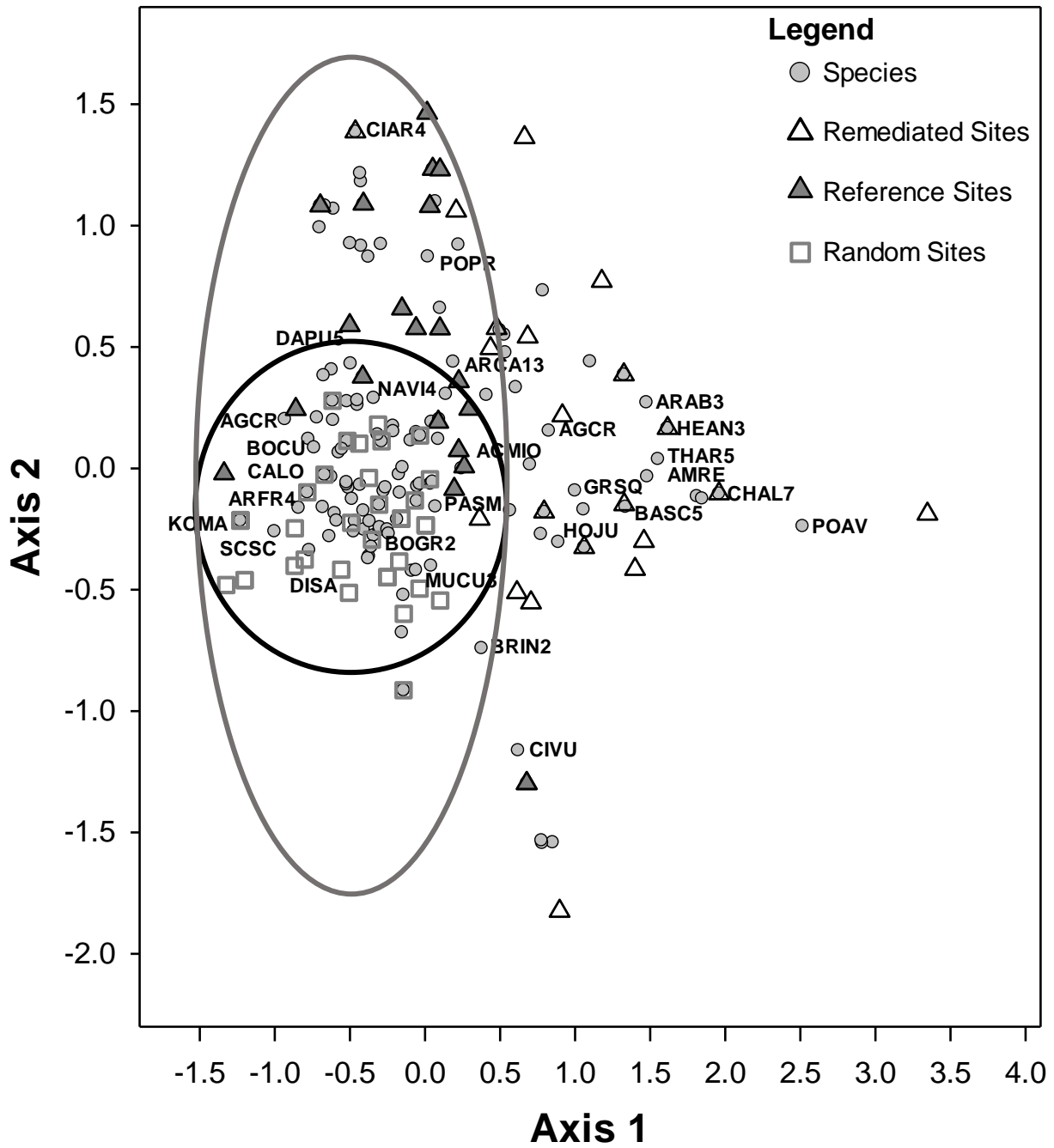
**Figure 9:** Non-metric multidimensional scaling ordination (NMS) displaying species and site scores on axis 1 and axis 2 across seven ecological sites on the Little Missouri National Grasslands in western North Dakota. Ovals circle reference (grey) and remediated (black) sites on the NMS graph. The area where the two ovals overlap include several halophytic species (e.g., PASM, HOJU, and AGCR).



**Figure 10:** Sørensen dissimilarity index comparing plant cover between chemical amendment and topsoil excavation techniques across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.



## NMS Ordination



**Figure 11:** Non-metric multidimensional scaling ordination (NMS) displaying species and sites scores on axis 1 and axis 2 across seven ecological sites on the Little Missouri National Grasslands in western North Dakota. Ovals circle random (left -side) and reference (left-side) sites on the NMS graph.

## Discussion

Discharges of brine onto the soil surface can create novel ecosystems in the absence of remediation techniques. Chemical amendment and topsoil excavation techniques aim to lower the abiotic gradient to levels suitable for plant growth. Therefore, we evaluated residual brine salts, and plant composition and productivity to determine the preferred remediation technique for semi-arid rangelands. The US Forest Service expects that after four years post-remediation of brine-impacted sites, the soil  $EC_e$  levels should be comparable to reference sites and plant community is comprised of 70% native species. Our study confirmed that both remediation techniques were successful at lowering soil  $EC_e$  to facilitate natural and assisted vegetation recovery, however, vegetation on the remediated sites were not similar to reference plant communities.

Since our sampled sites were small ( $\leq 45 \times 45 \text{ m}^2$ ), the bordering edge of remediated and native prairie played an important role in species recruitment and migration. We would expect secondary succession to occur at a faster rate on smaller sites because they are in closer proximity to seed sources of intact plant communities as compared to larger sites that are further away from prairie edges (Prach and Pyšek 2001; Limb et al. 2010).

Vegetation composition and productivity mirrored  $EC_e$  data, with vegetation being similar between remediation techniques, however, vegetation was different between remediated and reference sites. A study examining soil and vegetation on a chemically remediated ( $\text{CaCl}_2$ ) brine spill site in western North Dakota found that native plant establishment was higher on the remediated brine spill portion than on the non-remediated brine spill portion, but was not as diverse as the nearby reference site (Halvorson and Lang 1989). Residual brine salts attributed to differences in vegetation with the lowest  $EC_e$  being on the reference site and highest  $EC_e$  on the

non-remediation portion (Halvorson and Lang 1989). Our results were consistent with other findings that showed chemical amendments coupled with water effectively remediated brine-affected sites (Jong 1982; Halvorson and Lang 1989; Merrill et al.1990). Though chemical amendments were effective in these studies (Jong 1982; Halvorson and Lang 1989; Merrill et al.1990), topsoil excavation is the fastest method for reducing soil contaminants (Steele and Pichtel 1998). However, there is limited research examining soil and plant recovery on *ex situ* remediated brine spill sites. Soil removal and introduction of replacement soil rapidly transforms surface soils from once vegetated areas into bare ground areas (Um and Wright 1999). Our topsoil excavation sites had three times more bare ground than chemical amendment sites because they started with bare substrate.

Life history characteristics of grassland species play an important role in species migration and propagation of disturbed grassland sites (Mcintyre et al. 1995). Succession on bare substrate is likely to start with early successional species, such as ruderals and exotics, because the environmental conditions favor their establishment (Prach and Hobbs 2008). These species usually reproduce by seed and have high dispersal capabilities, which enable them to establish on disturbed soil and take advantage of available resources first (e.g., water, light, nutrients, and space resources), outperforming later successional species (Grime 1977; Prach and Pyšek 2001). Later LMNG successional species (e.g. blue grama, sideoats grama, western wheatgrass, little bluestem, and threadleaf sedge) primarily reproduce by vegetative means (Eriksson and Jakobsson 1998), which allow grass species to fill both above and belowground gaps in grassland communities given sufficient time (Karl et al. 1999). Soil quality tends to drive early succession, whereas nutrient dynamics and life histories drive later succession stages (Gleeson and Tilman 1990). The distinction between these succession phases may explain why native

grasses establishment was six times higher on chemical amendments than on topsoil excavation sites when compared to reference sites.

Natural and/or assisted perennial grass establishment is the first step in revegetating disturbed grassland communities followed by the expected natural migration of native forb species (Romo and Grilz 2002). Interestingly, native forb biomass was six times higher on topsoil excavation sites than on chemical amendment sites when compared to reference sites due to the presence of annual sunflower (*Helianthus annuus L.*) and curlycup gumweed (*Grindelia squarrosa* (Pursh) Dunal) (Limb et al. 2010). These two native annual forb species were not present on chemical amendment sites. Annual sunflower and curlycup gumweed produce greater aboveground biomass than the native forb species found primarily on the chemical amendment sites, which included wooly plantain (*Plantago patagonica* Jacq.), upright prairie coneflower (*Ratibida columnifera* (Nutt.) Wooton & Standl.), purple coneflower (*Dalea purpurea* Vent.), groundplum milkvetch (*Astragalus crassicaarpus* Nutt.), white heath aster (*Sympyotrichum ericoides* (L.) G.L. Nesom), and prairie sagewort (*Artemisia frigida* Willd.). Annual sunflower and curlycup gumweed are better at colonizing contaminated soils as opposed to characteristic LMNG forb species (Olson and Fletcher 2000; Alexander and Schrag 2003).

Native shrub patches, similar to the reproductive strategies of perennial graminoid species, can migrate laterally from prairie edges to colonize disturbed sites (Romo and Grilz 2002). Silver sagebrush (*Artemisia cana* Pursh) and western snowberry (*Symphoricarpos occidentalis* Hook.) comprised 87% of native shrub biomass across all sites. Chemical amendment sites had approximately nine times higher native shrub biomass than topsoil excavation sites when compared to reference sites. Silver sagebrush and western snowberry resprout by vegetative means, enabling these shrubs to occupy both undisturbed and disturbed

sites (Pelton 1953; Wambolt et al. 1990). Similarly, native shrubs can suppress perennial grass establishment and vice versa through competition of plant resources (Köchy and Wilson 2000; Peltzer and Köchy 2001).

Exotic species are highly resilient to human-disturbances and can readily invade sites, leading to potential permanent conversions of plant communities (Stylinski and Allen 1996; DeKeyser et al. 2015). Invasive species, such as crested wheatgrass and Kentucky bluegrass, are notorious invaders of North America grasslands, creating monotypic stands and displacing native prairie species (Henderson and Naeth 2005; DeKeyser et al. 2015). Kentucky bluegrass was present on 17 of the 21 study sites, and comprised 58% of our exotic grass biomass. The presence of Kentucky bluegrass is the reason why there was no difference in exotic grass biomass between remediated and reference sites. Exotic forb biomass on both chemical amendment and topsoil excavation sites were similar due to the presence of kochia (*Bassia scoparia* (L.) A.J. Scott) and Canada thistle (*Cirsium arvense* (L.) Scop.). Kochia and Canada thistle are both considered halophytic species and can germinate and establish under high saline (NaCl) conditions (Evetts and Burnside 1972; Wilson 1979), which enable these species to invade areas where recalcitrant brine salts are present. The presence of exotic species can stymie the reclamation project and make it difficult to reach management objectives. Stylinski and Allen (1996) found that exotic species on excavated and filled disturbed sites in California shrublands prevented plant communities from recovering to their pre-disturbed states (25 years; Stylinski and Allen 1996).

Plant establishment on remediated brine spill sites is a combination of both natural and assisted vegetation. The United States Forest Service (USFS) seed mix used for oil and gas reclamation includes four LMNG species (e.g., western wheatgrass, green needlegrass, prairie

sandreed (*Calamovilfa longifolia* (Hook.) Scribn.), and Canada wildrye). Green needlegrass and prairie sandreed were located on the left side of Axis 1 in closer proximity to reference sites (Figure 9). Western wheatgrass and Canada wildrye were located directly in the middle of Axis 1 and Axis 2 (Figure 9). Ideally, we would expect all four native species from the USFS seed mix to reside in the middle of the ordination. However, a variety of other species propagated our remediated sites indicating that natural revegetation exerted a stronger influence than assisted vegetation on remediated brine spill sites. The results of this study were similar to Robson et al. (2004) observations of natural migration and propagation of plant species such as, foxtail barley, western wheatgrass, kochia, annual sunflower, and curly cup gumweed, on hydrocarbon and brine contaminated sites in a semi-arid grassland.

Remediation helped to restore both the abiotic and biotic component of brine-affected sites in our study, but there are some pitfalls associated with each technique to consider before selecting a final remediation technique. Even though topsoil excavation and introduction of replacement soil does not fix the root of the problem but simply transfers it to a new location. Soil removal is an invasive method that destroys thousands of years of soil development (Bradshaw 1997), which is why *in situ* remediation has a general higher public acceptance among soil and plant ecologists than *ex situ* remediation (Khan et al. 2000). Heavy machinery associated with topsoil excavation can lead to soil compaction (Stylinski and Allen 2000), especially on soil that lacks adequate stability to resist change during mechanical compression (Angers and Caron 1998). Soil compaction further reduces soil porosity for water, gas, and nutrient fluxes to occur (Angers and Caron 1998; Bronick and Lal 2005), and prevent plant roots from penetrating through soil layers (Milchunas et al. 1999; Stylinski and Allen 2000). Replacement soil may also differ in soil properties (e.g., soil texture, organic matter, and nutrient

status) from the surrounding area and yield a different plant community than what previously existed. Soil texture influences plant and soil water relationships and is the primary factor in defining rangeland plant communities (Barnes et al. 1983; Milchunas et al. 1999). Soils dominated with coarser particles (i.e. sand) have larger soil pores and lower water holding capacity to support deep-rooted  $C_4$  species (Barnes and Harrison 1982). Whereas, soils dominated by finer, textured particles, such as silt and clay, have smaller soil pores and display higher water holding capabilities that benefit shallow-rooted  $C_3$  species (Barnes and Harrison 1982).

Soil replacement may also unintentionally contain an exotic seed bank, introducing new species that can germinate and migrate into the surrounding area. Replacement soil from a local or nearby source is desirable because it reduces the likelihood of introducing new species. However, excavation for replacement soil on public land is illegal, requiring replacement soil to come from private sources. Transportation and disposal of brine-contaminated soil can lead to social and ecological challenges, by requiring designated hazardous waste landfills to be within close proximity to excavated sites. The two designated hazardous waste landfills in North Dakota are not equipped to handle the high influx of soil contaminated by oil and gas activities, which has led to the illegal dumping of contaminated soil in municipal landfills. These illegal dumpings could lead to the migration of brine-contaminants from these landfills into adjacent areas. Despite its ecological implications, topsoil excavation is a common remediation technique even though it is an unsustainable method.

Alternatively, chemical amendments rely on intact soil structure to displace  $Na^+$  ions on soil cation exchange sites and replace it with  $Ca^{2+}$  ions. The main pitfalls to chemical amendments is that they are often intensive and ineffective without a supplemental water source

in semi-arid to arid climates (Jury and Weeks 1978). Leached brine salts can also travel upward during periods of dryness, leading to resalinization of surface soil (Thimm 1990; Harris et al. 2005), or perched salt-laden water may travel laterally with the curvature of the landscape and resurface in a new location (Murphy et al. 1988; Harris et al. 2005). However, these pitfalls are less cumbersome than trying to overcome the loss of original soil and/or mixing of soil horizons that occurred during topsoil excavation. Soil structure is important for plant productivity because it influences root distribution (Pardo et al. 2005). Plant roots that are able to penetrate through soil layers to extract water and nutrients from the soil matrix will continue to grow and develop, whereas compacted soil layers restrict plant roots from accessing water and nutrients (Bronick and Lal 2005). Most importantly, both above and belowground plant parts protect soil from erosional hazards, while contributing organic materials via species turnover to improve site conditions and increase soil fertility. We would expect plant succession to occur at a slower rate on soil that lacks adequate soil structure and presents an additional barrier for plants to overcome. Soils that start with bare substrate also tend to be nutrient poor due to lack of nitrogen, which limits plant productivity and is more in line with early succession processes (Gleeson and Tilman 1990). Additionally, external calcium ( $\text{Ca}^{2+}$ ) may facilitate higher potassium ( $\text{K}^+$ ) selectivity over sodium ( $\text{Na}^+$ ) ion uptake in the soil matrix, which can help plants gradually adapt to salt stress ( $\text{NaCl}$ ) (Liu and Zhu 1997).

### **Conclusions**

Successful brine spill remediation removes the majority of brine salts from the plant root zone to facilitate plant establishment. Since natural attenuation in semi-arid to arid areas is not effective, remediation via chemical amendments and topsoil excavation can ameliorate site conditions. Both remediation techniques were successful at lowering  $\text{EC}_e$  levels to allow



vegetation establishment to occur in semi-arid rangelands. Since soil  $EC_e$  was not a delimiting factor between remediation techniques in this study, we believe the use of chemical amendments are the preferable remediation option on small spill sites because it maintains soil integrity and does not generate soil waste. Although there are some pitfalls to this technique, chemical amendments isolate the problem on sites as opposed to topsoil excavation that translocate contaminated soil to a disposal location, where it can potentially have negative effects on the surrounding ecosystems. Efficiency and sustainability are two factors to consider before selecting a remediation technique.

Succession on oil and gas contaminated sites is slow in semi-arid to arid climates, where limited precipitation and hot temperatures can delay plant recovery on remediated brine spill sites. Ruderal and exotic species are likely to dominate remediated brine spill sites during early successional stages, especially on topsoil excavation sites that start with bare substrate. Some of these early successional species help stabilize soil and improve site conditions via species turnover to facilitate the establishment of later successional species. However, the presence of exotic species such as, Kentucky bluegrass, crested wheatgrass, and Canada thistle, can lead to permanent changes in plant cover and prevent the site from returning to pre-spill conditions. Although the act of brine discharged onto the soil surface is usually one discrete event, returning native plant productivity to these remediated brine spill sites is slow and in some cases highly unlikely. Future work should focus on preventing brine spills as opposed to reacting to them.

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## GENERAL CONCLUSIONS

Brine spills can create novel ecosystems in the absence of remediation techniques. Natural attenuation does not lessen the negative impacts of brine overtime in semi-arid to arid regions, at least not on a practical timescale. Limited precipitation in these regions prevents the downward leaching of salt ions below the plant root zone. Human intervention through topsoil excavation and chemical amendments is necessary to remove/lower the amount of brine salts to levels suitable for plant growth. Salt tolerant species (i.e. halophytes) are desirable for revegetating remediated brine spill sites because they are naturally present in the environment and can uptake residual brine salts through plant roots during dry periods when salt-laden water moves from upper to lower soil depths via capillary action to meet atmospheric demand.

Halophytes are adapted to completing their lifecycle in the presence of salt stress, and can desalinate and stabilize soil to restore the biotic component of salt-degraded sites. The results of the greenhouse experiment display the potential of salt sensitive species (i.e. glycophytes) and salt tolerant species (i.e. halophytes) for revegetating remediated sites. Glycophytes (e.g., *Poa pratensis*, *Nassella viridula*, *Bouteloua gracilis*, and *Schizachyrium scoparium*) exhibited lower brine thresholds (9.70 and 18.6 dS m<sup>-1</sup>) than known halophytic species (18.6 and 34.6 dS m<sup>-1</sup>; e.g., *Pascopyrum smithii*, *Hordeum jubatum*, *Puccinellia nuttalliana*, *Distichilis spicata*, and *Sporobolus airoides*). We detected no brine threshold for two halophytes (*Hordeum jubatum* L. and *Puccinellia nuttalliana* (Sault.) Hitchc.) at both growth stages in this greenhouse experiment. Declines in above-and-belowground weights mirrored increases in soil EC<sub>e</sub> concentrations, with lower biomass weights observed at the seedling stage for both halophytes and glycophytes. The results of the greenhouse study aid oil and gas personnel and environmental consultants in identifying EC<sub>e</sub> benchmarks for plant growth. Additionally, the

native halophytes examined in this study are suitable species for revegetating remediated brine spill sites and diversifying oil and gas reclamation seed mixes.

Brine spill remediation techniques may attribute to differences in plant community assembly and affect how sites undergo secondary succession. Our study concluded there was no difference ( $p > 0.05$ ) in soil  $EC_e$  between chemical amendments and topsoil excavation techniques to the 60 cm depth. Bare ground cover was different ( $p < 0.05$ ) between remediation techniques, but litter cover was not different between ( $p \geq 0.05$ ) remediation techniques. Biomass for all functional plant groups with the exception of native forbs was not different ( $p \geq 0.05$ ) between remediation techniques. Species richness and evenness, and Simpson's diversity was not different ( $p \geq 0.05$ ) between remediation techniques. Additionally, the Sørensen Dissimilarity Index was not different ( $p \geq 0.05$ ) between remediation techniques. Exotic species establishment was more prevalent on remediated brine spill sites as indicated by the NMS ordination and PerMANOVA. Native species comprised higher proportions of reference and random sites, random sites were more diverse than reference sites likely because they were further away from oil and gas related disturbances.

Our field study determined that chemical amendments and topsoil excavation are both effective techniques to remove/minimize brine salts across seven ecological sites with different soil properties (e.g., texture, nutrient status, and organic matter). Additionally, our research identifies ruderal and exotic species that are likely to establish on remediated brine spill sites and several invasive species (e.g., *Poa pratensis*, *Agropyron cristatum*, and *Cirsium arvense*) that may stymie the reclamation process. We also discuss some of the pitfalls associated with each remediation techniques so that oil and gas personnel and environmental consultants can

effectively evaluate each remediation technique and choose a remediation technique based on efficacy and sustainability.

## APPENDIX A. CHAPTER 2 TABLES

**Table A1:** List of sampled remediated brine spill sites on the Little Missouri National Grasslands in western North Dakota in the summer of 2015; list includes spill date, cause of spill, quantity of brine spilt (bbls), respective remediation techniques, data of remediation, and if reseeded occurred.

Remediated Site	Spill Date	Cause of Spill	Quantity (bbls)	Remediation Technique	Remediation Date	Re seeding Yes or No
1	11/4/2007	Tank Battery Overflow	60	Chemical Amendment	11/5/2007	No
2	Unk. <sup>†</sup>	Pipeline leak	Unk.	Chemical Amendment	6/14/2012	Yes
3	3/21/2008	Tank Battery Overflow	100	Chemical Amendment	7/22/2008	Yes
4	9/22/2008	Pipeline leak	690	Chemical Amendment	9/22/2008	Yes
5	4/14/2010	Pipeline leak	Unk.	Chemical Amendment	4/16/2010	Yes
6	9/1/2011	Pipeline leak	40	Chemical Amendment	9/2/2011	Yes
7	3/21/2011	Tank Battery Overflow	70	Chemical Amendment	3/28/2011	Yes
8	Unk.	Tank Battery Overflow	Unk.	Chemical Amendment	9/22/2014	No
9	10/23/2013	Pipeline Leak	150	Chemical Amendment	10/26/2013	Yes
10	12/14/2011	Tank Battery Overflow	220	Chemical Amendment	07/21/2011	No
11	12/14/2011	Tank Battery Overflow	Unk.	Topsoil Excavation	07/21/2011	No
12	06/22/2014	Tank Battery Overflow	10	Topsoil Excavation	6/30/2014	Yes
13	10/6/2011	Pipeline leak	3	Topsoil Excavation	10/08/2011	Yes
14	12/17/2011	Pipeline Leak	60	Topsoil Excavation	4/28/2012	Yes
15	8/24/2009	Pipeline Leak	22	Topsoil Excavation	8/27/2009	Yes
	11/26/2008		11		6/02/2009	
	11/26/2008		5		6/02/2009	
16	6/2/2013	Tank Battery Overflow	15	Topsoil Excavation	7/31/2013	Yes
17	4/22/2007	Pipeline Leak	40	Topsoil Excavation	5/01/2007	Yes
	10/7/2009		100		10/09/2009	
	10/31/2013		70		11/05/2013	
	8/18/2014		65		8/19/2014	
18	1/28/2013	Tank Battery Overflow	13	Topsoil Excavation	1/28/2013	Yes
19	11/25/2013	Pipeline Leak	16	Topsoil Excavation	11/27/2013	Yes
20	11/5/2012	Tank Battery Overflow	299	Topsoil Excavation	7/19/2013	Yes
21	10/23/2013	Pipeline Leak	150	Topsoil Excavation	12/01/2015	No

<sup>†</sup>=Unk. =Unknown information

**Table A2:** USDA soil texture classifications for topsoil excavation sites (REM) and the paired reference sites (REF) across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

Site	Percent Sand		Percent Silt		Percent Clay		USDA Textural Class <sup>†</sup>	
	REF <sup>‡</sup>	REM	REF	REM	REF	REM	REF	REM
11	48.0	30.3	32.4	38.7	19.6	31.0	Loam	Clay Loam
12	22.3	20.3	51.3	73.3	26.4	6.4	Silt Loam	Silt Loam
13	5.9	34.1	64.1	32.9	30.0	33.0	Silty Clay Loam	Clay Loam
14	25.4	58.6	52.8	25.2	21.8	16.2	Silt Loam	Sandy Loam
15	13.3	23.6	60.9	50.6	25.8	25.8	Silt Loam	Silt Loam
16	55.1	35.0	32.5	33.0	12.4	32.0	Sandy Loam	Clay Loam
17	5.8	52.5	56.0	28.3	38.2	19.2	Silty Clay Loam	Sandy Loam
18	38.5	13.1	43.9	49.9	17.6	37.0	Loam	Silty Clay Loam
19	10.8	22.5	57.6	53.9	31.6	23.6	Silty Clay Loam	Silt Loam
20	68.2	20.2	24.8	53.6	7.0	26.2	Sandy Loam	Silt Loam
21	40.7	30.5	39.5	47.9	19.8	21.6	Loam	Loam

<sup>†</sup>= Black, 1965

<sup>‡</sup>= REF= Reference site, REM= Remediation site

**Table A3:** Saturated Paste extract (EC<sub>e</sub>) electrical conductivity for topsoil excavation sites (REM) and the paired reference sites (REF) across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

Treatment	Site ID	EC <sub>e</sub> <sup>§</sup> (dS m <sup>-1</sup> )		
		0-15cm	15-30cm	30-60cm
Chemical	REF 1 <sup>‡</sup>	0.69 <sup>†</sup>	2.12	2.73
Amendment	REM 1 <sup>‡</sup>	0.68	0.97	3.19
Chemical	REF 2	1.30	1.15	2.94
Amendment	REM 2	1.58	1.43	1.71
Chemical	REF 3	0.87	0.75	6.21
Amendment	REM 3	1.68	3.68	6.33
Chemical	REF 4	0.94	0.97	3.88
Amendment	REM 4	1.78	2.49	8.39
Chemical	REF 5	0.65	0.54	0.97
Amendment	REM 5	3.91	6.48	8.95
Chemical	REF 6	0.78	0.78	3.54
Amendment	REM 6	8.89	15.2	12.8
Chemical	REF 7	0.88	0.68	0.65
Amendment	REM 7	1.69	1.48	1.83
Chemical	REF 8	0.97	2.43	6.93
Amendment	REM 8	4.93	6.76	8.85
Chemical	REF 9	0.30	0.24	0.21
Amendment	REM 9	2.14	5.31	13.7
Chemical	REF 10	0.90	1.18	2.51
Amendment	REM 10	10.6	8.40	9.40
Topsoil	REF 11	0.90	1.18	2.51
Excavation	REM 11	2.51	4.08	8.34
Topsoil	REF 12	1.41	9.13	18.5
Excavation	REM 12	8.00	18.1	25.6
Topsoil	REF 13	1.23	1.25	6.65
Excavation	REM 13	1.62	3.67	6.77
Topsoil	REF 14	0.46	3.79	27.1
Excavation	REM 14	3.90	5.95	14.5
Topsoil	REF 15	1.86	2.19	8.17
Excavation	REM 15	1.48	3.28	9.15
Topsoil	REF 16	0.31	0.34	0.98
Excavation	REM 16	2.32	5.58	3.14
Topsoil	REF 17	0.85	0.80	1.58
Excavation	REM 17	1.48	1.41	1.34
Topsoil	REF 18	0.85	0.84	0.85
Excavation	REM 18	3.51	6.79	7.13
Topsoil	REF 19	0.85	0.77	1.23
Excavation	REM 19	4.53	2.12	3.33
Topsoil	REF 20	0.38	0.79	3.17
Excavation	REM 20	4.19	13.1	21.6
Topsoil	REF 21	3.63	19.0	28.7
Excavation	REM 21	2.14	6.40	27.6

<sup>§</sup>= Electrical conductivity from a saturated paste extract

<sup>†</sup>= Klaustermeier et al. 2016

<sup>‡</sup>= REF= Reference site, REM= Remediation site

**Table A4:** Biomass and ground cover data for topsoil excavation sites (REM) and the paired reference sites (REF) across seven ecological sites on the Little Missouri National Grasslands in western North Dakota.

Treatment	Site ID	Native Grass	Exotic Grass	Native Forb	Exotic Forb	Native Shrub	Litter	Bare Ground
		-----kg/ha-----					-----%-----	
Chemical Amendment	REF 1 <sup>‡</sup>	473	38.2	123	0.60	110	7.40	0.9
Chemical Amendment	REM 1	80.2	42.4	87.8	4.20	740	9.00	33.5
Chemical Amendment	REF 2	149	123	130	11	204	7.53	6.13
Chemical Amendment	REM 2	5.25	517	6.2	1040	5.65	16.0	7.28
Chemical Amendment	REF 3	965	46.9	76.3	30.4	75.1	2.13	2.13
Chemical Amendment	REM 3	112	867	38.6	130	156	16.6	3.56
Chemical Amendment	REF 4	860	297	28.1	0.00	0.00	11.7	8.97
Chemical Amendment	REM 4	833	2.33	22.13	0.00	6.20	1.2	35.7
Chemical Amendment	REF 5	326	0.00	234	0.00	1853	3.85	0.50
Chemical Amendment	REM 5	1224	212	4.70	0.80	23.6	22.0	0.50
Chemical Amendment	REF 6	967	0.00	16.2	0.00	400	10.0	4.00
Chemical Amendment	REM 6	478	0.00	2.40	492	102	8.60	51.0
Chemical Amendment	REF 7	16.3	99.4	117	129	704	30.0	0.50
Chemical Amendment	REM 7	0.00	162	46.6	205	821	3.63	9.75
Chemical Amendment	REF 8	87.1	1099	55.5	138	23.9	19.8	0.50
Chemical Amendment	REM 8	30.0	1.53	0.60	1365	0.00	0.50	40.4
Chemical Amendment	REF 9	1037	4.30	173	1.30	48.4	6.94	0.63
Chemical Amendment	REM 9	97.6	1338	20.0	4.6	0.00	28.2	0.75
Chemical Amendment	REF 10	1780	453	41.3	18.4	0.00	12.8	2.54
Chemical Amendment	REM 10	3523	62.8	0.00	2.33	0.00	24.2	0.50
Topsoil Excavation	REF 11	1780	453	41.3	18.4	0.00	12.8	2.54
Topsoil Excavation	REM 11	75.1	65.8	615	414	0.00	4.50	41.7
Topsoil Excavation	REF 12	110	858	4.40	2.80	0.00	8.10	0.50
Topsoil Excavation	REM 12	0.20	0.00	0.00	75.4	0.00	0.50	90.4
Topsoil Excavation	REF 13	507	10.0	54.0	0.00	259	1.70	15.2
Topsoil Excavation	REM 13	1116	4950	3248	1458	0.00	0.50	37.0
Topsoil Excavation	REF 14	0	1208	58.8	0.00	42.6	24.0	0.50
Topsoil Excavation	REM 14	23.0	116	0.00	258	0.00	0.50	72.7
Topsoil Excavation	REF 15	1007	53.2	69.8	0.00	174	7.70	1.70
Topsoil Excavation	REM 15	49.0	647	15.4	8.20	237	5.40	12.9
Topsoil Excavation	REF 16	1154	4.30	106	0.00	18.3	1.30	4.85
Topsoil Excavation	REM 16	198	30.2	204	381	0.00	1.20	54.0
Topsoil Excavation	REF 17	301	175	42.6	1.50	683	6.46	0.75
Topsoil Excavation	REM 17	35.3	0.25	0.75	1215	0.00	0.50	57.3
Topsoil Excavation	REF 18	510	24.2	139	0.00	97.2	5.40	0.50
Topsoil Excavation	REM 18	117	5.60	271	614	0.00	1.10	58.7
Topsoil Excavation	REF 19	848	1.10	29.0	0.00	145	6.79	2.50
Topsoil Excavation	REM 19	199	41.7	0.00	155	0.00	0.50	64.6
Topsoil Excavation	REF 20	725	6.63	42.1	12.0	142	6.00	10.3
Topsoil Excavation	REM 20	292	0.00	3.75	0.63	0.00	0.50	73.8
Topsoil Excavation	REF 21	157	1093	6.40	0.00	3.60	27.0	0.50
Topsoil Excavation	REM 21	0.00	217	0.00	8.60	0.00	1.70	82.1

<sup>‡</sup>= REF= Reference site, REM= Remediation site