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Phytoremediation of Saline-Sodic Soils in East Central South Dakota Utilizing Perennial Grass Mixtures

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PHYTOREMEDIATION OF SALINE-SODIC SOILS IN EAST CENTRAL SOUTH DAKOTA UTILIZING PERENNIAL GRASS MIXTURES

BY

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

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This thesis is dedicated to my parents, Chad and Kari, and to my siblings, Austin and Jacqueline.

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ABBREVIATIONS

°C	Degrees in Centigrade
CDT	Central Daylight Time
CH_4	Methane
CH_4-C	Methane-Carbon
CO_2	Carbon Dioxide
CO_2-C	Carbon Dioxide-Carbon
DAT	Days After Treatment
$EC_{1:1}$	Electrical Conductivity 1:1 Soil:Water Dilution
ESP	Exchangeable Sodium Percentage
GHG	Greenhouse Gas
H_2O	Water
ha	Hectare
hr	Hour
N	Nitrogen
N_2O	Nitrous Oxide
N_2O-N	Nitrous Oxide-Nitrogen
NH_3	Ammonia
NH_3-N	Ammonia-Nitrogen
NH_4	Ammonium
NH_4-N	Ammonium-Nitrogen
NO_3	Nitrate
NO_3-N	Nitrate-Nitrogen

pH	$-\log[H^+]$
PLS	Pure live seed
PVC	Polyvinyl Chloride
SAR	Sodium Adsorption Ratio
sec	Second

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ABSTRACT

PHYTOREMEDIATION OF SALINE-SODIC SOILS IN EAST CENTRAL SOUTH DAKOTA UTILIZING PERENNIAL GRASS MIXTURES

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Several decades of above average precipitation in South Dakota has increased the area of saline and sodic soils, which reduce crop yields and inhibit sensitive plant growth. Saline and sodic soils are difficult to remediate using traditional agricultural crops. Establishing salt tolerant perennial species may restore productivity to salt affected areas. Two perennial grass mixtures (mix 1: slender wheatgrass, beardless wildrye; mix 2: slender wheatgrass, western wheatgrass, green wheatgrass, creeping meadow foxtail) were dormant frost seeded along a topographic gradient in Clark Co., SD. Soils were Forman-Cresbard loam and a Cresbard-Cavour loam with surface electrical conductivity ($EC_{1:1}$) that ranged from <0.5 to >15 dS m^{-1} and sodium <400 to >2500 ug g^{-1}. Perennial grass and corn biomass, soil chemical properties, weed cover, and greenhouse gas emissions were quantified in two growing seasons (2018 and 2019). By 2019 perennial grass mixtures and corn reduced soil $EC_{1:1}$ and sodium similarly. Slender wheatgrass was the dominant species in mix 1, comprising up to 65% of September 2018 total biomass (246-1705 kg ha^{-1}) and 83% in 2019 (6400-9700 kg ha^{-1}). AC Saltlander was the dominant species in mix 2 comprising up to 61% of 2018 total September biomass (604-2646 kg ha^{-1}) and 81% in July, 2019 (5853-10663 kg ha^{-1}). In July, 2019 mix 1 and mix 2 saline plots had 16% and 3% weed cover (kochia/foxtail barley), respectively, compared to 75% in corn. Over a 7 d period in July, 2018 and 2019 non-fertilized barren saline soils

emitted 2.09 and 4.89 g N_2O-N ha^{-1} hr^{-1}, respectively; and 611 and 324 g CO_2-C ha^{-1} hr^{-1}, respectively. During the same time period, grass vegetated non-saline soil emitted 0.38 and 0.46 g N_2O-N ha^{-1} hr^{-1} in 2018 and 2019, respectively; and 1589 and 2538 g CO_2-C ha^{-1} hr^{-1}, respectively. Corn vegetated non-saline soil emitted 0.01 and 0.62 g N_2O-N ha^{-1} hr^{-1} in 2018 and 2019, respectively; and 1821 and 1812 g CO_2-C ha^{-1} hr^{-1}, respectively. Urea application (224 kg^{-1} ha^{-1}) increased CO_2 emissions in all treatments both years from 19-155%, but increased N_2O emissions by 102-704% in 2019 only. Simulated root exudates, plant residue decomposition, simulated root respiration, and barley growth increased greenhouse gas emissions compared with nontreated controls in laboratory studies on a saline Cresbard-Cavour loam. Growing barley plants reduced soil $EC_{1:1}$ from 6.3 dS m^{-1} to 5.9 dS m^{-1} and reduced soil NO_3^- from 509 ug g^{-1} to 428 ug g^{-1} after 7 weeks and increased N_2O-N and CO_2-C flux by 224% and 244%, respectively, from baselines of 0.359 ug N_2O-N kg^{-1} hr^{-1} and 206 ug CO_2-C kg^{-1} hr^{-1}. Revegetating salt affected soils with perennial grasses may reduce soil $EC_{1:1}$, NO_3^-, Na, and weed cover and also may improve soil microbial activity and nutrient cycling.

CHAPTER 1: INTRODUCTION TO SOIL SALINITY AND LITERATURE REVIEW

1.1 SOIL SALINITY: PROBLEMS AND CAUSES

Soil salinity and sodicity are important soil properties which, if not managed correctly, can have damaging impacts on crop yield and establishment. Soils in South Dakota are classified as saline when saturated paste electrical conductivity (EC) soil test level is ≥ 4 dS m^{-1} (Clay et al., 2015). Sodic soils are defined as having sodium adsorption ratio (SAR) levels ≥ 4 $mmol_c$ $L^{-0.5}$ (Clay et al., 2015). Saline-sodic soils have EC and SAR values that meet both criteria (Clay et al., 2015).

In recent decades, soil salinity and sodicity have increased in both area affected as well as severity in South Dakota. Approximately 3,442,000 ha of land in South Dakota is impacted by soil salinity and over 2 million ha impacted by sodicity in North Dakota and South Dakota (Millar, 2003; Seelig, 2000). Multiple Northern Great Plains (NGP) states are also impacted by soil salinity, including Minnesota, Nebraska, North Dakota, South Dakota, Montana, and Wyoming with an estimated 10.6 million ha affected (Carlson et al., 2013; Hopkins et al., 2012; Millar, 2003; Seelig, 2000; Soil Survey Staff, 2018).

The area of NGP land impacted by salinity has increased over time. Between 2008 and 2012, 13.4% of corn and soybean ha in eastern South Dakota increased in salinity by at least 4 dS m^{-1} (Kharel, 2016). Beadle, Brown, and Spink counties in SD lose $26.2 million in crop revenue annually due to soil salinity (USDA-NRCS, 2012). In the Red River Valley of ND, losses attributed to soil salinity were estimated at $150 million on 485,000 ha of land (Hadrich, 2011). If the number of affected acres continues to

increase, so will economic losses. The widespread prominence of saline and sodic soils indicates the need for researching management methods to minimize economic and ecological loses.

In order to manage the issue, one must first understand the causes of salinity. The overall underlying cause for soil salinity in the NGP is the soil parent material. However, other factors exacerbate the problem including increased precipitation, temperature, and land conversion (Kharel, 2016).

During the Cretaceous and Paleogene time periods, the Western Interior Seaway covered much of the Great Plain states, including nearly all of South Dakota. When this ocean receded, shale formations high in salt content were left behind, becoming the subsurface parent material for much of the state. In areas where glaciation occurred, this parent material can be very close to the soil surface and is impermeable to water (George, 1978). When the water table rises, it brings soluble salts closer to the soil surface (George, 1978). These salts can travel further up the soil profile through capillary action with higher evaporation increasing salinity in these areas (Seelig, 2000). Therefore, factors that favor water table rise or increase evaporation can increase soil salinity in these at-risk areas.

The Northern Great Plains climate in recent decades has experienced precipitation increases (Seelig, 2000). Between 1980 and 2018, South Dakota had 25 years with above average precipitation and 13 years below average, based on 1901-2000 baseline (NOAA, 2019). During the 1990's eight out of ten years had above average precipitation (NOAA, 2019). This pattern of wetter years has thus brought more soluble salts to the soil surface.

Another factor influencing the salt problem is evaporation due to increased temperature. Like precipitation, South Dakota temperatures have increased. From 1980-2018, 26 of the years had above average mean temperatures during the growing season (May-October) based on 1901-2000 as a baseline (NOAA, 2019). These higher temperatures can increase evaporation rates and increase salinity due to capillary action.

Land use change in the 1800's also contributed to the problem (George, 1978). South Dakota native prairie consisted of mixed annual and perennial grass and forb species. Perennial grasses have large, deep root systems capable of removing water from deep in the soil profile throughout the growing season (George, 1978). This helped keep the water table lower than annual cropping systems which only provide an actively growing plant a few months of the frost-free season. Annual crops also have a limited size root system compared to perennial grasses. Joshi (2018) reported between 2006 and 2014 there were 910,000 ha of grassland converted to cropland. This is 25% more than estimated by Reitsma et al. (2015) who calculated 730,000 ha for the same time period. Between 2006 and 2012, it was found that about 700,000 ha of grassland was converted to cropland in South Dakota and between 2012 and 2014 210,000 ha were converted (Joshi, 2018). This allows more water to percolate to the water table and thus decrease the depth to the water table which carries soluble salts. Additionally, without the yearlong coverage of the soil with perennial species, soil evaporation may increase. Higher evaporation rates increase water movement through capillary action. This capillary action also brings salts up the soil profile similar to a rise in water tables (Seelig, 2000).

When salt levels become too high, plant growth is reduced (Lauchli and Grattan, 2011). When salts are dissolved in the soil-water solution, it reduces the soil osmotic

potential making it more difficult for roots to extract water (Lauchli and Grattan, 2011). Therefore, high levels induce drought-like symptoms in plants and can result in plant death (Seelig, 2000).

Likewise, high sodium (Na) levels in soil also have negative impacts (Davis et al., 2012; Rengasamy and Olsson, 1991). Sodium causes soil particles to disperse rather than aggregate (Davis et al., 2012; Seelig, 2000). This creates a soil with poor water and air permeability, little structure, dense layers, and crusting, all of which inhibit plant growth (Davis et al., 2012; Seelig, 2000; Lauchli and Grattan, 201). Na can also have specific ion effects on plant nutrition, such as preventing adequate uptake of calcium from soil (Lauchli and Grattan, 2011).

1.2 DRAINAGE AND CHEMICAL REMEDIATION STRATEGIES

For areas in the western United States with saline conditions, the recommendation is to install tile drainage and use irrigation with water of a similar EC to leach soluble salts from the profile. This successful western state tactic has been unsuccessful in the Northern Great Plains environment (Davis et al., 2012; Seelig, 2000). In the NGP, there is little irrigation in many regions and locally high sodium levels limit infiltration through the soil profile, which results in tile lines which do not function as intended. There also are limited tile outlets available in South Dakota so off site drainage is not always possible.

Other efforts to remediate saline sodic or sodic fields have included chemical remediation as a first step. Application of calcium (Ca) based amendments, such as gypsum, to the soil is one such tactic (Davis et al., 2012; Seelig, 2000). The added Ca is supposed to replace Na on clay exchange sites, allowing Na to be leached as soil structure

improves (Davis et al., 2012; Seelig, 2000). However, in SD high levels of calcium carbonate are already present in the soil (Franzen et al., 2006). Therefore, elemental sulfur is suggested as an amendment to lower the soil pH and solubilize the free lime (Davis et al., 2012). However, these chemical amendments have limited success.

A study done by Birru (2016) on two South Dakota saline sodic soils found that applications of gypsum (4975kg ha^{-1}) or elemental sulfur (922 kg ha^{-1}) did not impact soil pH or EC. SAR was not impacted by amendments at the White Lake location (Beadle-Dudley complex, Delmont-Talmo complex, and Houdek and Ethan loams), but sulfur reduced SAR at the Redfield location (Harmony-Aberdeen silty clay loam, Winship-Tonka silt loam, and Great Bend-Beotia silt loam) (Birru, 2016).

In a Pakistan study, 25 Mg ha^{-1} gypsum did not affect soil hydraulic conductivity 6 months after application unless plants were grown or 7.5 Mg ha^{-1} residue was added with the gypsum (Ilyas et al., 1993). After one year however, gypsum increased hydraulic conductivity, although weeds growing in the treatment plots may have contributed to this (Ilyas et al., 1993).

Ilyas et al. (1997) applied 25 Mg ha^{-1} of gypsum to a saline sodic fine-loamy, mixed thermic Typic Natrustalf in Pakistan with a SAR of 49 dS m^{-1} at the 0- to 20- cm depth. The authors reported that after one year, gypsum amendment reduced pH to 7.9 compared to 8.5 in non-treated control plots. Differences in SAR were not seen unless perennial alfalfa (*Medicago sativa* L.) or sesbania [*Sesbania bispinosa* (Jacq.)W.F. Wright]) was grown or wheat residue was added to the treated plots at 7.5 Mg ha^{-1} (Ilyas et al., 1997).

Gypsum applied at 5000 kg ha^{-1} and allowed to incubate under laboratory conditions for one-month improved soil chemical conditions by reducing pH from 9.75 to 8.22, EC from 12.35 to 1.98 dS m^{-1}, and exchangeable sodium percentage (ESP) from 44.75 to 6.61% (Hanay et al., 2004). However, soil physical and biological properties were not improved by the gypsum (Hanay et al., 2004). When gypsum was applied to leaching columns at 10 t ha^{-1} to a Hyperthermic Salic Calciorthids, soil EC did not decrease quicker than non-treated soil (Khosla et al., 1979). The SAR however, decreased quicker with gypsum, but the non-gypsum treated soil had similar results when an additional 16 cm of leaching water was added (Khosla et al., 1979). Gypsum was not required for sodium or salt leaching but may quicken the process in heavy textured soils with low infiltration (Khosla et al., 1979).

An 11-week field study in Pakistan applied 12,700 kg ha^{-1} gypsum on a sandy clay loam saline sodic soil (Arshad et al., 2015). The gypsum reduced pH from 8.55 to 7.23 compared to the control of 7.62 in the top 15-cm of the profile. EC was lowered from 8.72 dS m^{-1} to 4.15 dS m^{-1} compared to the control of 8.2 dS m^{-1} and SAR was lowered from 12.53 to 2.75 compared to the control of 11.39 (Arshad et al., 2015).

At the Soil Salinity Research Institute in Pindi Bhattian (Pakistan), the application of gypsum at the required rate equivalent to the total CO_3 and HCO_3 in the soil did not impact EC but decreased pH from 8.94 to 8.5 after 30 days (Hussain et al., 2001). The gypsum also decreased SAR from 40.66 to 27.27 (Hussain et al., 2001).

Gypsum was applied to a Halic Camborthid sandy clay loam soil in leaching columns, which were leached four times over a year (Qadir et al., 1996a). Gypsum treated soil removed more Na (1884 $mmol_c$) compared to the control (393 $mmol_c$) (Qadir

et al., 1996a). Gypsum application also reduced EC from 4.3 dS m^{-1} to 1.3 dS m^{-1}, SAR was reduced from 42.3 to 12.1, and pH was reduced from 9.0 to 8.8 (Qadir et al., 1996a).

Although chemical amendments can be successful in some cases, there are known problems with gypsum applications in the north-central region of the United States. First, fields may already have high amount of free lime in the soil, thus adding more calcium may not be effective (Franzen et al., 2006). Many soils in the Northern Great Plains also have naturally high amounts of gypsum in the soil, making the small amounts of added gypsum insignificant (Franzen et al., 2006). Additionally, soil structure takes time to rebuild and could take decades for the sodium to leach out in certain field conditions unless tile drainage is also installed, which is only an option if outlets are available and water can infiltrate the soil. Also, in many of these studies, the soil amendments are tilled into the soil for maximum effectiveness. Forty-five percent of SD fields are in no till management, meaning the practice would have to be abandoned for gypsum to be incorporated (Franzen et al., 2006; USDA-NRCS, 2017; Sharma et al., 1974). Additionally, many of the successful studies were done in Pakistan or the Western United States in areas under irrigation. The studies done in SD show no benefit of applying gypsum on sodic soils in the NGP (Birru, 2016).

1.3 SPECIES SALT TOLERANCE

Phytoremediation is another proposed method to restore productivity to salt affected soils. However, selecting tolerant species is important in order for this strategy to be successful. Tolerant species are able to grow in soil with high salt content for several reasons. These can include the formation of compatible solutes, ion absorption, compartmentalization, formation of antioxidants, and genetics (Liang et al., 2018; Parida

and Das, 2005). Salt tolerant plants are better adapted in one or several of these tactics of tolerance than sensitive plants.

Compatible solutes are substances formed by the plant that do not interfere with normal plant functions but are used to alleviate the osmotic differential between the plant cells and soil solution due to salinity and thus allow water uptake by roots (Liang et al., 2018; Parida and Das, 2005). These substances include proline, sugars, betaine, and glycine (Liang et al., 2018; Parida and Das, 2005).

In cells, high levels of Na can be toxic, so maintaining high K^+/Na^+ ratios is another trait important for salt tolerance when dealing with higher Na levels in the intracellular region (Liang et al., 2018; Parida and Das, 2005). Some species store excess Na in the cell vacuole to mitigate cell damage (Liang et al., 2018). During salinity stress, there is an increase in reactive oxygen species (ROS). These can include hydrogen peroxide, singlet oxygen, and hydroxyl radicals (Parida and Das, 2005). It is important for plants to form the proper enzymes to remove ROS (Liang et al., 2018; Parida and Das, 2005). All of these tolerance abilities are controlled by a multitude of genes in plants, and when upregulated these genes enable some species to be more salt tolerant than others (Liang et al., 2018).

Selecting species to plant on saline fields depends on the degree of salinity, as different species are impacted at different levels of salinity. Different tolerance levels are reported in the literature, although the tolerance may be method specific, and often has high variance. Corn, a dominant crop in South Dakota, is considered to be moderately salt tolerant of EC up to 4 dS m^{-1} (Alberta Agriculture and Rural Development, 2001). Although some reports have threshold levels of 1.7 dS m^{-1} with a 12% yield decrease

with each unit increase in EC, making corn a moderately sensitive crop (Grieve et al., 2012). Corn also has 50% germination reduction between EC levels of 6-10 dS m^{-1} (Allison et al., 1954). Barley (*Hordeum vulgare*) in comparison, is rarely planted in SD due to low commodity prices but is rated as tolerant up to 8 dS m^{-1} with a 5% yield decrease with each EC unit increase (Alberta Agriculture and Rural Development, 2001; Grieve et al., 2012). Barley has 50% germination reductions at EC levels at 16 dS m^{-1} making it much more salt tolerant that corn (Allison et al., 1954). Slender wheatgrass (*Elymus trachycaulus*), a perennial grass, is rated tolerant at EC levels up to 16 dS m^{-1} (Alberta Agriculture and Rural Development, 2001) but growth can be highly variable based on soils and locations (Tilley et al., 2011). Beardless wildrye (*Leymus triticoides*) can tolerate EC levels up to 20 dS m^{-1} as reported by Alberta Agriculture and Rural Development (2001) but may also only tolerate EC levels up to 2.7 dS m^{-1} and a 6% decrease in yield with each unit increase in EC depending on soils (Grieve et al., 2012). The Natural Resources Conservation Service (NRCS) rates beardless wildrye for EC tolerances in excess of 15 dS m^{-1} (Young-Matthews and Winslow, 2010). Western wheatgrass (*Pascopyrum smithii*) has 50% germination reduction at EC levels between 12-18 dS m^{-1} (Allison et al., 1954). Creeping meadow foxtail (*Alopecurus arundinaceus*) has EC tolerances up to 12 dS m^{-1} according to the NRCS and green wheatgrass (*Elymus hoffmannii*) is rated tolerant to EC levels up to 12.9 dS m^{-1} (Hybner et al., 2014; Tilley et al., 2004).

1.4 GREENHOUSE GAS EMISSIONS IN AGRICULTURE

Greenhouse gases (GHG) are defined by the Environmental Protection Agency (EPA) as “gases that trap heat in the atmosphere” (EPA, 2018) and by Merriam-Webster

as “any of various gaseous compounds (such as carbon dioxide or methane) that absorb infrared radiation, trap heat in the atmosphere, and contribute to the greenhouse effect” (Merriam-Webster, 2019). The primary greenhouse gases that contribute to the greenhouse effect and are monitored include carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (EPA, 2018).

CO_2 is produced from soil primarily through biological activity, both from plant and microbial respiration (USDA-NRCS, 2019). Because of this, CO_2 is often used as an index of soil microbial activity and to some extent microbial populations (USDA-NRCS, 2019). CO_2 from soil respiration is strongly related to temperature, where a two-fold increase in respiration occurs with every 10 °C increase in temperature, up to 35 °C (USDA-NRCS, 2019). Because CO_2 is driven by microbial and plant respiration, which are indicators of soil health, it would not be ideal to attempt reducing GHG emissions from agriculture by focusing on CO_2 emissions from soil sources alone.

N_2O has a global warming potential (GWP) of 298, partly because N_2O lasts 114 years in the atmosphere (EPA, 2018; IPCC, 2001). In the United States, 77% of all N_2O emissions are from agricultural soils, with an additional 5% from manure (EPA, 2018). N_2O is produced from soil by several processes, with the primary contributors being nitrification and denitrification (Davidson et al., 1986; Hu et al., 2015; Stevens et al., 1997, Liu et al., 2016). A major factor determining the source of N_2O production in soil is water filled pore space (WFPS). When WFPS is 30-70%, the primary contributor to N_2O production is nitrification and when WFPS is 80-90% denitrification dominates (Hu et al., 2015; Braker and Conrad, 2011; Huang et al., 2015).

Nitrification forms N_2O through several pathways. One pathway is through the chemical decomposition of hydroxylamine (Butterbach-Bahl et al., 2013). Hydroxylamine is formed as an intermediate product of nitrification (Bremner, 1997). When soil pH is acidic to neutral, hydroxylamine oxidation produces N_2O, whereas when pH is higher (7.8-8.2) the main product is N_2 gas (Bremner et al., 1980). Nitrification can also produce N_2O when oxygen is limited (eg. high WFPS) and there is an elevated level of NO_2^- in the soil which is then used as an alternate electron acceptor (Khalil et al., 2004; Snyder et al., 2009). During this process, NO_2^- is converted to NO and subsequently N_2O. Denitrification produces N_2O under anaerobic conditions by facultative anaerobic bacteria when nitrate NO_3^- is converted to nitrite NO_2^- and finally to N_2O or N_2 (Snyder et al., 2009).

Methane (CH_4) has a GWP of 25, meaning CH_4 is 25 times more potent than an equivalent amount of CO_2 (EPA, 2018; IPCC, 2001). Soils can either be a sink or source of CH_4 depending on soil conditions. Because CH_4 is the most reduced form of carbon, it is only produced in soils under highly reducing conditions with very limited oxygen (Topp and Pattey, 1997). This could be the case in SD saline-sodic soils where soil is dispersed and often saturated forming reducing conditions. CH_4 is produced by methanogen bacteria in the absence of oxygen by reducing oxidized forms of carbon to CH_4 as a source of energy when soils have very low levels of nitrate, sulfate, and ferric iron (Topp and Pattey, 1997). Because of these conditions needed for CH_4 production in soil, the main source of CH_4 is wetlands or rice fields where the soils are saturated for long periods of time (Lelieveld et al., 1993). In South Dakota due to high water tables in

ditches and drainage ways near saline-sodic areas, these conditions may occur for most of the spring and early summer.

Although the largest sink for methane is the atmosphere, where it reacts with the hydroxyl radical, soils account for 10% of the total CH_4 sinks across the globe, fixing approximately 30±25 Tg yr^{-1} of CH_4 (Lelieveld et al., 1993). CH_4 is removed from the atmosphere through soil due to methanotrophic bacteria. Methanotrophic bacteria obtain energy by oxidizing reduced forms of carbon such as CH_4 into CO_2 (Topp and Pattey, 1997). During this process, oxygen is required for use as the terminal electron acceptor, meaning methanotrophic bacteria only oxidize CH_4 under aerobic conditions, typically near the air-soil interface at the soil surface (Topp and Pattey, 1997). Because of the requirement of oxygen for soil to act as a sink for CH_4, soil structure and moisture have a large impact on CH_4 emissions. If soil has good porosity, structure, and drainage, the soil will likely act as a sink (Dunfield et al., 1995). However, if a soil is compacted and saturated it will have a limited ability to remove CH_4 and in some cases may emit CH_4 (Dunfield et al., 1995).

Temperature also influences CH_4 fluxes. Methanogen bacteria are more sensitive to temperature fluctuation than methanotrophs (Dunfield et al., 1993). Therefore, soils which typically act as a sink for CH_4 have little fluctuation in flux throughout the day; whereas methanogenesis will typically occur during the warmest time of day and be reduced during cooler temperatures (Dunfield et al., 1993; Mosier et al., 1991). The balance between soil moisture and temperature thus determines if CH_4 is released from or removed by the soil.

1.5 SOIL GREENHOUSE GAS EMISSIONS AND UREA FERTILIZER IMPACT

Urea application to soil has several known impacts on GHG emissions. Urea is broken down in soil by the urease enzyme (Sigurdarson et al., 2018). This enzyme hydrolyzes urea into two ammonia molecules and carbonic acid which is broken into water and carbon dioxide (Sigurdarson et al., 2018). Because of hydrolysis, urea application increases CO_2 emissions. Urea also increases CO_2 indirectly by providing nitrogen to soil microbes and plants for growth, which release CO_2 through respiration. Nitrogen fertilizers, including urea, have also been shown to inhibit CH_4 oxidation in soil (Mosier et al., 1991; Bosse et al., 1993; Hansen et al., 1993; Hutsch et al., 1993; Bronson and Mosier, 1994; Dunfield et al., 1995; Dunfield and Knowles, 1995). This phenomenon is thought to be the result of two reactions. First, ammonia that is released from urea is oxidized by the enzyme methane monooxygenase (MMO) into hydroxylamine, then to nitrite and nitrous oxide (Bedard and Knowles, 1989). MMO is the same enzyme used by methanotrophs to oxidize methane, thus when there is a sudden increase in ammonia there is a reduction in available MMO for methanotrophs (Bedard and Knowles, 1989). Second, during this previously mentioned process, the nitrite formed may be toxic to methanotrophs and reduce their ability to oxidize methane by inhibiting the formate dehydrogenase enzyme (Jollie and Lipscomb, 1990; King and Schnell, 1994). The lack of formate oxidation then results in a shortage of reductant for MMO and thus inhibition of methane oxidation (Jollie and Lipscomb, 1990; King and Schnell, 1994; Topp and Pattey, 1997). The end result of these processes means soil treated with urea fertilizer may not be an efficient CH_4 sink a short period of time following application.

Perhaps the largest impact urea is on N_2O emissions. As previously mentioned, urea hydrolyzes into NH_3 in the soil which equilibrates with NH_4^+ depending upon soil pH (Sigurdarson et al.,2018). This NH_3 then undergoes nitrification by soil bacteria from *Nitrosomonas* and *Nitrobacter* species to form NO_3^- (Signor and Cerri, 2013). During the process of nitrification, N_2O gas is released into the atmosphere. Although usually in small amounts, losses can be higher when there is higher soil moisture or platy soil structure (Signor and Cerri, 2013). Likewise, the increase in soil NO_3^- from urea increases the available soil N for N_2O losses due to denitrification (Bremner, 1997).

Although little research has been done on GHG emissions from saline soils in SD, there is extensive publications on GHG emissions from other soil types in the region as well as limited studies on saline soils in other parts of the world. CO_2 emissions from a non-saline Brandt silty clay loam soil averaged 1318 g ha^{-1} hr^{-1} over a 7-day study based on the mean of measurements taken 6 times per day in mid-June (Thies, 2018). When urea was applied at 224 kg ha^{-1} CO_2-C emissions decreased by 30% to 1010 g ha^{-1} hr^{-1} (Thies, 2018). Soil N_2O-N emissions averaged 0.81 g ha^{-1} hr^{-1} with no urea and decreased to 0.35 g ha^{-1} hr^{-1} when urea was added (Thies, 2018).

A continuous wheat field had mean CO_2 emissions of 488 g ha^{-1} hr^{-1}, N_2O emissions of 0.2 g ha^{-1} hr^{-1}, and CH_4 emissions of 0.06 g ha^{-1} hr^{-1} when averaged over the growing season from mid-June through mid-October (Lai, 2017). A long-term study done in Mandan, ND on a Werner–Sen–Chama complex soil reported that a grazed western wheatgrass [*Pascopyrum smithii* (Rybd) Love] prairie had peak flux rates of 2542 g CO_2-C ha^{-1} hr^{-1} when averaged over multiple years, and a non-grazed mixed grass prairie had an average peak flux rate of 2417 g CO_2-C ha^{-1} hr^{-1} (Frank et al., 2002). Another

experiment in Nesson Valley, ND reported a Lihen sandy loam no-till field planted with malt barley (*Hordeum vulgaris* L.) had a flux of 6042 g CO_2-C ha^{-1} hr^{-1} with no nitrogen applied and 6833 g CO_2-C ha^{-1} hr^{-1} when 64 kg N ha^{-1} was applied when averaged from May to November (Sainju et al., 2008). In Rasmussen, MT on a Williams loam the mean flux from May through November was 3158 g CO_2-C ha^{-1} hr^{-1} when 78 kg N ha^{-1} was applied (Sainju et al., 2008). During the same time period, in a no till fallow field at the Montana site with no nitrogen applied the flux was 1567 g CO_2-C ha^{-1} hr^{-1} (Sainju et al., 2008).

A laboratory study done on saline-sodic soils from Australia reported CO_2-C emissions were negatively correlated with soil EC, SAR, and bulk density (Setia et al., 2011). The mean CO_2 emissions of 0.47 g CO_2-C kg $soil^{-1}$ with saline soils at an $EC_{1:5}$ of 2.5 dS m^{-1} was ~50% lower than a non-saline soil with $EC_{1:5}$ of 0.5 dS m^{-1} (Setia et al., 2011).

Methane is another GHG that also can be emitted from soils, although usually at very low levels. A study done by Le Mer and Roger (2001) reported aerobic upland soils are mainly a sink for CH_4 and rarely have fluxes higher than 1 g CH_4 ha^{-1} hr^{-1}. Orthic Black Chernozemic Loam near Saskatoon, Saskatchewan, Canada had a flux of 0.27 g CH_4 ha^{-1} hr^{-1} in July after a 79 mm rain event (Wang and Bettany, 1995). A fine sandy loam in Mandan, ND with switchgrass (*Panicum virgatum* L.) vegetation and 67 kg ha^{-1} N fertilizer had mean peak emissions of 620-2500 g ha^{-1} hr^{-1} for CO_2, 0.01-0.36 g ha^{-1} hr^{-1} for CO_2 equivalent of N_2O, and -0.16-0.1 g ha^{-1} hr^{-1} for CO_2 equivalent of CH_4 (Schmer et al., 2012). Nitrogen fertilizer addition increased CO_2 hourly peaks, but not cumulative growing season totals; whereas it did increase season long cumulative CO_2 equivalent

N_2O emissions from 27600 g ha^{-1} in non-fertilized plots to 86300 g ha^{-1} in fertilized plots (Schmer et al., 2012).

A study conducted in Bozeman, MT on an Amsterdam silt loam reported that a perennial grass-alfalfa (*Medicago sativa* L.) mix averaged 0.014 g N_2O-N ha^{-1} h^{-1} over the year (Dusenbury et al., 2008). Nitrogen additions increased cumulative N_2O-N emissions in a wheat-wheat no till system from 145 kg N_2O-N ha^{-1} yr^{-1} to 656 kg N_2O-N ha^{-1} yr^{-1} when 245 kg N ha^{-1} was applied (Dusenbury et al., 2008). A laboratory study done on a sandy loam soil with an EC of 2.3 and a pH of 8.5 from Lake Texacoco, Mexico reported urea additions of 200 mg N kg^{-1} dry soil increased CO_2 emissions by 170% and N_2O emissions by ~765% when compared to nonamended soils at 100% water filled pore space (WFPS) (Silva et al., 2008).

A review of 25 studies concluded no-till fields with poor aeration had higher N_2O emissions when compared to tilled soils by 2 kg N_2O-N ha^{-1}, whereas there was little difference in no-till fields with good or medium aeration (Rochette, 2008). These results suggest that saline sodic areas, which have poor aeration due to soil dispersion, may have high N_2O emissions after nitrogen fertilizer addition.

1.6 PLANT-SOIL INTERACTION AND SALINITY IMPACT ON SOIL CHEMICAL PROPERTIES AND GREENHOUSE GAS EMISSIONS

Impact of Root Exudates on Soils

Root exudates are an important component of plants because they are a direct link between the plant and the soil rhizosphere. Root exudates are compounds such as saccharides, amino acids, organic acids, and secondary metabolites (proteins) released by the plant roots into the rhizosphere for the purpose of nutrient uptake, chemical signaling,

promotion of microbial growth among numerous other functions (Gargallo-Garriga et al., 2018; Badri and Vivanco, 2009). Up to 30-40% of a plant's fixed carbon can be excreted during seedling stages (Whipps, 1990). Crested wheatgrass (*Agropyron cristatum*) exuded 5.6-10.4 mg C per plant over a 70-day lab experiment when grown from seed (Henry et al., 2007). Whereas wheat plants exuded between 2.6-22.5 mg C per plant over a 2-month period when grown from seedlings (Harmsen and Jager, 1962).

Root exudates impact soil physical, chemical, and biological properties. For example, Traoré et al. (2000) reported that both a modelled soluble exudate and natural root mucilage from corn (*Zea mays*) increased the amount of stable soil aggregates by 3.8-4.2 fold after 30 days compared to a non-treated control. This increase in soil structure would be especially important in sodic soils to help limit the effects of sodium dispersion and erosion. Root exudates also influence soil CO_2 emissions (Traoré et al., 2000). Simulated exudates added at 2 g C kg^{-1} soil mineralized the added carbon in 2 days, and after a 30-day incubation the mineralized carbon totaled 2.82 g C (Traoré et al., 2000). Compared to a glucose solution that released 1.81 g C, a maize mucilage that released 2.07 g C, and a control soil released 0.42 g C after 30 days (Traoré et al., 2000). In total, 87% of added C from the modelled exudate solution was mineralized as measured by CO_2 evolution in the first 75 hrs. The addition of a simulated exudate solution in another study reported 85% of added C released as CO_2 in the first 76 hours (Kunc and Macura,1966). These studies demonstrate the relatively quick metabolism of added carbon sources by soil microbes.

Additions of glucose and amino acids can increase gram negative bacteria populations, which are the primary colonizers of plant roots (Rovira and Brisbane, 1967).

The addition of 100 μg C g^{-1} day^{-1} of synthetic exudate solution increased bacterial colony forming units (CFU) by 1.5 Log CFU g^{-1} dry soil after 14 days (Baudoin et al., 2003). Therefore, improving plant growth on saline soils also has the potential to help recolonize microbially "dead" saline soil through root exudation with subsequent microbial population increases.

Impact of Root Respiration on Soil pH

Another component associated with plant growth in soil is CO_2 respiration from plant roots. Roots respire CO_2 as a result of metabolism. The buildup of CO_2 in the soil from plant root respiration impacts soil chemical properties, especially soil pH. As plants respire CO_2, the CO_2 combines with water molecules and forms H_2CO_3, carbonic acid. Over time H_2CO_3, a weak acid, can reduce soil pH. This is favorable in high pH sodic soils where soil pH can be 8 or greater. The decrease in pH can help dissolve free lime and gypsum providing increased calcium to exchange with sodium. Increased calcium also can help rebuild soil structure. However, research has had mixed results in attempting to prove this hypothesis.

A field experiment in a soil with 0.82% carbonate in which CO_2 was injected 60 cm below the soil surface at 1.0 L min^{-1} for 8 weeks reported that soil pH increased from 6.31 to 6.7 at the 15- to 30-cm depth and from 5.89 to 6.39 at the 45- to 60-cm depth (Biose et al., 2016). However, in pasture and fallow field plots the soil pH decreased from ~6.4 to ~6.0 after 21 weeks compared to controls at the 0- to 30-cm depth (Patil et al., 2010). A greenhouse experiment percolated CO_2 at 400 mL min^{-1} for 36 days in construction grade mineral soil and increased soil pH from 6.8 to 7.4, whereas N_2 increased pH to 7.5 and no gas treatment final pH was 7.0 (He et al., 2019).

Others argue that CO_2 respired by roots may diffuse through soil pores too easily to change pH (Nye, 1981). It is unlikely for root respiration to change pH in acidic soils, as H_2CO_3 has its first pKa value at 6.36 (Hinsinger et al., 2003; Lindsay, 1979) However, there is a greater potential for CO_2 to lower pH in alkaline soils when present in high enough concentrations (Hinsinger et al., 2003; Lindsay, 1979). When plants were cultivated for 5 months in alkaline saline-sodic soil pH decreased from ~8.5 to ~8.2, depending on plant species present (Qadir et al., 1996b). This difference was hypothesized to be the result of plant-induced increases in CO_2 from root respiration (Qadir et al., 1996b). Thus, under the right conditions CO_2 respiration from plant roots has the potential to induce chemical changes in soil properties such as pH.

Impacts of Plant Tissue Residue Additions to Saline Soil

As a result of plant growth in soil, plant residue and organic materials will be added to the soil and may impact soil properties. Plant residue is a source of carbon addition to the soil and depending on the plant residue makeup, nutrient addition as well. The incorporation of kangaroo grass at 10 t ha^{-1} over 12 weeks to highly alkaline saline soils decreased pH from ~10.1 to ~9.2 at the 10- to 20-cm depth but did not impact the pH at 0- to 10-cm depth and increased $EC_{1:5}$ from ~1.7 dS m^{-1} to ~5.9 dS m^{-1} in the 0- to 5-cm depth (Wong et al., 2009). In an acidic saline soil, the pH did not change, which remained ~4.5 from 0- to 40-cm. However, $EC_{1:5}$ at the 0- to 5-cm depth increased from ~1.7 dS m^{-1} to ~3.4 dS m^{-1} (Wong et al., 2009). In both soils, the addition of residue increased cumulative soil CO_2-C respiration throughout the 12 week study at the 0- to 5-cm depth in the alkaline soil from ~500 mg CO_2-C kg^{-1} in control to ~1700 mg CO_2-C kg^{-1} and in the acidic soil from ~700 mg CO_2-C kg^{-1} in control to ~2000 mg CO_2-C kg^{-1}

(Wong et al., 2009). After 12 weeks, soil microbial carbon in the alkaline soil increased from <100 mg C kg^{-1} in control to ~500 mg C kg^{-1} and in the acidic soil increasing to ~700 mg C kg^{-1} (Wong et al., 2009). In the alkaline soil, the chemical differences were attributed to residue decomposition processes, which produced organic acids, thereby reducing the pH (Wong et al., 2009). It was proposed this difference was not seen in the acidic soil because the pH was already low and thus not easily influenced by weak acids (Wong et al., 2009). The increase in EC was attributed to the increase of ions present due to mineral dissolution or organic acids produced (Wong et al., 2009). The increases in microbial biomass and respiration were likely results of short-term substrates provided by the residue which relieved osmotic stresses (Wong et al., 2009).

The addition of 1% *Hemp sesbania* residue to artificially salinized soils increased CO_2 evolution and microbial activity at saturated paste EC levels of up to 26 dS m^{-1} (Rao and Pathak, 1996). However, after the 90-day study there were no significant differences in soil microbial biomass in saline soils treated with residue compared to control (Rao and Pathak, 1996). After 5 months and 4 pore volumes of leachate, the addition of pistachio residue at 50 g kg^{-1} reduced Na levels from ~25 meq L^{-1} in the control to ~15 meq L^{-1} and reduced EC from of 4.0 dS m^{-1} initially, to 3.6 dS m^{-1} (Mahmoodabadi et al., 2013).

The addition of corn residue at 20 g kg $soil^{-1}$ to artificially salinized soil incubated at soil moisture of 0.17 or 0.25 g g^{-1} for 47 days induced changes in the soil respiration (Li et al., 2006). During the first 3 days, soils with higher salinity levels had lower CO_2 evolution at both moisture contents compared to lower salinity levels. During days 4-32 the higher salinity level soils had higher CO_2 evolution. However, from days 33-47 CO_2

evolution from both soil types was similar (Li et al., 2006). It was hypothesized that the 3 day delay in the saline soil was a function of microbial acclimation to saline soils and once acclimated corn residue was metabolized (Li et al., 2006). Additionally, the higher salinity soils had increased SAR levels up to 4.97 which could cause aggregate dispersion and release organic carbon that had been protected inside the aggregates (Li et al., 2006). The cumulative CO_2 respired during the 47 day study was 15% higher in the 0.25 g g^{-1} moisture treatment compared to the 0.17 g g^{-1} treatment when averaged across salinity levels (Li et al., 2006). The 0.17 g g^{-1} moisture soil cumulative CO_2 emission was higher in the elevated saline soils at 739 mg CO_2 (50 g soil)$^{-1}$ [9.57 dS m^{-1} using saturated paste] compared to 693 mg CO_2 (50 g soil)$^{-1}$ [5.35 dS m^{-1}]; whereas in the 0.25 g g^{-1} moisture level the elevated saline soils were similar to the control in cumulative emissions averaging ~821 mg CO_2 (50 g soil)$^{-1}$ (Li et al., 2006).

Other studies have investigated the impacts of residue additions to soil pH. When faba beans (*Vicia faba*) were cultivated for 45 days and the accumulated biomass was added back to the soil, pH increased from 5.64 to 6.29 as a result of organic anions and nitrogen released during residue decomposition (Yan et al., 1996). The increase in pH due to residue additions was also reported when 19 g faba bean or wheat (*Triticum aestivum*) residues were added to 4.95 kg soil and pH increased from 6.2 to 6.6 with faba bean and to 6.4 with wheat residue. However, residue decomposition also can decrease pH, if nitrification is an active process taking place (Yan et al., 1996).

An experiment quantified the impacts of maintaining either canola (*Brassica napus* L.) or wheat straw residue on the soil surface during the growing season on soil quality and GHG emissions and reported that during both years cumulative N_2O-N

emissions were similar in residue and no residue treatments (Malhi et al., 2006). When 8.23 Mg ha^{-1} of residue over 3 yrs were left on the soil surface in a no-till corn-soybean rotation, CO_2 emissions were 24% less than when residue was removed (Al-Kaisi and Yin, 2005). This is hypothesized to have occurred due to minimal residue decomposition on the soil surface which also acted as a barrier between the soil surface and the atmosphere, so gas exchange was minimized (Al-Kaisi and Yin, 2005). Residues reduced soil temperatures slowing mineralization as well (Al-Kaisi and Yin, 2005). Maintaining plant residue on the soil surface can also help relieve salt issues by reducing water evaporative losses, minimizing capillary rise (Cardon et al., 2014).

Impact of Plant Systems Growth on Saline Soils

To fully understand how plant growth impacts saline soils and GHG emissions, all the individual plant components must be combined. When a plant establishes in a saline soil it will exude carbon substances from its roots into the soil and also respire CO_2 into the soil atmosphere. Additionally, as the plant matures it will drop matured leaves and add organic material to the soil which can return ions and Na to the soil that the plant had taken up. This full plant system is important to understand as a whole, as different components of plant growth interact with each other and may produce net neutral impacts on soil.

One of the ways plant growth may influence saline soils is through nutrient uptake. Nitrogen is a key element that plants use in the soil for growth and in the process changes in pH can occur. When plants extract nutrients from the soil, they must also exude an equally charged ion to maintain electrical balance within the plant (Youssef and Chino, 1989). Saline soils found in crop production areas can be high in nitrogen as a

result of over-fertilization and lack of plant growth to utilize the nitrogen. Therefore, if plants were to be reestablished on these soils, there may be an abundance of nitrogen in the soil. This can result in high plant tissue nitrate levels, which in high enough concentrations can be toxic to livestock (Vermunt and Visser, 1987; O'Hara and Fraser, 1975).

Depending on the form of the nitrogen present, the uptake of excess nitrogen could result in soil pH changes. Since NO_3^- is an anion, the uptake by plants would likely result in the addition of HCO_3^- or OH^- ions from roots and increase soil pH, whereas the uptake of nitrogen in the form of NH_4^+ would result in H^+ ion additions and thus lower pH (Walker, 1960; Kirkby, 1968; Grinsted et al., 1982; Nye, 1981). Several studies have observed the effects of pH change due to nitrogen uptake.

When corn was grown in pots for 50 days, the soil pH increased with a concomitant decrease in EC due to nitrate and ion uptake by plant roots (Yanai et al., 1995). When rape (*Brassica napus*, var. Emerald) with high root density was grown in phosphorus deficient soil, pH decreased as much as 2.4 units from day 14 to day 28 (Grinsted et al., 1982). This was attributed to an imbalance of cation-anion uptake by the plant. During the time of pH decrease, there was more calcium uptake than nitrate uptake resulting in an imbalance and a net increase in H^+ to the soil. This hypothesis was confirmed, as the amount of H^+ required to result in the observed pH change matched the difference of milliequivalents between cations and anions during the same time period (Grinsted et al., 1982).

Different nitrogen sources can likewise influence the impact that plants have on soil pH changes as a result of plant N uptake (Marschner and Römheld, 1983). When

nitrate was applied as a nitrogen source in corn, soil pH increased from 6.0 to 7.5 (Marschner and Römheld, 1983). However, when ammonium was used the pH decreased to 4.0 (Marschner and Römheld, 1983). These results vary however; when applied to legume crops such as chickpea (*Cicer arietinum*) and white clover (*Trifolium repens*) pH changes were opposite of those found in corn (Marschner and Römheld, 1983). In soybean (*Glycine max* (L.) Merr.), when nitrate initially composed 40% of total anions in the soil, and bicarbonate 0%, the rhizosphere soil increased in pH from 5.9 to 6.3 after 2 weeks of soybean growth (Riley and Barber, 1969). The bicarbonate levels also increased to ~20% of anions in rhizosphere soil during this time. However, when initial nitrate anion composition was increased to 75%, the rhizosphere soil increased in pH from 6.3 to 7.0. Also, bicarbonate levels in the 75% nitrate experiment increased to ~55% which was the result of higher anion uptake than cations by the plants (Riley and Barber, 1969).

Other studies however, observed that the initial bulk soil pH, and not nitrogen source, determine how plants influence soil pH. For example, Youssef and Chino (1989) reported that after 8 weeks of growth, both soybeans and barley increased pH from 5 to 7 in both a sandy and clay loam soil. This study also reported a decrease in soil pH around the root zone to ~7 when the initial bulk soil pH was 8.5. Therefore, depending on nutrient uptake and initial soil pH levels, plant revegetation could either increase or decrease soil pH in soils (Youssef and Chino, 1989).

In addition to pH, other soil chemical properties can be influenced by plants. The use of kallar grass (*Leptochloa fusca*) as a cropping treatment in a leaching study on a highly saline sodic soil with an EC of 9.8 dS m^{-1}, pH of 9.1, SAR of 103, and Na of 88 $mmol_c$ kg^{-1} reduced soil EC to 2.9 dS m^{-1} compared to 4.3 dS m^{-1} in the control with no

plant growth after 4 leaching cycles totaling 13.7 L of leachate (Qadir et al., 1996a). Additionally, SAR was reduced to 20 compared to 42.3 in the control and the pH was reduced to 8.2 compared to 9.0 in the control (Qadir et al., 1996a). The changes could have been due to root dissolution of free lime in the soil which then replaced sodium on clay exchange sites, making the sodium leachable through root channels (Qadir et al., 1996a). Also, the sodium reductions could have been the result of plant uptake (Qadir et al., 1996a; Abdullah, 1985). Another experiment used three crop treatments which included sesbania (*Sesbania aculeata* Pers.), sordan [*Sorghum bicolor* (L.) Moench × *Sorghum Sudanese* (Piper) Stapf], and kallar grass grown on field plots ranging in EC from 7.4-8.8 dS m^{-1} and SAR from 55.6-73.0 (Qadir et al., 1997). After two growing seasons and 2 harvests where biomass was removed, the non-planted control plot EC decreased by 1.0 dS m^{-1} whereas the cropping treatments of sesbania, sordan, and kallar grass had reductions of 3.1, 1.8, and 2.5 dS m^{-1}, respectively. Sesbania, sordan, and kallar grass also reduced SAR by 25.5, 22.3, and 25.4 units, respectively, compared to control of 8.9 units (Qadir et al., 1997). These reductions were attributed to both increased ion exchanges with sodium and leaching of sodium as well as plant uptake in aboveground biomass (Qadir et al., 1997). The effectiveness of salt and sodium leaching however depends on the permeability of the salt-affected soil.

A review done by Jesus et al. (2015) identified several methods by which plants grown in saline or saline-sodic soils could remediate the soil. Methods for salt removal included direct plant uptake by roots and improving soil structure which could improve salt leaching. Identified methods for sodium removal included the two aforementioned pathways, as well increases of the pCO_2 in the soil. Increases in pCO_2 is can result from

plant root respiration as well as microbial respiration, which can be stimulated directly by plants themselves as well as indirectly through increased organic matter (Jesus et al., 2015; Qadir et al., 2000; Qadir et al., 2006b; Rabhi et al., 2009). These methods were also proposed by a review done by Qadir et al. (2006a); where proposed methods of phytoremediation for saline-sodic soils included increased pCO_2 to increase dissolution of $CaCO_3$, release of H^+ by roots to reduce pH as well as directly exchange with Na^+ on clay exchange sites, and uptake by plants directly.

Plants may also be able to alter GHG emissions associated with saline soils as well. Saline soils in SD are often wet due to poor drainage and occurrence in areas with a high-water table. A 10-day study done by Adviento-Borbe et al. (2006) investigated the impacts of salinity and water content of soils under corn growth. As soil EC increased from 0.5 dS m^{-1} to 2.0 dS m^{-1}, cumulative soil CO_2 emissions decreased from 50.6 kg CO_2-C ha^{-1} to 36.6 kg CO_2-C ha^{-1} and N_2O emissions decreased from 20 g N_2O-N ha^{-1} to 8.6 g N_2O-N ha^{-1} when soil moisture was at 60% WFPS. This was hypothesized to be the result of inhibited microbial activity and respiration (Adviento-Borbe et al., 2006). However, when WFPS was 90%, N_2O emissions were 2 to 40 times higher than when soils were at 60% WFPS (Adviento-Borbe et al., 2006). When EC increased from 0.5 dS m^{-1} to 2.0 dS m^{-1} at 90% WFPS, N_2O emissions increased from 37.7 g N_2O-N ha^{-1} to 343 g N_2O-N ha^{-1}; whereas CO_2 emissions decreased from 43.9 kg CO_2-C ha^{-1} to 30.6 kg CO_2-C ha^{-1} (Adviento-Borbe et al., 2006). It is hypothesized in the 90% WFPS treatment, denitrifying bacteria tolerated higher salinities than nitrifying bacteria, hence why N_2O increased with higher moisture content and EC (Adviento-Borbe et al., 2006).

A review found saline soils have reduced CO_2 emissions as a result of reduced microbial and enzymatic activities (Rastogi et al., 2002). This was documented by Pathak and Rao (1998) as well, where Sesbania residue was added to soils with different EC values and monitored for 90 days. Soils with crops had 2-3 fold higher CO_2 emissions compared to barren soils (Rastogi et al., 2002). Residue amended soils had less cumulative CO_2 emissions ranging from 2.1 g kg soil $^{-1}$ to 0.89 g kg soil^{-1} with increasing EC_e values from 1.1 dS m^{-1} to 96.7 dS m^{-1} (Pathak and Rao, 1998). Unamended saline soils from Australia had cumulative CO_2-C emissions of 0.6 g kg soil^{-1} at $EC_{1:5}$ of 0.5 dS m^{-1} but only 0.3 g kg soil^{-1} at $EC_{1:5}$ of 2.5 dS m^{-1} after 120 days (Setia et al., 2011). This was attributed to the saline soils having inhibited microbial activity due to osmotic stresses.

Based on the information presented there are several knowledge gaps, especially for South Dakota saline soils. These include how various practices and processes may produce different outcomes in Northern Great Plains soils. Salt-affected soil in South Dakota commonly has low infiltration and permeability, but high gypsum and free lime. This may decrease the effectiveness of leaching salts down the soil profile, especially where the water table is high, as well as gypsum application to improve soil structure and sodium removal. Phytoremediation may have potential in South Dakota, however there are very limited publications which go beyond species tolerance studies and into the effectiveness of phytoremediation at reducing salinity and sodicity in the Northern Great Plains. Additionally, while greenhouse gases have been well documented in non-saline soils, there are no publications which have measured saline soil greenhouse gas emissions

in the Northern Great Plains and how revegetating saline soils may impact these emissions.

The overall goal of this research is to better understand how saline soils in South Dakota function and respond to phytoremediation as a reclamation method. In these research studies, specific information regarding saline soils in South Dakota were conducted to determine which salt tolerant species were best suited for Eastern South Dakota, quantify how vegetation and fertilizer management practices influence greenhouse gas emissions from saline soil, and what impacts plants have on soil properties in response to root respiration, root exudates, and residue additions.

CHAPTER 2: PERENNIAL GRASS MIXTURES AND CORN IMPACTS ON SALINE-SODIC SOIL CHEMICAL AND VEGETATIVE PARAMETERS IN SOUTH DAKOTA

2.1 ABSTRACT

A multitude of research had been done on the use of phytoremediation in salt affected soil. However, most of these studies are from the Middle East, with soils different from those in South Dakota. This study was conducted in 2018 and 2019 to determine if establishing perennial grasses on salt affected soils in the Northern Great Plains could improve soil chemical properties, weed control, and biomass production. Two grass mixes (mix 1: beardless wildrye and slender wheatgrass; mix 2: AC Saltlander green wheatgrass, creeping meadow foxtail, western wheatgrass, and slender wheatgrass) were established on a non-saline Forman-Cresbard loam ($EC_{1:1}$ <1.3 dS m^{-1}) as well as a transitional ($EC_{1:1}$ 2.3-3.0 dS m^{-1}) and saline ($EC_{1:1}$ >3.5 dS m^{-1}) Cresbard-Cavour loam and compared to corn. Corn and perennial grass mixtures influenced soil electrical conductivity ($EC_{1:1}$), pH, sodium (Na), Na:$EC_{1:1}$, NO_3^-, and NH_4^+ similarly. In 2018 mix 1 produced the least biomass at ~2000 kg ha^{-1}, whereas in 2019 both grass mixtures produced more biomass (10500 kg ha^{-1}) than corn (1800 kg ha^{-1}). In 2019 saline soil, weed cover and weed biomass were lower in grass treatments (mix 1=16%, mix 2=3% weed cover)(mix 1=850 kg ha^{-1}, mix 2=150 kg ha^{-1} weed biomass) than corn (75% weed cover)(2475 kg ha^{-1} weed biomass). Results indicated that >1 year may be required to quantify potential benefits of perennials to soil EC and Na reductions. Also, results may be weather dependent, as 2019 had significantly higher precipitation than 2018. Grass

mixtures, once established can revegetate marginal saline soils, suppress weeds better than corn, and produce more biomass without additional fertilizer.

2.2 INTRODUCTION

Saline soils are in need of remediation efforts because elevated salt concentrations inhibit plant growth which leaves the soil barren and at risk for compaction and erosion. In addition, salt moves by wind to new areas, increasing salinity in areas at higher elevations. Phytoremediation is the use of plants to renovate poor soil conditions. Phytoremediation of saline soils requires the establishment of salt tolerant crops to solubilize calcite and improve soil physical properties by root respiration (Qadir and Oster, 2002). Reducing soil pH through formation of carbonic acid from root respiration can increase dissolution of free lime in the soil, making more calcium available for exchange with sodium (Qadir et al. 2000, 2006a; Rasouli et al. 2013; Walker et al. 2014). In addition, plant roots would create pores for air and water infiltration and root exudates could help rebuild soil structure. These benefits would restore soil health quicker than chemical remediation alone. Plant growth would also add organic matter to the soil through residue helping build soil structure and provide soil microbes food sources to stimulate activity. Perhaps most importantly, if perennial plants are established, a deep root system would utilize excess water from both surface inputs and subsurface (shallow aquifer) sources.

Plants can also remove salts, specifically sodium, through accumulation in biomass. However, the effectiveness of plant uptake is debated, with some authors suggesting little success in reducing sodium levels as plant litter would return the sodium to the soil unless physically removed (Qadir et al., 2000; Minhas et al. 2007; Shekhawat

et al. 2006; Gharaibeh et al. 2011). However, others reported reductions in soil sodium levels by plant accumulation (Rabhi et al., 2009, 2010; Ammari et al. 2013; Shelef et al. 2012). Jesus et al. (2015) proposed a hypothetical scenario and found that in a medium textured soil, with a goal of reducing the EC from 20 dS m^{-1} to 4 dS m^{-1}, that using *Sesuvium portulacastrum* (the species found to remove the most salt in the study review) could reduce the 10.725 ton ha^{-1} of salt in 2 years strictly through plant removal to attain the goal of 4 dS m^{-1}. Jesus et al. (2015) also reviewed eight studies looking at the impact of plant growth and chemical amendments had on reducing soil EC and SAR. Jesus et al. (2015) found trends indicating that phytoremediation and chemical amendments had similar results in EC reduction; whereas phytoremediation, with a few exceptions, had higher reductions in SAR. A review found that after just 1 year, Kallar grass (*Laptochloa fusca*) reduced soil EC from 22.0 dS m^{-1} in the top 20 cm to 12.6 dS m^{-1} (Ashraf et al., 2010). After 5 years there was an 87% reduction from the starting EC compared to an uncropped control (Ashraf et al., 2010). This same study found a reduction in pH from 10.4 to 8.9 after 5 years and a reduction in SAR from 185.5 to 20.7 (Ashraf et al., 2010).

Qadir et al. (2006b) reviewed several phytoremediation studies and reported a general trend that phytoremediation reduced EC and SAR at rates similar to chemical amendments such as gypsum but also provided the benefits of no chemical purchase, income provided by the sown crop, added soil stability and porosity, improvements at greater soil depths, higher carbon sequestration, and productive use of marginal lands. A study done on a calcareous soil in Pakistan found that after one year of growth, Sesbania (*Sesbania aculeate*) and Kallar grass both reduced soil EC greater than gypsum treatment compared to the control (Ahmad et al., 1990). Sesbania and Kallar grass reduced the EC

by 47.4% and 38.5%, respectively, compared to gypsum which reduced EC by 25.5% (Ahmad et al., 1990). However, all three of the treatments had similar reduction in SAR after two years and none of the treatments reduced soil pH (Ahmad et al., 1990).

A 2-month laboratory study done by Qadir et al. (2003) on an artificially calcareous saline–sodic soil compared gypsum application to alfalfa (*Medicago sativa*) growth in soil columns that were then subjected to 10 days/cycles of leaching (wetted to 130% water holding capacity once per day). The authors reported that gypsum was more effective at sodium removal in the earlier leaching cycles, but at the end of the experiment the chemical and phytoremediation treatments removed similar amounts of sodium in leachate at ~37 mmol Na, whereas plant uptake in tissue only accounted for 1.6% of Na removal from the soil.

Another study done by Ilyas et al. (1997) found that after one-year alfalfa treatment and crop rotation treatment (sesbania-wheat-sesbania) reduced SAR to ~29, compared to fallow control at 46. Singh et al. (2013) found that rhizospheric soil samples from Bermuda grass in a salt affected soil had significantly lower pH, EC, ESP, and SAR compared to non-rhizospheric samples. These differences were attributed to plant soil interactions such as CO_2 root respiration, root exudates, and increases in organic carbon and enzyme activity (Singh et al., 2013).

Most of these studies attribute the soil improvement and reductions in soil salinity to increased leaching of salts through root channels and dissolution of free lime in the soil from root respiration (Ashraf et al., 2010; Qadir and Oster, 2002; Qadir et al. 2000, 2006a; Rasouli et al. 2013; Walker et al. 2014). However, many of the species in these studies are not ideal for the Northern Great Plains soil types.

Salt tolerant species that are suited for the Northern Great Plains include species such as slender wheatgrass, beardless wildrye, green wheatgrass, western wheatgrass, and creeping meadow foxtail. Slender wheatgrass is a cool season bunchgrass with a relatively short lifespan of 3-5 years (Tilley et al., 2011). It is suitable for grazing with high protein content and establishes quickly with high vigor to prevent erosion while slower growing species establish such as beardless wildrye (Tilley et al., 2011).

Beardless wildrye is a cool season, sod forming salt tolerant species which is strongly rhizomatous and can tolerate saline and wet soil conditions as well making it well suited for saline soils (Young-Mathews and Winslow, 2010). It is a good candidate for soil stabilization as it is sod forming and can tolerate erosion sedimentation up to 12 inches and high waterflow (Young-Mathews and Winslow, 2010). Beardless wildrye is palatable to livestock when is young and can provide valuable waterfowl habitat (Young-Mathews and Winslow, 2010).

Creeping meadow foxtail is a cool season introduced species with very large rhizomes forming a dense sod (Tilley et al., 2004). Creeping meadow foxtail does not undergo dormancy in the summer and provides a good source of forage for haying or grazing with minimal recovery time (Tilley et al., 2004). Additionally, creeping meadow foxtail is tolerant of wet soils, very winter hardy, and provides beneficial wildlife habitat (Tilley et al., 2004).

Western wheatgrass is a slow to establish cool season native species with a sod forming rhizomatous growth pattern (Ogle, 2000). Western wheatgrass usually has poor germination and does not compete well against vigorous introduced species and therefore

may take up to four years to establish well (Ogle, 2000). Western wheatgrass provides a good forage source with high protein early in the season before it matures (Ogle, 2000).

AC Saltlander green wheatgrass is a hybrid wheatgrass cultivar created from crossing Eurasian bluebunch wheatgrasses with quackgrass (Hybner et al., 2014). AC saltlander is a cool season species with high salinity tolerance and moderately aggressive rhizomes (Hybner et al., 2014). It is a long-lived perennial that stays green longer in the season than other wheatgrass species making for high forage quality and recovers quickly from haying or grazing (Hybner et al., 2014). AC saltlander is well suited for erosion control and also competes strongly against weeds (Hybner et al., 2014).

Objectives

The objectives of this experiment were to measure and compare two different perennial grass mixtures and corn based on establishment and growth in a saline-sodic field over two growing seasons. The influence of these treatments on soil chemical properties, biomass production, and weed suppression also was determined.

2.3 MATERIALS AND METHODS

Study Site

The experiment was conducted in a field setting located at 44° 42' 11.6388" N, 97° 52' 43.8312" W in Clark County, SD in the James River watershed. The field had summit positions on the east and west sides of the field and a depression running down the middle going north and south. The soil classification for the elevated east and west sides of the field was a Forman-Cresbard loam (Soil Survey Staff, 2018). The Forman series is considered well drained and is a fine-loamy, mixed, superactive, frigid Calcic Argiudoll. The Cresbard series is considered moderately well drained and is a Fine,

smectitic, frigid Glossic Natrudoll. The soil classification for the depression running north and south was a Cresbard-Cavour loam. The Cavour series is a Fine, smectitic, frigid Calcic Natrudoll (Soil Survey Staff, 2018). According to the National Oceanic and Atmospheric Administration (NOAA) (2019) the 30-year average annual precipitation (1981-2010) for Clark, SD is 60.4 cm and the average annual temperature is 6.2 °C.

Experimental Design

The overall experimental design was a split block design with four replications within each zone. Zones were low salinity, moderate salinity, and high salinity. Each zone had four treatment levels: corn planted in spring, mix 1 and mix 2 which were over seeded as a dormant seeding in both year 1 and 2, and areas where no vegetation was planted.

Field Management

The field was dormant seeded with two perennial grass mixtures in 13.7 m wide strips on December 15, 2017 with a Truax Company, Inc. FLEX-II drill (Traux Company, Inc., New Hope, MN) at 6 mm depth. The first mixture, designated mix 1, consisted of Shoshone beardless wildrye (*Leymus triticoides* (Buckl.) Pilger) planted at 3.9 kg ha^{-1} of pure live seed (PLS) and Certified First Strike slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners) planted at 3.9 kg ha^{-1} PLS. The second mixture, designated mix 2, consisted of AC Saltlander green wheatgrass (*Elymus hoffmannnii*) planted at 3.6 kg ha^{-1} PLS, Garrison creeping meadow foxtail (*Alopecurus arundinaceus* Poir) planted at 2.2 kg ha^{-1} PLS, western wheatgrass (*Agropyron smithii* Rydb) planted at 6.7 kg ha^{-1} PLS, and Certified First Strike slender wheatgrass planted at 2.2 kg ha^{-1} PLS. DeKalb DKC45-65RIB (Monsanto Co, St. Louis, MO) 95-day Smartstax corn (*Zea mays*

L.) on May 17, 2018 at a rate of 79,000 seeds ha^{-1} in 0.76 m wide rows. However, during planting there was a planter error and seed was planted 2.5-cm deep rather than 5-cm, resulting in a reduced stand by ~45%.

The treatments were repeated in 2019, however the grass mixtures were seeded over each other in the same 2018 strips of existing grass stand on October 24, 2018 as a dormant seeding at the same rate and depth as in 2017. Corn was planted at a 5-cm depth on May 31st, 2019 at a rate of 79,000 seeds ha^{-1} in the same area as 2018, with rows offset from the 2018 rows. Variety was DKC40-77RIB (Monsanto Co, St. Louis, MS) which is a 90 day Smartstax hybrid. No fertilizer was applied in any treatments.

Weed management was required both years due to high weed densities of kochia (*Kochia scoparia* L.). The plot area was sprayed on June 6, 2018 with Engenia® herbicide [3,6-Dichloro-o-anisic acid (0.56 kg ae ha^{-1})] at a rate of 935 mL ha^{-1} with water as carrier applied at a rate of 187 L ha^{-1} with TT11003 nozzles (TeeJet Technologies®, Wheaton, IL) at 207 kPa. On June 21, 2018 the area, except corn plots, was mowed to a 12-cm height using a King Kutter® lift rotary mower model L-60-40-P (King Kutter®,Winfield, AL). On June 27, 2018 the field was sprayed with a tank mix of Callisto® , BROCLEAN®, and Destiny HC® with 8002VS nozzles (TeeJet Technologies®, Wheaton, IL) at 276 kPa. Callisto® (mesotrione)[2-(4-thysulfonyl-2-nitrobenzoyl)-1,3-cyclohexanedione] was applied at 219 mL ha^{-1} (0.105 kg ai ha^{-1}). BROCLEAN® (bromoxynil)(3,5-dibromo-4- hydroxybenzonitrile) was applied at 438 mL ha^{-1} (0.072 kg ae ha^{-1}). Destiny HC® (high surfactant oil concentrate) was applied at a rate of 0.25% v/v. Water carrier for this tank mix was applied at 122 L ha^{-1}. The entire plot area, excluding corn, was mowed on September 12, 2018 to a height of 17-cm.

In 2019, the plot area was sprayed on June 6th with Starane NXT® (fluroxypyr) [((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid, 1-methylheptyl ester] (0.102 kg ae ha^{-1}) plus bromoxynil [2,6-dibromo-4-cyanophenyl octanoate] (0.41 kg ae ha^{-1}). This was applied with water carrier at 150 L ha^{-1} with 8002VS nozzles (TeeJet Technologies®, Wheaton, IL) at 276 kPa. The fallow and corn plots were sprayed a second time on July 26, 2019 with 2338 ml ha^{-1} of Roundup Powermax® (glyphosate, N-(phosphonomethyl) glycine) (1.26 kg ae ha^{-1}), Chemsurf™ at 0.5 % v/v, ammonium sulfate at 2% w/v, and Callisto® was applied at 219 mL ha^{-1} (0.105 kg ai ha^{-1}). This was applied with water carrier at 150 L ha^{-1} with 8002VS nozzles (TeeJet Technologies®, Wheaton, IL) at 276 kPa.

Data Collection

Three zones were designated in each strip of vegetation in early July 2018 after plant emergence: 1) Good: plant growth did not appear limited by soil conditions, 2) Transition: plant growth was stunted with lower stand establishment, and 3) Saline: plant growth was severely impacted with few or no emerged plants. During the growing seasons of 2018 and 2019, multiple parameters were measured in each zone.

Soil samples were collected from each zone in all vegetation treatments from 0- to 15- cm and 15- to 30-cm depths in mid-July (VT corn growth stage). Samples were dried at 37.8 °C, ground, and sieved to < 2 mm. Samples were tested for $EC_{1:1}$ (electrical conductivity), pH, NO_3^-, NH_4^+, Na, and Na:EC. $EC_{1:1}$ was measured using an Orion Star A215 (Thermo Scientific, Waltham, MA) and pH was measured using a 1:1 soil to water ratio using an Accumet Excel XL60 pH meter with the same soil slurry, with EC measured first (Fisher Scientific, Hampton, NH) following methods described by Grafton

(2015). This was performed with 10 g oven dried soil and 10 mL nano-pure filtered water which was stirred immediately and after 15 min. Measurements were taken 30 min after water addition. NO_3^- and NH_4^+ was measured using an Astoria Nutrient Analyzer (Astoria-Pacific, Inc. Clackamas, OR) following methods described in Maynard and Karla (1993) in which extraction was performed with 10 g soil using 100 ml 1.0 *M* KCl, shaken for 1 hr, and filtered through fine porosity Ahlstrom filter paper. Na was extracted using 1 M ammonium acetate at pH 7.0 following methods in (Grafton, 2015) of a 1:10 soil to extract ratio which is shaken for 5 min at 200 evolutions per minute (epm) and filtered through Whatman No. 2 filter paper and measured on a Jenway PFP7 flame photometer (Cole Parmer, Staffordshire, UK).

In grass mixtures the % ground cover and % species composition of desired ground cover were visually evaluated in mid-July 2018 and 2019. Samples for total biomass were collected and separated by species on September 10, 2018 and July 11, 2019 at species maturity. Species matured earlier in 2019 due to greater precipitation, higher temperature, and less weed competition. Biomass samples were collected in 2018 from two 1-m^2 areas and in 2019 from two 0.1 m^2 areas from representative areas of each zone. The larger sample area in 2018 was used due to sparse growth for a more representative measurement.

Two stover biomass samples were collected from each corn plot zone on September 11, 2018 and August 29, 2019 when corn was at R5 growth stage. In 2018 the stover biomass samples were collected from 5.3 m of row and in 2019 from 2.7 m of row. Grain yield data were collected in 2018 but not 2019 due to weed pressure and no fertilization.

The % weed ground cover in early June was estimated in 2018 and 2019 from a saline and non-saline site for each block and treatment. In 2019 weed biomass was sampled on July 11, 2019.

All biomass samples were placed in paper bags and dried at 60°C for 72 hours, or until constant weight was achieved, in a forced air drier. Samples were weighed for dry matter.

Statistics

For statistical analysis of vegetation biomass as a function of specific soil properties, a linear regression approach was used. The linear regression model was as follows:

$$y = \beta_0 + \beta_1 x + \varepsilon$$

Where β_0 is the biomass of the soil test parameter, β_1 is the change in biomass with each unit change in x which is the soil test parameter being modeled; this would include soil $EC_{1:1}$, Na, and $Na{:}EC_{1:1}$. ε is the random error. For each linear regression, bootstrapping was performed with 5000 replications to generate 90% confidence intervals for each model's intercept and slope using the resampling package (Wu, 2019) in R (ver. 1.1.383) (R Core Team, 2017).

ANOVA was performed in R (ver. 1.1.383) (R Core Team, 2017) for statistical analysis of total grass, corn, and weed biomass, grass species biomass, % ground cover, and % weed cover on plots. The ANOVA model is as follows:

$$y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

Where y_{ij} is the outcome of the measured variable (biomass, % ground cover, etc.). μ is the overall grand mean of the measurements. τ_i is the treatment effect of the *i*th

treatment (species, vegetation plot). β_j is the blocking effect of the jth observation and ε_{ij} is the random error. Following ANOVA, if the P value was <0.05, a LSD post-hoc test was done for mean comparisons using the package agricolae in R (De Mendiburu, 2009).

2.4 RESULTS AND DISCUSSION

Environmental Conditions

The total annual precipitation in 2018 was 536 mm, which was 68 mm less than the 30-year normal (1981-2010) of 604 mm (Table 2.1). In 2018, the first half of the year had below average precipitation during the months of January through July (Table 2.1). The latter half of the year from August through December generally had more precipitation than normal with the exception of September and December which were also below normal (Table 2.1). Average monthly temperatures during 2018 were below normal from January through May and October through November (Table 2.1). July through September and December average monthly temperatures were above average in 2018 (Table 2.1).

In 2019, January through August had above average precipitation with the exception of June which was 25 mm below normal. Average monthly temperatures in 2019 were below normal from January through May and the month of August (Table 2.1). June and July had above average monthly temperatures by ~1°C (Table 2.1).

Table 2.1. Monthly Mean Precipitation and Average Temperature for Clark, South Dakota for 2018-2019 and Historical 30-Year Average (1981-2010).

Month	2018	2019	30 Year Normal	2018	2019	30 Year Normal
	-------Precipitation-------			----Average Temperature (°C)----		
	---------- mm ----------			---------- °C ----------		
January	0	25	13	-11.1	-12.2	-10.8
February	14	31	14	-13.9	-16.7	-8.2
March	7	58	31	-2.2	-5.3	-1.7
April	37	78	50	-0.6	6.1	6.4
May	51	197	75	10.6	10.8	13.4
June	54	75	100	21.7	19.7	18.7
July	81	171	89	22.2	22.5	21.9
August	83	139	72	21.1	20.3	20.7
September	58	-	71	16.7	-	15.2
October	59	-	52	5.6	-	7.6
November	79	-	23	-3.9	-	-1.2
December	14	-	14	-4.3	-	-8.8
Total	536	-	604	-	-	-

Collected from Clark, South Dakota weather station ID CLARK NUMBER 2, SD US located at 44° 52' 54.84" N, -97° 44' 3.12" W. Data obtained from the NOAA (National Oceanic and Atmospheric Administration).

Soil Tests

0- to 15- cm Depth

There were minimal differences among soil test results in vegetation treatments. Therefore, soil data were averaged across vegetation treatments within corresponding soil zones, which were then compared.

In 2018 the pH, Na:EC, and NO_3-N soil test levels were similar among the three sampling zones, averaging 7.35, 415, and 51 ug g^{-1}, respectively (Table 2.2). Soil $EC_{1:1}$

was highest in the saline zone as expected and measured 3.97 dS m^{-1}, whereas non-saline and transition zones were 1.18 and 2.62 dS m^{-1}, respectively (Table 2.2). Sodium was highest in the saline zone at 1744 ug g^{-1} wheras the non-saline zone was 388 ug g^{-1} (Table 2.2).

In 2019 pH decreased similarly across all zones by an average of 0.12 to a pH of 7.22 (Table 2.2). The Na:EC content decreased similarly across all zones compared to 2018 by an average of 157 (Table 2.2). Soil NO_3-N also decreased similarly across zones by 36 ug g^{-1} to an average value of 15 ug g^{-1} (Table 2.2). Soil $EC_{1:1}$ decreased similarly across all zones by an average of 0.66 dS m^{-1} (Table 2.2). The saline zone still had the highest soil $EC_{1:1}$ reading of 3.03 dS m^{-1} (Table 2.2). Soil sodium content decreased most in the transition and saline zones by 605 and 729 ug g^{-1}, respectively, whereas the non-saline zone decreased 243 ug g^{-1} compared to 2018 (Table 2.2).

15- to 30- cm Depth

In 2018, the pH and NO_3-N was similar among zones and averaged 7.91 and 22 ug g^{-1}, respectively (Table 2.3). The saline and transition zones had similar in $EC_{1:1}$ levels averaging 3.41 dS m^{-1}, which was higher than the non-saline zone of 2.23 dS m^{-1} (Table 2.3). The soil Na content highest to lowest was in the saline zone (1633 ug g^{-1}) > transition zone (1157 ug g^{-1}) > non-saline zone (714 ug g^{-1}) (Table 2.3). The Na:EC ratio was highest in the saline zone at 443, whereas the non-saline and transition zones were similar to each other and averaged 338 (Table 2.3).

In 2019 the pH decreased similarly among all zones compared to 2018 by 0.39 to an average pH of 7.52 (Table 2.3). Soil $EC_{1:1}$ in 2019 changed similarly from 2018 among zones and the saline and transition zones had the highest $EC_{1:1}$ of 4.07 dS m^{-1} and

3.12 dS m^{-1}, respectively (Table 2.3). Compared to 2018, soil sodium content in 2019 decreased similarly among zones by 206 ug g^{-1} and was highest in the saline zone at 1424 ug g^{-1} (Table 2.3). Soil Na:EC in 2019 decreased similarly among zones by an average of 96 compared to 2018 and was highest in the saline and transition zones which were 327 and 279, respectively (Table 2.3). In 2019, soil NO_3-N was highest in the transition and saline zones at 10 and 24 ug g^{-1}, respectively. The saline zone NO_3-N remained unchanged compared to 2018 (Table 2.3).

Soils Discussion

It was anticipated that the perennial grass mixtures would reduce soil EC and Na more than corn due to earlier season growth, a more extensive root system, and greater vegetative biomass. However, this was not substantiated in either year as these measurements were similar by vegetation treatments within a zone. The results of this research did not show similar reductions in soil $EC_{1:1}$ or Na as those of previous studies (Qadir et al., 2006b; Ahmad et al., 1990). Ashraf et al. (2010) reported rapid reductions in soil $EC_{1:1}$ from 22.0 dS m^{-1} to 12.6 dS m^{-1} after 1 year of Kallar grass growth and an 87% reduction in $EC_{1:1}$ compared to a fallow non-cropped treatment. However, our study did not find any advantage to perennial grass mixtures over corn in such a short time period.

One possible reason for these results could be the time it took for the grass mixtures to establish in 2018. Although the grass mixtures were dormant seeded the previous fall, emergence did not readily occur until June of 2018 due to soil crusting and weed competition, whereas corn established soon after planting. This additional time of fallow in the grass mixture treatments could have allowed more salts to accumulate through evaporation and capillary rise in the surface soil compared to the corn treatment.

The first year of strong establishment in the grass mixture treatments was in 2019. Additionally, 2019 was 76% wetter than normal from April-August so that standing water was present in lower elevation saline zones on several occasions. Since the beneficial effects of perennial grasses is attributed to improved soil structure and porosity to increase salt leaching (Qadir and Oster, 2002; Qadir et al., 2003), the high water table and lack of drainage could have mitigated any beneficial effects of the cropping treatments. Over several years of different environmental conditions and further development of a perennial root system, differences among treatments may occur and should be monitored for future research.

Table 2.2. Soil Test Results from Mid-July 2018 and 2019 for 0- to 15- cm Depth Averaged Across Vegetative Treatments.

	pH			Na:EC			NO_3^--N			EC			Na			NH_4-N		
							ug g^{-1}			dS m^{-1}			ug g^{-1}			ug g^{-1}		
Zone	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change
Non-Saline	7.18	7.12	-0.07	340	195 b	-146	37	8	-29	1.18 c	0.82 c	-0.36	388 c	145 b	-243 a	6	11	5
Transition	7.31	7.12	-0.17	467	252 b	-215	40	13	-27	2.62 b	1.95 b	-0.68	1193 b	522 b	-605 b	2	13	11
Saline	7.56	7.42	-0.13	437	328 a	-109	75	25	-51	3.97 a	3.03 a	-0.94	1744 a	1015 a	-729 b	3	8	5
P-Value	0.337	0.212	0.880	0.085	0.002	0.167	0.129	0.071	0.409	<0.001	<0.001	0.447	<0.001	<0.001	0.008	0.090	0.642	0.514

Table 2.3. Soil Test Results from Mid-July 2018 and 2019 for 15- to 30- cm Depth Averaged Across Vegetative Treatments.

	pH			Na:EC			NO_3^--N			EC			Na			NH_4-N		
							ug g^{-1}			dS m^{-1}			ug g^{-1}			ug g^{-1}		
Zone	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change	2018	2019	Change
Non-Saline	7.72	7.42	-0.30	312 b	225 b	-87	17	3 b	-14	2.23 b	1.96 b	-0.27	714 c	516 b	-197	5	14	9
Transition	7.92	7.44	-0.48	364 b	279 ab	-84	24	10 ab	-14	3.16 a	3.12 a	-0.04	1157 b	909 b	-314	2	9	7
Saline	8.09	7.71	-0.38	443 a	327 a	-116	24	24 a	0	3.65 a	4.07 a	0.42	1633 a	1424 a	-208	2	8	6
P-Value	0.097	0.314	0.670	<0.001	0.020	0.599	0.578	0.035	0.235	<0.001	0.003	0.196	<0.001	0.003	0.726	0.107	0.576	0.878

Vegetation Performance

Grass Mixture Species Ground Cover Composition

Grass species composition was measured as it indicates which species in the mixtures were better adapted for different field soil conditions. The first year of the study (2018), mix 1 had low total ground cover. In the non-saline zone, mix 1 ground cover was 54%, whereas the transition zone and saline zones had 28% and 5%, respectively (Table 2.4). In 2019, the non-saline, transition zone and saline zones had 86%, 90%, and 61% total ground covered by mix 1, respectively (Table 2.4). The lower % ground covered by mix 1 in 2018 was the result of it being the establishment year and early season weed competition. In 2019, a second year seeding may have improved coverage in addition to less weed competition and adequate moisture (Table 2.4). There was lower % ground cover in the saline zones during both years compared to other zones, likely the result of increased $EC_{1:1}$ and Na level in the soil which increased stress on the plants.

Both years of the study had similar species compositions in mix 1. Slender wheatgrass was the dominant species both years in the non-saline (61-70%) and transition (65-72%) zones of the field where soil $EC_{1:1}$ was <2.6 dS m^{-1}, whereas in the saline zone beardless wildrye and slender wheatgrass composed equal amounts of the total mix composition (Table 2.4; Table 2.2).

Mix 2 followed a similar trend to mix 1 in terms of total ground covered in 2018 and 2019. In 2018, mix 2 covered 54%, 25%, and 4% of the plots in the non-saline, transition, and saline zones, respectively (Table 2.5). This is in contrast to 2019 where mix 2 ground cover increased to 100%, 83%, and 51% in non-saline, transition, and saline plots, respectively (Table 2.5).

Within mix 2, species performed differently and had clear niches. In mix 2, the least productive species were slender wheatgrass and western wheatgrass which comprised <20% of mix 2 ground cover in 2018 and <10% in 2019 (Table 2.5). In the non-saline zone AC Saltlander and creeping meadow foxtail comprised 41% and 31% of the mix 2 ground cover, respectively (Table 2.5). Similarly, in the transition zone in 2019, AC Saltlander and creeping meadow foxtail were similar in % ground cover at 52% and 41%, respectively (Table 2.5). However, as the salinity gradient increased, AC Saltlander increased in ground cover, whereas creeping meadow foxtail decreased. In the saline zone, AC Saltlander comprised 71% and 84% of mix 2 ground cover in 2018 and 2019 respectively; whereas creeping meadow foxtail made up 1% and 6% in 2018 and 2019, respectively (Table 2.5).

Table 2.4. Mix 1 Species Composition in Mid-July 2018 and 2019 as a Percent of Total Ground Cover.

	2018			2019		
	Non-Saline	Transition	Saline	Non-Saline	Transition	Saline
% Total Ground Covered	54	28	5	86	90	61
Species	% Composition of Desired Ground Cover			% Composition of Desired Ground Cover		
Beardless Wildrye	39 b	35 b	49	30 b	28 b	47
Slender Wheatgrass	61 a	65 a	51	70 a	72 a	53
P-Value	0.002	<0.001	0.921	0.003	0.004	0.775

Table 2.5. Mix 2 Species Composition in Mid-July 2018 and 2019 as a Percent of Total Ground Cover.

	2018			2019		
	Non-Saline	Transition	Saline	Non-Saline	Transition	Saline
% Total Ground Covered	54	25	4	100	83	51
Species	% Composition of Desired Ground Cover			% Composition of Desired Ground Cover		
Slender Wheatgrass	19 c	19 bc	11 b	1 b	4 c	7 b
Western Wheatgrass	9 d	9 c	17 b	6 b	0 c	3 b
AC Saltlander	41 a	49 a	71 a	52 a	59 a	84 a
Creeping Meadow Foxtail	31 b	23 b	1 b	41 a	37 b	6 b
P-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Grass Mixture Species and Corn Biomass

In 2018 the most biomass produced in mix 1 was in the non-saline zone followed by the transition and saline zone with 1705, 801, and 246 kg ha^{-1}, respectively (Table 2.6). In the non-saline zone and transition zone, slender wheatgrass comprised 64% and 60% of the mixture 1 biomass weight, respectively, whereas in the saline zone beardless wildrye and slender wheatgrass both produced ~120 kg ha^{-1} (Table 2.6). Similar to ground cover discussed earlier, this low biomass in the saline zone in the first year of the study was most likely due to soil crusting, early season weed pressure, and saline conditions.

In 2019 mix 1 biomass increased in all three zones to 9038, 9700, and 6400 kg ha^{-1} from the non-saline, transition, and saline zones, respectively (Table 2.6). In the non-saline zone slender wheatgrass produced 7225 kg ha^{-1} of biomass compared to 1813 kg ha^{-1} from beardless wildrye (Table 2.6). Similar results were found in the transition zone. In the saline zone in 2019, slender wheatgrass and beardless wildrye produced similar levels of biomass, producing 4250 and 2150 kg ha^{-1}, respectively (Table 2.6).

Mix 2 had lower 2018 biomass levels in all three zones compared to 2019. In 2018 mix 2 produced 2646, 1368, and 604 kg ha^{-1} from non-saline, transition, and saline zones, respectively (Table 2.7). In the non-saline zone AC Saltlander produced the most biomass at 1045 kg ha^{-1}, which was 25%, 95%, and 358% more than creeping meadow foxtail, slender wheatgrass, and western wheatgrass, respectively (Table 2.7). In the transition zone, AC Saltlander produced the most biomass at 690 kg ha^{-1}, which was 124-452% more than the other species in the mix which were all similar to each other (Table 2.7). The same trend was found in the saline zone where AC Saltlander produced 367 kg

ha^{-1}, which was 198-1012% greater than the other species in the mixture, those of which were also similar to each other (Table 2.7).

In 2019 total mix 2 biomass was 10663, 9775, and 5853 kg ha^{-1} from non-saline, transition, and saline zones, respectively (Table 2.7). AC Saltlander produced the most biomass in all three zones compared to the other species in the mixture with 6625, 6775, and 4763 kg ha^{-1} from non-saline, transition, and saline zones, respectively (Table 2.7). Creeping meadow foxtail was the next highest biomass producer in 2019 and out produced slender wheatgrass and western wheatgrass in the non-saline and transition zones with 3550 and 2675 kg ha^{-1}, respectively (Table 2.7). Slender wheatgrass and western wheatgrass produced similar amounts of biomass in 2019 in the non-saline and transition zones with <400 kg ha^{-1} (Table 2.7). In the saline zone, creeping meadow foxtail, slender wheatgrass, and western wheatgrass all produced similar amounts of biomass ranging from 263-438 kg ha^{-1} (Table 2.7).

In 2018 corn produced 3952 kg ha^{-1} of stover and 6881 kg ha^{-1} grain in the non-saline plots (Table 2.8). The saline plots produced the least stover biomass with 943 kg ha^{-1} of stover and 1302 kg ha^{-1} of grain (Table 2.8). In 2019 due to the heavy weed pressure and lack of nitrogen, there was no grain yield. Stover biomass in 2019 was similar in non-saline and transition plots with ~2000 kg ha^{-1} (Table 2.8). Saline plots produced 1489 kg ha^{-1} of stover (Table 2.8).

Ground Cover and Biomass Discussion

In mix 1 slender wheatgrass composed more of the mix in the low to moderate salinities. However, in the most saline zone, slender wheatgrass and beardless wildrye had similar biomass. Slender wheatgrass is classified as tolerant of $EC_{1:1}$ levels up to 16

dS m^{-1} by Alberta Agriculture and Rural Development (2001) and beardless wildrye up to 20 dS m^{-1}. Young-Matthews and Winslow (2010) rated beardless wildrye highly salt tolerant up to over 15 dS m^{-1}. However, beardless wildrye has also been regarded as not highly salt tolerant by others (Grieve et al., 2012). Our research indicated that slender wheatgrass and beardless wildrye were equally tolerant of $EC_{1:1}$ levels of 3.2-4.1 dS m^{-1}. However, slender wheatgrass was more competitive, based on biomass, than beardless wildrye at $EC_{1:1}$ levels below 3.0 dS m^{-1}. Thus, using a mix of the two species, as in mix 1, has the advantage of a competitive species (slender wheatgrass) providing high cover on low to moderate salinity soils and the two species filling the niche in higher salinity soils. The findings in this study infer two other main points for mix 1: First, the establishment year of converting cropland to perennial grass mixtures may produce little overall biomass and the focus should be achieving a strong stand rather than getting a hay crop or grazing livestock on the field. Second, species will perform differently depending on salinity. In the case of mix 1, slender wheatgrass outcompeted beardless wildrye in both the non-saline and transition zones and produced similar biomass levels in the saline zones.

The results of mix 2 show that in the non-saline regions of the field, creeping meadow foxtail and AC Saltlander comprised the majority of mix 2 ground cover. However, creeping meadow foxtail was more sensitive to increasing salinity gradients and growth was reduced as salinity increased, whereas AC Saltlander was more salt tolerant and dominated the mixture in the saline regions. This result could be expected, as AC Saltlander was developed as a wheatgrass hybrid intended for high salinity tolerance. However, past research rated creeping meadow foxtail and AC Saltlander as equally

tolerant of salinity with tolerances of 12.0 and 12.9 dS m^{-1}, respectively (Tilley et al., 2004; Hybner et al., 2014). Western wheatgrass was rated tolerant up to 18 dS m^{-1} (Allison et al., 1954). The species which tolerated higher salinity in past studies would be expected to perform better than low tolerance species at moderate salinities as well. This was not the case with the findings in this research however. Although our study did not measure absolute tolerance limits, there were clear superior species in mix 2. Even though creeping meadow foxtail and AC Saltlander were classified as equally salt tolerant, in this study creeping meadow foxtail was clearly not as tolerant as AC Saltlander. Also, western wheatgrass had the highest seeding rate of mix 2 and also the highest salt tolerance (Allison et al., 1954). However, western wheatgrass was consistently one of the poorest species in terms of ground cover percentage and biomass produced. In the case of mix 2, AC Saltlander produced the most ground cover and biomass in all the zones measured. Creeping meadow foxtail performed well in the non-saline and transition zones, however it produced little biomass in the saline zone with $EC_{1:1}$ values >3 dS m^{-1} (Table 2.2; Table 2.7). The results of this study indicate that a mixture is a good option to use of saline soils, as different species occupied different niches. Thus, mixtures of species could help to maximize ground cover and biomass production while minimizing weed pressure.

Corn plots in this experiment did not perform well in biomass production likely due to soil fertility not meeting the high nitrogen demands of corn, and weed pressure suppressing growth. If fertilizer were applied and other weed control measures utilized, corn yields and biomass would likely have been higher. However, when all treatments were treated equally as in this experiment corn produced the least biomass of the

treatments in 2019. While corn can certainly establish in the some of the same soils as the grass mixtures tested, it requires extra inputs such as herbicides, fertilizer, and annual seed costs to produce equal amounts of biomass. There are additional challenges to corn in saline soils. For example, 2018 was drier in spring so corn was planted at an ideal time. However, 2019 was very wet in spring and the earliest date corn could be planted was after the crop insurance final plant date. Even then, only the hillsides were dry enough to plant in 2019 and the central lowland portion of the field plots were never planted to corn due to wet soil conditions. This is in contrast to perennial grasses which were able to be planted in drier conditions in fall and once established will not need future plantings and less trips across the field with equipment.

Table 2.6. Mix 1 Species Dry Biomass on September 10, 2018 and July 11, 2019.

	Estimated 2018 Biomass			2019 Biomass		
	Non-Saline	Transition	Saline	Non-Saline	Transition	Saline
Species	----------kg ha^{-1}----------					
Beardless Wildrye	685 b	290 b	134	1813 b	1675 b	2150
Slender Wheatgrass	1020 a	511 a	112	7225 a	8025 a	4250
P-Value	0.024	0.014	0.535	<0.001	<0.001	0.145
Total Biomass	1705	801	246	9038	9700	6400

Table 2.7. Mix 2 Species Dry Biomass September 10, 2018 and July 11, 2019.

	Estimated 2018 Biomass			2019 Biomass		
	Non-Saline	Transition	Saline	Non-Saline	Transition	Saline
Species	----------kg ha^{-1}----------					
Slender Wheatgrass	536 c	245 b	123 b	138 c	325 c	438 b
Western Wheatgrass	228 d	125 b	81 b	350 c	0 c	389 b
AC Saltlander	1045 a	690 a	367 a	6625 a	6775 a	4763 a
Creeping Meadow Foxtail	837 b	308 b	33 b	3550 b	2675 b	263 b
P-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total Biomass	2646	1368	604	10663	9775	5853

Table 2.8. Corn Grain Yield @ 15.5% Moisture and Stover Dry Biomass at Maturity in 2018 and 2019.

Crop Parameter	2018			2019		
	Non-Saline	Transition	Saline	Non-Saline	Transition	Saline
	kg ha^{-1}					
Corn Stover Biomass	3952	2468	943	2003	1926	1489
Corn Grain Yield	6881	5164	1302	-	-	-

Biomass Reduction Regression

Biomass was regressed as a function of $EC_{1:1}$, Na, and $EC_{1:1}$:Na ratio (Figure 2.1). In 2018 corn and mix 2 had the highest intercepts of 3655 and 2916 kg ha^{-1}, respectively, indicating that in low $EC_{1:1}$ soils these treatments had more biomass than mix 1 (Table 2.9). However, the slopes of the regressions were similar for all treatments in 2018 at ~-481 kg ha^{-1}, indicating that for each unit increase in $EC_{1:1}$, all three treatments lost similar amounts of biomass (Table 2.9). The same trend was found in the regression for Na soil levels, with corn and mix 2 having the highest intercept, averaging 3031 kg ha^{-1} with similar slopes among all treatments (Table 2.9). Using Na:$EC_{1:1}$ ratio as the regression parameter, corn and mix 2 again had the highest intercept at 4602 and 4716 kg ha^{-1}, respectively, and mix 1 was lowest at 1626 kg ha^{-1} (Table 2.9). However, in this regression slopes differed among treatments with mix 1 losing the least biomass with each unit increase in Na:$EC_{1:1}$ at -1.75 kg ha^{-1} whereas corn and mix 2 both lost ~6.51 kg ha^{-1} (Table 2.9).

In 2019, corn had the lowest intercept when $EC_{1:1}$ was fitted in the regression at 1807 kg ha^{-1}, whereas mix 1 and mix 2 had intercepts of 9890 and 11279 kg ha^{-1} (Table 2.9). In 2019 corn had more weed competition compared to grass mixtures which likely lowered the intercept. The slopes of the $EC_{1:1}$ regression in 2019 also differed among

treatments, with corn having the lowest slope of -0.6 kg ha^{-1} and mix 2 having the greatest at -1018 kg ha^{-1} (Table 2.9). However, the regression slope for $EC_{1:1}$ in 2019 should be interpreted with caution. Since corn saline zones had a large reduction in $EC_{1:1}$ compared to 2018, the regression is only fitted to an $EC_{1:1}$ up to 2.26 dS m^{-1}, whereas the grass mixtures are fitted up to 4.5-5.2 dS m^{-1}. This means there was not as large of a detectable biomass reduction in corn at higher $EC_{1:1}$ levels because there were no high $EC_{1:1}$ levels to be fitted. Thus, the intercept data for 2019 $EC_{1:1}$ is of good quality, but slope may be skewed. When Na was used for the regression, corn had the lowest intercept of 1858 kg ha^{-1} compared to mix 1 and mix 2 at 9084 and 10977 kg ha^{-1} (Table 2.9). This also indicated that at lower sodium levels, both grass mixtures produce more biomass than corn in this study. However, the slopes for Na were similar among treatments. The final parameter, $Na:EC_{1:1}$, indicated similar results as Na and $EC_{1:1}$ alone, where corn had the lowest biomass in low $Na:EC_{1:1}$ soils. Again, all treatments were similar for slope when fitted with $Na:EC_{1:1}$.

Biomass Reduction Discussion

The results of the regression analysis for biomass as a function of soil $EC_{1:1}$, Na, and $Na:EC_{1:1}$ suggest several things. First, in the establishment year of perennial grass plantings the biomass may be lower compared to a corn cash crop depending on the mixture. In this study, mix 1 was lower in biomass production compared to corn whereas mix 2 was similar to corn. In the second year after grass mixtures establish, they can outperform corn biomass production under some circumstances; 2019 was a wetter than average year which may have had an impact. Both mixtures produced more biomass than corn in 2019 and had similar losses in biomass for each unit increase in Na and $Na:EC_{1:1}$.

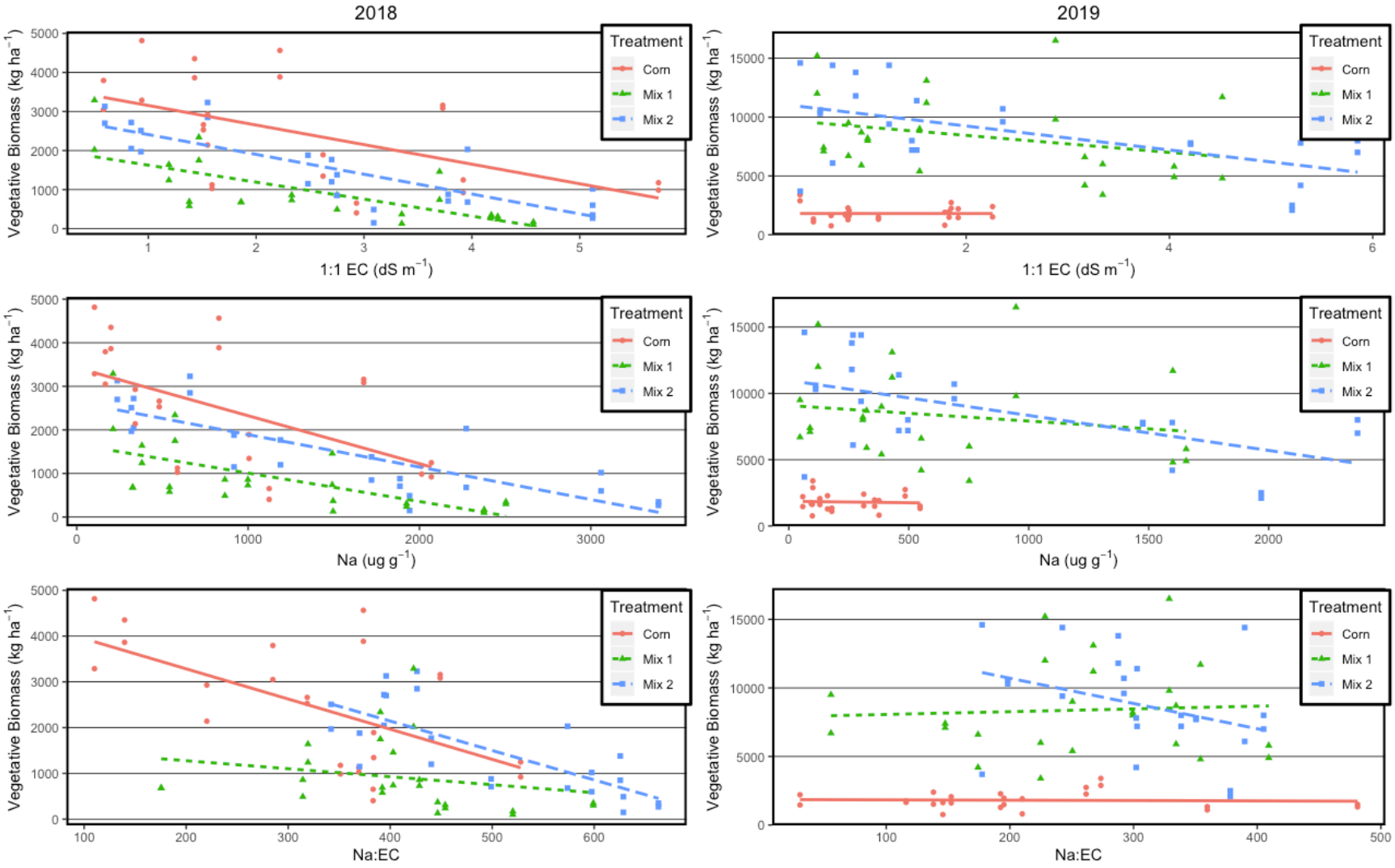
The intercepts showed that when salinity and sodicity parameters were zero, some treatments performed better than others depending on the year. Likewise, the slopes showed differences among treatments in biomass lost per unit increase in parameters. However, this slope can be misleading.

For example, let's assume mix 1 has a low intercept and mix 2 has a high intercept, but both stopped growing at the same $EC_{1:1}$ level in the soil. Although in this example both tolerated similar $EC_{1:1}$ levels, the slope of mix 1 would be lower because of an initially lower biomass. This would lead to the interpretation that mix 1 produced less biomass but was more tolerant to a salinity increase. But in reality, mix 1 produced less biomass and they both tolerated the salinity increase similarly.

Table. 1.9. Regression Intercept and Slope of Dry Basis Biomass as Function of Soil Parameters in 2018 and 2019.

Treatment	2018						2019					
	EC		Na		Na:EC		EC		Na		Na:EC	
	dS m^{-1}		ug g^{-1}		----------		dS m^{-1}		ug g^{-1}		----------	
	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
	Biomass kg ha^{-1}											
Corn	3655 a	-501	3425 a	-1.10	4602 a	-6.59 ab	1807 b	-0.6 a	1858 b	-0.22	1860 b	-0.26
Mix 1	2060 b	-435	1662 b	-0.65	1626 b	-1.75 a	9890 a	-723 ab	9084 a	-1.17	7863 a	2.02
Mix 2	2916 ab	-508	2636 a	-0.74	4716 a	-6.43 b	11279 a	-1018 b	10977 a	-2.64	14390 a	-18.42
	Adj R^2	p value	Adj R^2	p value	Adj R^2	p value	Adj R^2	p value	Adj R^2	p value	Adj R^2	p value
Corn	0.25	0.007	0.29	0.004	0.30	0.003	-0.05	0.999	-0.04	0.797	-0.04	0.829
Mix 1	0.51	<0.001	0.39	<0.001	0.01	0.285	0.04	0.168	-0.01	0.395	-0.04	0.794
Mix 2	0.63	<0.001	0.63	<0.001	0.54	<0.001	0.29	0.004	0.29	0.004	0.09	0.082

Figure 2.1 Vegetative Biomass Regressions as a Function of Soil Test Parameters from 2018 and 2019.



Weed Suppression

Weeds were present in both years of the study with the primary species being kochia (*Kochia scoparia*) and foxtail barley (*Hordeum jubatum*). In early June of 2018 the percent of plots covered in weeds was similar among treatments in both the saline (20%) and non-saline (24%) zones of the field (Table 2.10). This was expected because no crop had emerged at this time, so the field was uniform among treatments. In early June of 2019 the weed cover was similar among treatments in both the saline and non-saline zones (Table 2.10). At this point in time grasses in the mixtures were beginning to break dormancy from overwintering and emerge but there was not yet enough competition to reduce weed cover compared to other treatments.

After a broadleaf herbicide application between early June and mid-July in 2019 measurements, there were % weed cover differences mainly composed of foxtail barley. In non-saline plots, corn and no crop treatments had 100% weed cover (Table 2.11). This was higher than mix 1, which had 14% weed cover, and mix 2, which had no weeds in non-saline plots (Table 2.11). In the transition plots corn had the highest weed cover of 89% and no crop treatment had the next highest at 66% weed cover (Table 2.11). Mix 1 and mix 2 had the lowest weed cover at 7% and 0%, respectively (Table 2.11). In the saline plots, corn again had the highest weed presence with 75% of the plots covered by weeds whereas mix1 and mix 2 were lower with 16% and 3% weed cover, respectively (Table 2.11). No crop treatment comparison in the saline soil is not valid due to drastically higher $EC_{1:1}$ levels, which violated assumptions of the analysis. However, the no crop treatment in the saline plots averaged 20% weed cover (Table 2.11).

In addition to percent cover, weeds present in the study were also measured for biomass in 2019. In the non-saline zone, the no crop treatment had the highest weed biomass of 6713 kg ha^{-1} compared to corn, mix 1, and mix 2 which had 1650, 1300, and 0 kg ha^{-1} of weeds, respectively (Table 2.12). In the transition zone, no crop treatment again had the highest weed biomass of 5625 kg ha^{-1} (Table 2.12). Corn and mix 1 had the next highest weed biomass in the transition zone at 2150 and 562 kg ha^{-1} weeds, whereas mix 2 had no weeds present, although mix 1 was also similar to mix 2 (Table 2.12). In the saline zone corn had the highest weed biomass with 2475 kg ha^{-1} weeds and mix 1 and mix 2 were similar with 850 and 150 kg ha^{-1} weeds (Table 2.12). No crop treatment was not compared for the same reasons as in Table 2.11 but did average 775 kg ha^{-1} weed biomass in saline plots (Table 2.12).

Weed Suppression Discussion

To the author's knowledge, no publications have been produced examining how perennial grasses influence weed pressure in saline soils of the Northern Great Plains. The results of the weed data collected show strong differences among treatments. Although all treatments had similar percentages of the ground covered by weeds in spring, by the summer of 2019 the grass mixtures had much less weed presence. Both mix 1 and mix 2 were able to keep weeds suppressed after an herbicide application in all three zones compared to corn and no crop treatments. This result was evident in the weed biomass data where mix 1 and mix 2 both had less weed biomass than no crop treatment in non-saline zones and less weed biomass than corn in saline zones. The advantage of the grass mixes was the fact they were already established from the previous year, which allowed them to emerge early and provide a dense stand cover over the soil much earlier

than corn canopy. Additionally, grass mixtures were drilled at 0.2 m spacing, whereas corn was planted on 0.76 m row spacing. This means there is more time required to canopy the wider row spacing in corn. This allowed the grass mixtures to compete with weeds and suppress them much better than corn by mid-summer. Thus, our findings indicate that although the first year of grass establishment may face heavy weed pressure, but once established they have high weed suppression potential.

Table 2.10. Percent of Plots Covered by Weed Species* in Early June of 2018 and 2019 in Good and Saline Soil Zones of Corn, Grass, and No Crop Soil Treatments.

	Spring 2018 % Plot in Weed Cover		Spring 2019 % Plot in Weed Cover	
	Non-Saline	Saline	Non-Saline	Saline
Treatment	----------%----------		----------%----------	
Corn	16	13	76	13
Mix 1	38	39	51	16
Mix 2	24	14	31	4
No Crop	19	13	75	18
P-Value	0.551	0.377	0.199	0.668

*Weed species included primarily foxtail barley and kochia.

Table 2.11. Percent of Plots Covered by Weed Species (primarily foxtail barley) in Mid-July of 2019 in Good, Transition, and Saline Soil Zones of Corn, Grass, and No Crop Soil Treatments.

	Summer 2019 % Plot in Weed Cover		
	Non-Saline	Transition	Saline
Treatment	----------%----------		
Corn	100 a	89 a	75 a
Mix 1	14 b	7 c	16 b
Mix 2	0 c	0 c	3 b
No Crop	100 a	66 b	20 b*
P-Value	<0.001	<0.001	<0.001

* Value lower than expected due to higher EC of the saline zone in No Crop strips

Table 2.12. Weed Biomass (primarily foxtail barley) in Mid-July of 2019 in Corn, Grass, and No Crop Soil Treatments.

	Weed Biomass		
	Non-Saline	Transition	Saline
Treatment	----------kg ha^{-1}----------		
Corn	1650 b	2150 b	2475 a
Mix 1	1300 b	562 bc	850 b
Mix 2	0 b	0 c	150 b
No Crop	6713 a	5625 a	775 b*
P-Value	<0.001	<0.001	<0.001

* Value lower than expected due to higher EC of the saline zone in No Crop strips

CHAPTER 3: GREENHOUSE GAS EMISSIONS OF A SALINE-SODIC SOIL WITH NO VEGETATION AND A NON-SALINE SOIL UNDER CORN AND GRASS VEGETATION

3.1 ABSTRACT

Greenhouse gas (GHG) emissions (CO_2, N_2O, CH_4) of saline soils in South Dakota has not been studied, although over 3.4 million ha are impacted in the state. These areas are intertwined with non-saline areas in the landscape and are often managed similarly for convenience. This two-year study quantified and compared GHG emissions from adjacent saline and non-saline soils without (0 kg N ha^{-1}) or with urea (224 kg N ha^{-1}). The saline soil was barren, whereas corn or grass was growing in the non-saline soil. Urea was applied immediately prior to GHG collection, which was measured every 4 hrs for 7 consecutive days in July (2018 and 2019) using a near continuous automated system. All treatments were replicated twice each year. Methane (CH_4-C) flux was near zero for all treatments both years. In the 0 N treatment, average CO_2-C flux from saline soil was 611 and 324 g CO_2-C ha^{-1} hr^{-1} in 2018 and 2019, respectively, which was 36% and 16% of the flux measured in vegetated soils in 2018 and 2019, respectively. Urea increased CO_2-C flux in 2018 by ~35% from all areas, but in 2019 by 19% (grass), 74% (corn), and 155% (saline). N_2O emissions in the 0 N treatment in saline soil were 2.09 and 4.89 g N_2O-N ha^{-1} hr^{-1} in 2018 and 2019, respectively, which was 450% and 963% higher than soils with grass vegetation. Urea did not increase N_2O flux in 2018 due to cool temperatures and drier conditions, however, urea increased N_2O-N flux in 2019 by 704%, 602%, and 102% in grass, corn, and saline soils, respectively. Saline soils disproportionately contributed to N_2O emissions. Low CO_2 emissions from saline soils

imply decreased biological activity. Revegetating barren salt-affected soils may increase soil health and reduce N_2O emissions.

3.2 INTRODUCTION

In SD, saline and sodic soils are scattered across the landscape in regions where they occur. Typically, a salt affected area starts small but may spread throughout the field due to salt movement with wind and water. Because of this, without the use of precise GPS and variable rate technology, it is often an inconvenience or extra burden to manage these small areas separate from the rest of the field unless the impacted area is large. Variable rate technology also adds to production costs if custom applied by a retailer. Higher costs may further decrease the incentive to spend more money on unproductive marginal land that does not produce a profit unless the decision to implement the technology will reduce fertilizer input costs enough to offset the variable rate cost. Therefore, fertilizer is often applied to small saline or sodic areas at the same rate as the rest of the field. Baseline GHG emissions have not been quantified from saline soils in South Dakota and past studies' results from non-saline soils may not be applicable to saline soils, as saline soils have drastically different biology and chemistry. These lack of data warrant attention and was the basis for this research study.

Objectives

This research quantified GHG emissions of CO_2, CH_4, and N_2O from a saline sodic soil with no vegetation and compared results to an adjacent non-salt affected area that was vegetated with either corn or mixed perennial grass vegetation. These data can be used to determine the impact that revegetating saline soils could have on GHG

emissions. Additionally, urea was applied to each area to quantify the impact of urea nitrogen fertilizer addition to GHG emissions from soils in SD.

3.3 MATERIALS AND METHODS

Study Site

This experiment was conducted in a field setting in Clark County, South Dakota at 44° 42' 11.6388" N, 97° 52' 43.8312" W and was selected because it had elevated electrical conductivity and sodium levels which limit plant growth and establishment. The area of the field where the experiment was conducted was planted with DeKalb DKC45-65RIB (Monsanto Co, St Louis, MO) corn (*Zea mays* L.) on May 17, 2018 in 0.762 m rows at a rate of 79,000 seeds ha^{-1} at 2.5-cm depth. Adjacent to the corn, a 13.7 m wide strip of a perennial grass mixture was dormant seeded on December 15, 2017 using a Truax Company, Inc. FLEX-II drill (Traux Company, Inc., New Hope, MN) at 6 mm depth. This grass mixture consisted of Shoshone beardless wildrye (*Leymus triticoides* (Buckl.) Pilger) planted at 3.9 kg ha^{-1} pure live seed (PLS) and Certified First Strike slender wheatgrass [*Elymus trachycaulus* (Link) Gould ex Shinners] planted at 3.9 kg ha^{-1} PLS. The grass mixture was over seeded in the same strip of existing grass stand on October 24, 2018 at the same rate and depth as done in 2017 to strengthen the stand. Corn was planted in the same strips of the field on May 31st, 2019 at a rate of 79,000 seeds ha^{-1}. Corn was planted at a depth of 5 cm with variety DKC40-77RIB (Monsanto Co, St. Louis, MS). At the time of measurements corn was at the tassel growth stage both years. The grass mix was at the boot stage in 2018 and seed stage in 2019.

The field sloped downhill to the East; the further down the slope, soil properties become more unfavorable for plant growth as seen visually and by data collected. The

uphill region of the field is a Forman-Cresbard loam on a 3-6% slope. The Forman series is a fine-loamy, mixed, superactive, frigid Calcic Argiudoll. The Cresbard series is a Fine, smectitic, frigid Glossic Natrudoll. The downhill region of the field is a Cresbard-Cavour loam on a 0-3% slope. The Cavour series is a Fine, smectitic, frigid Calcic Natrudoll and has a natric restrictive feature 13- to 36- cm below the surface (Soil Survey Staff, 2018). According to the National Oceanic and Atmospheric Administration (2019) the 30-year average annual precipitation (1981-2010) for Clark, SD is 60.4 cm and the average annual temperature is 6.2 °C.

Soil Tests

Soil samples were collected at the start of the experiment directly outside the chambers and at the end of the experiment, from inside each chamber PVC ring. All samples taken were from 0- to 15- cm and 15- to 30-cm depths, dried at 37.8 °C, and ground and sieved to < 2 mm. Samples were tested for $EC_{1:1}$ (electrical conductivity), pH, NO_3^-, NH_4^+, Na, and Na:EC. $EC_{1:1}$ was measured using a 1:1 soil to water ratio with an Orion Star A215 (Thermo Scientific, Waltham, MA) and pH was measured using an accumet excel XL60 pH meter (Fisher Scientific, Hampton, NH) and followed methods described by Grafton (2015). NH_4^+ and NO_3^- were extracted using 1.0 *M* KCl and measured using an Astoria Nutrient Analyzer (Astoria-Pacific, Inc. Clackamas, OR) following methods described in Maynard and Karla (1993). Na was measured using ammonium acetate extraction using flame photometry (Grafton, 2015).

Equipment

Soil flux was measured in the field using a LI-COR 8100A (LI-COR, Lincoln, NE) system to collect gas samples and a Picarro G2508 (Picarro Inc., Santa Clara, CA)

system for gas sample analyzation. Twelve LI-COR 8100-104 Long Term Chambers (LI-COR, Lincoln, NE) where used for gas collection from the soil. These chambers were placed over a PVC (polyvinyl chloride) ring that was inserted approximately 5 cm into the soil surface and had an inside diameter of 20.1 cm and a total area of 317 cm^2 with total chamber volume of 4244 cm^3. PVC rings and chambers were placed according to LI-COR protocols (LI-COR, Lincoln, NE). Special attention was given to leveling the PVC rings and chamber placement over PVC rings; ensuring the PVC ring did not extend into the chamber headspace. The chambers used mixed air within the chamber and were vented to maintained ambient air pressure. Vegetation was removed from PVC rings before measurements began in the grass mix, whereas in the corn rings were placed between rows.

The collected gas sample was measured for CO_2 using a LI-8100A Analyzer Control Unit (LI-COR, Lincoln, NE). The gas sample is then analyzed using a Picarro G2508 cavity ringdown spectrometer for CO_2, N_2O, and CH_4 measurements. A LI-8150 multiplexer (LI-COR, Lincoln, NE) controlled chamber sequence to automatically open and close chambers to collect data. During chamber measurement, the system measured gas concentration every second for 15 minutes. Before each chamber closed to take measurements, an automatic 45 second pre-purge was conducted through the gas lines. Likewise, after each chamber was done sampling gas, an automatic 45 second post-purge was conducted through the gas lines. A 45 second deadband was used for flux computations. To measure soil moisture, LI-COR 8150-205 Soil Moisture Probes (LI-COR, Lincoln, NE) were placed to 5 cm depth to determine volumetric soil moisture content. Soil temperature was measured at 5 cm depth using LI-COR 8150-201 Soil

Temperature Probes (LI-COR, Lincoln, NE). Air temperature was measured from the LI-COR 8100-104 chambers (LI-COR, Lincoln, NE) with a built in sensor which measured the air temperature inside the chamber during the time of gas collection.

Flux Calculation

SoilFluxPro™ ver 4.0.1 software was used to calculate exponential flux of all gases. Flux was calculated using the following equations:

$$G' = G'_s + [G'_0 - G'_s]e^{-at} \qquad \text{(Equation 1)}$$

where G' is the instantaneous water vapor dilution-corrected chamber gas mole fraction, G'_s is the water vapor dilution-correction gas concentration in the soil surface layer under the chamber, and a is a rate constant. The flux is calculated using the initial slope ($\frac{\partial G'}{\partial t}$ at $t = 0$) of the function at the time of chamber closing when G' is close to the ambient level (G'_0). $\frac{\partial G'}{\partial t}$ is calculated by the equation

$$\frac{\partial G'}{\partial t} = a[G'_s - G'_0]e^{-at}. \qquad \text{(Equation 2)}$$

Flux is then calculated using the equation

$$F_c = \frac{VP_0(1-W_0)}{RS(T_0+273.15)} \frac{\partial G'}{\partial t} \qquad \text{(Equation 3)}$$

where F_c is the soil gas flux, V is the volume, P_0 is the initial pressure, W_0 is the initial water vapor mole fraction, S is soil surface area inside the chamber, T_0 is the initial air temperature, and $\frac{\partial G'}{\partial t}$ is the initial rate of change in water vapor dilution-corrected gas mole fraction (LI-COR, 2019). For flux calculation of CO_2, gas collected from 45-165 seconds after chamber closing was used. For all other gases, 45-900 seconds was used. Equations were automatically modified for each gas for molar weights.

Design and Treatments

The overall experimental design was a 3x2 factorial with two replications. The first factor was vegetation, with three levels: corn, grass mixture, and no vegetation. The corn and grass mixture levels were positioned in the uphill land position were soil properties were less saline. The no vegetation level was positioned further downhill in saline soil. The second factor was urea application rate, with two levels: 0 kg ha^{-1} N and 224 kg ha^{-1} N. Nitrogen application was performed by dissolving urea in 10 mL H_2O and then evenly dripped on the soil within the PVC ring. Urea was applied to all treated areas directly before measurements began. Four LI-COR 8100-104 Long Term Chambers were placed in each vegetation level; with two chambers each treated with urea, and two chambers with no urea. The experiment was replicated over 2 years. The was conducted in 2018 from July 17 to July 24 and 2019 from July 16 to July 23.

Statistics

All statistics for flux and soil data were conducted in R (ver 1.1.383) (R Core Team, 2017). Before analysis was done, any outliers were removed from the dataset using DFFITS in R (Hebbali, 2017) and confirmed using SoilFluxPro™. ANOVA analysis was performed between among treatments with the following model:

$$\mathcal{Y}_{ij} = \mu + \tau_i + \epsilon_{ij} \qquad \text{(Equation 4)}$$

Where $\mathcal{Y}_{ij}$= i^{th} observed sample value from the i^{th} population, μ= the overall mean, τ_i= vegetation and urea treatment effect in the i^{th} treatment level which is the difference between the mean of the i^{th} treatment level and the overall mean, and ϵ_{ij} is the random error. If Pr(>F) was found to be less than a=0.05 significance level, a Fisher Least Significant Difference (LSD) post-hoc test was conducted in R to determine

differences among treatments using library agricolae (de Mendiburu,2009). Graph figures were generated using the ggplot2 package (Wickham and Winston, 2016).

3.4 RESULTS AND DISCUSSION

Overall Environmental Conditions

2018

This experiment took place in July, which is historically the warmest month of the year in Clark, SD according to 30-year normal temperature data (1981-2010) with average daily high temperatures of 28.0°C, and average daily low temperatures of 15.8°C. During the week of the experiment, the average temperature was 21.5°C, with an average high of 27.1°C and an average low of 16.0°C (Table 3.1). Precipitation during the study consisted of two rain events. The major rainfall event occurred on July 18, 2018 totaling 31 mm (Table 3.1). This rain fell during the evening and throughout the night for approximately 8 hours. The other totaled 1 mm on July 20, 2018 overnight (Table 3.1)

2019

The temperatures were slightly higher in 2019 compared to 2018 with an average low temperature of 16.5°C and an average high temperature of 28.8°C (Table 3.2). This was also higher than the 30-year normal low and high temperatures by 0.7°C and 0.8°C, respectively. There were three precipitation events during the 2019 study totaling 100 mm (Table 3.2). The first occurred on July 17, 2019 from 2:15 to 9:00 am CDT and totaled 13 mm (Table 3.2). The second precipitation event occurred on July 18, 2019 from 1:15 to 1:45 am CDT and totaled 3 mm (Table 3.2). The final precipitation event occurred on July 20, 2019 from 5:50 to 7:30 am CDT and totaled 84 mm (Table 3.2). This final precipitation event brought strong wind and heavy rain forcing the shutdown of

equipment for one measurement cycle at 7:30 am CDT and resumed measurements at 12:00 pm CDT. The heavy rain filled the chamber rings in the saline zone due to no infiltration and prevented GHG measurements; this water was manually removed at 4:40 pm CDT the same day.

Table 3.1. Daily Precipitation and Temperature Data During 2018 Experimental Run. Precipitation collected on field site and temperature data collected from Clark, SD weather station ID CLARK NUMBER 2, SD US USC00391740 located at 44° 52' 54.84" N, -97° 44' 3.12" W as obtained from the NOAA (National Oceanic and Atmospheric Administration).

Date	Precipitation	Minimum Temperature	Maximum Temperature
	---mm---	----------°C----------	
July 17, 2018	0	15.0	28.9
July 18, 2018	31	15.6	29.4
July 19, 2018	0	17.2	26.7
July 20, 2018	1	14.4	23.9
July 21, 2018	0	14.4	26.7
July 22, 2018	0	16.1	26.7
July 23, 2018	0	19.4	27.2
July 24, 2018	0	15.6	27.2
Total	32	-	-
Average	-	16.0	27.1

Table 3.2. Daily Precipitation and Temperature Data During 2019 Experimental Run. Precipitation collected on field site and temperature data collected from Clark, SD weather station ID CLARK NUMBER 2, SD US USC00391740 located at 44° 52' 54.84" N, -97° 44' 3.12" W as obtained from the NOAA (National Oceanic and Atmospheric Administration).

Date	Precipitation	Minimum Temperature	Maximum Temperature
	---cm---	----------°C----------	
July 16, 2019	0	20.6	32.2
July 17, 2019	13	19.4	30.0
July 18, 2019	3	17.8	28.9
July 19, 2019	0	18.9	31.7
July 20, 2019	84	13.3	31.7
July 21, 2019	0	14.4	25.0
July 22, 2019	0	13.9	24.4
July 23, 2019	0	13.9	26.1
Total	100	-	-
Average	-	16.5	28.8

Zone Environmental Conditions

2018

The mean air temperature as measured with field equipment was similar between all three vegetation zones throughout the study, ranging from 21.5°C to 25.9°C (Table 3.3).

Unlike air temperature, soil temperature differed by zones with averages 24.1°C (saline) and 19.0°C (corn) (Table 3.3). The cooler soil temperature in corn was most likely the result of 100% vegetative canopy. The saline zone was barren, allowing direct solar radiation to heat the bare soil. The first two days in the grass zone had temperatures

lower than the saline zone but higher than the corn zone due to less shading provided by the grass vegetation (~50% canopy) (Table 3.3).

Mean volumetric soil moisture contents at the start of the experiment for corn, grass, and saline treatments were 0.13 cm^3 cm^{-3} (corn), 0.20 cm^3 cm^{-3} (grass), and 0.46 cm^3 cm^{-3} (saline) (Table 3.3). Soil moisture increased on day 2 after the rainfall. The saline zone was consistently higher in moisture throughout the study due to poor drainage and the lower landscape position. Over the 7 days, grass, corn, and saline zone soil moisture averaged 0.31, 0.23, and 0.47 cm^3 cm^{-3}, respectively (Table 3.3).

2019

Similar to 2018, chamber air temperature did not differ between zones throughout the study. The average air temperatures for the grass, corn, and saline zones over the 7 days was 23.8, 23.9, and 24.5°C (Table 3.4). Days 1-4 of the study were ~5 to 10°C warmer than days 5-7 (Table 3.4).

Soil temperature also showed similar trends seen in 2018 with the saline zone being warmer than the corn and grass treatments on days 1 and 3 of the study as well as when averaged throughout the 7 days (Table 3.4). Corn and grass soil temperatures were similar on all 7 days and averaged 21.4 and 23.1°C, respectively (Table 3.4). In contrast, the saline zone averaged 25.7°C during the study.

Soil moisture content was higher in all treatments compared to 2018 due to a wetter season and more precipitation events. Corn and grass zone soil moisture averaged 0.41 and 0.42 cm^3 cm^{-3}, respectively, over the 7 days (Table 3.4). The saline zone had a higher moisture content throughout the study compared to corn and grass zones and

averaged 0.58 cm^3 cm^{-3} over the 7 days (Table 3.4). Soil moisture did not change substantially with rainfall in 2019 due to saturated conditions already present.

Table 3.3. Mean Chamber Air Temperature, Soil Temperature, and Soil Moisture at 2018 Experiment Site as Measured in Each Vegetation Zone.

Treatment	2018							
	Day							
	1	2	3	4	5	6	7	1-7
	Mean Chamber Air Temperature (°C)							
Grass Mixture	25.5	21.3	21.6	22.9	24.4	25.2	21	23.2
Corn	25.4	21.5	20.7	22.5	24	24.8	20.5	22.9
Saline No Vegetation	25.9	21.5	21.3	23.7	25.2	25.9	22.2	23.7
	Mean Soil Temperature (°C)							
Grass Mixture	24.9	19.2	-	-	-	-	-	22.3 a
Corn	21.7	17.8	16.8	18.2 b	19.3 b	20.5 b	18.1 b	19.0 b
Saline No Vegetation	26.2	21.3	20.7	24.2 a	25.5 a	25.8 a	24.6 a	24.1 a
	Mean Soil Moisture (cm^3 cm^{-3})							
Grass Mixture	0.22 b	0.37 b	0.37 b	0.34 b	0.32 b	0.31 b	0.28 b	0.31 b
Corn	0.13 c	0.31 b	0.30 c	0.23 c	0.23 c	0.19 c	0.19 c	0.23 c
Saline No Vegetation	0.46 a	0.48 a	0.48 a	0.47 a	0.47 a	0.45 a	0.44 a	0.47 a

Table 3.4. Mean Chamber Air Temperature, Soil Temperature, and Soil Moisture at 2019 Experiment Site as Measured in Each Vegetation Zone.

Treatment	2019							
	Day							
	1	2	3	4	5	6	7	1-7
	Mean Chamber Air Temperature (°C)							
Grass Mixture	26.9	25.9	28.3	25.8	21.1	19.6	18.1	23.8
Corn	27.2	25.9	28.9	25.3	20.7	20.0	18.4	23.9
Saline No Vegetation	27.0	26.0	29.5	26.2	21.9	20.1	19.6	24.5
	Mean Soil Temperature (°C)							
Grass Mixture	23.8 b	24.1	25.7 b	25.4	22.2	19.9	20.5	23.1 b
Corn	23.5 b	23.1	24.9 b	22.7	19.5	17.7	17.7	21.4 b
Saline No Vegetation	27.1 a	26.8	29.4 a	28.3	24.1	20.8	23.1	25.7 a
	Mean Soil Moisture (cm^3 cm^{-3})							
Grass Mixture	0.41 b	0.42 b	0.42 b	0.42 b	0.43 b	0.43 b	0.42 b	0.42 b
Corn	0.41 b	0.42 b	0.40 b	0.39 c	0.41 c	0.41 c	0.40 c	0.41 c
Saline No Vegetation	0.60 a	0.58 a	0.58 a	0.58 a	0.57 a	0.56 a	0.56 a	0.58 a

Soil Tests

2018

Analysis was examined by year due to the different environmental conditions and because the objective was to use annual soil data to help support findings in the flux data collected rather than to determine soil changes over time. During the 2018 study, soil $EC_{1:1}$ in the grass and corn chambers in both urea and non-urea treatments had similar values in the 0- to 15- cm depth (Table 3.5). Saline chambers had a higher $EC_{1:1}$ value at the 0- to 15- cm depth, with a mean $EC_{1:1}$ of 3.87 dS m^{-1} (Table 3.5). Compared to a mean $EC_{1:1}$ of 1.01 dS m^{-1} in the corn and grass chambers (Table 3.5). At the 15- to 30- cm depth, corn treatments had the lowest $EC_{1:1}$ of 0.52 to 0.80 dS m^{-1}, whereas grass and saline zones were greater and averaged 2.11 dS m^{-1} (Table 3.5).

Soil pH values had differences among treatments in the 0- to 15- cm depth. The grass area had an average pH of 7.31 (Table 3.5). This was similar to the soil pH of 7.34

in the corn area (Table 3.5). The saline zone had the lowest pH 6.84 (Table 3.5). pH in the 15- to 30- cm depth was similar across all zones averaged 7.53 (Table 3.5).

Most N in the soil profile was in the NO_3^- form and in the top 0- to 15- cm of the profile (Table 3.5). The beginning soil total N baseline tests for the corn, grass, and saline areas were 78, 181, and 169 ug g^{-1} at the 0- to 15- cm depth, respectively, and 63, 28, and 20 ug g^{-1} at the 15- to 30- cm depths, respectively. In the 0- to 15- cm depths the highest NO_3^- was found in the saline urea treatment with 137 ug g^{-1} (p=0.1) (Table 3.5), whereas the corn area had the lowest NO_3^- level of 6 ug g^{-1} in the non-urea treatment and 22 ug g^{-1} in the urea treatment (Table 3.5). The high NO_3^- values found in the saline zone could be due to lack of plants available to remove the nitrogen from the soil. In the 15- to 30- cm depths, all treatments had similar NO_3^- values with the exception of the saline urea treatment which had the highest NO_3^- value of 25 ug g^{-1} (Table 3.5).

Na in the 0- to 15- cm depth was highest in the saline area, with the saline urea treatment chambers averaging 1804 ug g^{-1} (Table 3.5) The lowest Na levels were found in the corn area, averaging 72 ug g^{-1} Na across the treatments (Table 3.5). The grass zone averaged 343 ug g^{-1} in Na levels. The 15- to 30- cm depth Na levels were highest in the saline area averaging 1026 ug g^{-1} across treatments (Table 3.5). Similar to $EC_{1:1}$ data, Na values also indicate the grass zone was on a transitional zone with slightly elevated levels of Na and $EC_{1:1}$ (Table 3.5).

The Na:$EC_{1:1}$ ratio helps depict how much of the soil $EC_{1:1}$ is a result of Na levels, as Na is a salt in the soil that increases $EC_{1:1}$. This ratio showed that in the 0- to 15- cm depth the saline area had the highest ratio value averaging 432 (Table 3.5) Corn

and grass areas had similar values averaging 189 (Table 3.5). Urea treatment did not impact the ratio.

2019

In the 0- to 15- cm depth the highest $EC_{1:1}$ was in the saline area at 4.96 dS m^{-1} when averaged between urea treatments (Table 3.6). Urea addition did not impact $EC_{1:1}$ in all treatments for both depths (Table 3.6). The grass area averaged an $EC_{1:1}$ of 1.12 dS m^{-1} and corn area treatments averaged an $EC_{1:1}$ of 0.41 dS m^{-1} (Table 3.6). At the 15- to 30- cm depth the saline zone had the highest in $EC_{1:1}$, averaging 2.29 dS m^{-1} (Table 3.6). Averaged across urea treatments, grass zone subsoil averaged 1.31 dS m^{-1} while the corn zone averaged 0.45 dS m^{-1}.

Soil pH was similar across all treatments for both depths tested (Table 3.6). When averaged across urea treatments, the pH for the 0- to 15- cm depth in grass, corn, and saline areas was 6.83. In the 15- to 30- cm depth the pH in grass, corn, and saline areas averaged 7.20.

Most soil N was in the nitrate form in 2019 as it was in 2018. Baseline NO_3^- levels before urea application were 5, 6, and 169 ug g^{-1} for corn, grass, and saline areas, respectively, at the 0- to 15- cm depth and 1, 1, and 34 ug g^{-1} at the 15- to 30- cm depth, respectively. NO_3^- content was similar across treatments for both depths tests. Averaged across treatments, soil NO_3^- was 55 ug g^{-1} at the 0- to 15- cm depth at the end of the experiment. At the 15- to 30- cm NO_3^- levels averaged 21 ug g^{-1} across treatments (Table 3.6).

Soil Na levels in the 0- to 15- cm depth was not affected by urea application and were highest in the saline area which averaged 1351 ug g^{-1}. The corn area averaged 67 ug

g^{-1} and the grass area averaged 191 ug g^{-1} (Table 3.6). The Na levels in the 15- to 30- cm depth in the saline area averaged 886 ug g^{-1} and was higher than the corn and grass treatments (Table 3.6). The grass urea treatment Na levels were higher than the no urea treatment with 236 ug g^{-1} and 383 ug g^{-1} Na, respectively, and is likely due to inherent variability (Table 3.6). Corn had the lowest Na levels at 98 ug g^{-1} averaged across urea treatments (Table 3.6).

Soil Na:$EC_{1:1}$ values were only different among treatments and the 0- to 15- cm depth. Corn no urea and both saline treatments had the highest Na:$EC_{1:1}$ levels of 266 and 273, respectively. The grass area averaged 166 Na:$EC_{1:1}$ which was similar to the corn urea treatment (Table 3.6). These results were similar to 2018 where the saline zone had the highest Na:$EC_{1:1}$ ratio as well.

Table 3.5. Soil Test Values for 2018 Study in Clark, SD on July 23.

	EC		pH		NO_3^--N		NH_4-N		Na		Na:EC	
	-------dS m^{-1}-------				----------ug g^{-1}----------							
Treatment	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm
Corn No Urea	0.37 b	0.80 bc	7.49 a		6	5 b			72 b	141 b	198 b	175 b
Corn Urea	0.40 b	0.52 c	7.18 ab		22	3 b			71 b	131 b	182 b	264 b
Grass No Urea	1.80 b	1.81 ab	7.37 a	7.53	61	8 b	6	4	438 b	346 b	209 b	188 b
Grass Urea	1.47 b	1.86 a	7.25 a		64	5 b			248 b	307 b	167 b	165 b
Saline No Urea	3.66 a	2.37 a	6.85 bc		78	17 ab			1555 a	1070 a	421 a	452 a
Saline Urea	4.07 a	2.40 a	6.83 c		137	25 a			1804 a	982 a	443 a	407 a
P-value	0.006	0.017	0.015	0.275	0.098	0.043	0.540	0.640	0.002	0.001	0.004	0.003
Corn vs Grass	*	***	ns	ns	ns	ns	ns	ns	ns	*	ns	ns
Corn vs Saline	***	***	***	ns	**	***	ns	ns	***	***	***	***
Saline vs Grass	***	ns	***	ns	ns	**	ns	ns	***	***	***	***

ns=not significant

* Significant at α=0.1

** Significant at α=0.05

*** Significant at α=0.01

Table 3.6. Soil Test Values for 2019 Study in Clark, SD on July 24.

	EC		pH		NO_3^--N		NH_4-N		Na		Na:EC	
	-------dS m^{-1}-------		----------ug g-1----------									
Treatment	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm	0- to 15- cm	15- to 30- cm
Corn No Urea	0.24 c	0.32 e						4 a	64 c	81 d	266 a	310
Corn Urea	0.57 bc	0.58 de						4 a	70 c	114 cd	127 b	
Grass No Urea	0.88 bc	1.02 cd	6.83	7.20	55	21	9	3 ab	136 bc	236 c	157 b	
Grass Urea	1.36 b	1.60 bc						2 ab	245 b	383 b	175 b	
Saline No Urea	4.95 a	2.42 a						1 b	1350 a	935 a	273 a	
Saline Urea	4.98 a	2.16 ab						1 b	1352 a	873 a	273 a	
P-value	<0.001	<0.001	0.369	0.206	0.256	0.231	0.757	0.029	<0.001	<0.001	0.004	0.577
Corn vs Grass	**	***	*	ns	ns	ns	ns	**	*	***	ns	ns
Corn vs Saline	***	***	ns	ns	**	ns	ns	***	***	***	ns	ns
Saline vs Grass	***	***	*	ns	*	ns	ns	**	***	***	**	*

ns=not significant

* Significant at α=0.1

** Significant at α=0.05

*** Significant at α=0.01

Flux

2018

Carbon Dioxide

All CO_2 emissions can be observed as a time series that shows changes in flux across treatments during the study (Figure 3.1). Corn soil treated with urea had the highest mean CO_2-C flux of 2,553 g CO_2-C ha^{-1} hr^{-1} (Table 3.7). This was higher than grass zone urea, corn zone no urea, grass zone no urea, saline zone urea, and saline zone no urea by 19%, 40%, 61%, 215%, and 318%, respectively (Table 3.7). The saline zone had the lowest CO_2-C flux throughout the study, whether or not it was treated with urea (Table 3.7, Figure 3.1). The grass zone produced slightly lower CO_2-C than the corn zone and was occasionally similar such as days 2-4.

When days 1-2 were averaged together, representing the time period before the 31 mm precipitation event, corn and grass zones treated with urea at 224 kg N ha^{-1} had the highest CO_2-C emissions, averaging 2,348 g CO_2-C ha^{-1} hr^{-1} (Table 3.7). Corn and grass zones with no added urea had the second highest emissions during this interval with flux

averaging 1412 CO_2-C ha^{-1} hr^{-1}(Table 3.7). Saline soil had the lowest CO_2-C flux rate of 446 g CO_2-C ha^{-1} hr^{-1} when no urea was added, and 669 g CO_2-C ha^{-1} hr^{-1} when urea was added (Table 3.7).

These results indicate the soil under saline conditions had lower CO_2-C emissions compared to both the corn and grass areas. Soil CO_2 is produced primarily due to biological respiration from plant roots and soil microbes (USDA-NRCS, 2019). Because the saline zone had no established plants, there were no roots to respire CO_2.

Differences were not only due to vegetation however, as differences within zones were also seen due to urea application. The addition of urea increased CO_2-C flux in grass zone chambers by 34%, corn zone by 40%, and saline zone by 33% over the 7 days (Table 3.7). Both plant roots and microbes in the soil utilize nitrogen, which may also result in higher respiration and thus increase CO_2-C flux. Additionally, the hydrolysis reaction of the urea molecule in soil also produces CO_2. The addition of urea at the rate of 224 kg N ha^{-1} would correspond to 40750 g CO_2-C ha^{-1}.

The general conclusions from CO_2 analysis are that CO_2 emissions increased after a rainfall and declined as soil moisture decreased (Figure 3.1, Figure 3.2). Also, saline affected soil had the lowest CO_2 emissions. While this could be interpreted as beneficial to mitigating the additions to global GHG concentrations, it must be noted that CO_2 emissions are an indicator of soil health and microbial activity. Also, these soils are not sequestering any carbon from the atmosphere through plant growth meaning there is likely a higher net loss of carbon from the saline soil when compared to vegetated soils.

When relating the soil test data to the flux data from non-treated chambers, it can be seen that the soils with higher $EC_{1:1}$, Na, and Na:$EC_{1:1}$ values had lower CO_2-C

emissions; further displaying that high soil salinity may inhibit biological populations, both plant and microbial.

Table 3.7. 2018 Mean Flux of CO_2-C, N_2O-N, and CH_4-C as Observed for 7 Days from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N.

Treatment	Day 1	2	3	4	5	6	7	1-7
	Daily Mean Soil CO_2-C Flux (g ha^{-1} hr^{-1})							
	---g ha^{-1} hr^{-1}---							
Corn No Urea	1547 c	1233 bc	1961 b	2075 bc	2146 b	2082 b	1733 b	1821 c
Corn Urea	2205 a	2728 a	3123 a	2802 a	2471 a	2443 a	2081 a	2553 a
Grass No Urea	1411 c	1455 b	1853 b	1860 c	1606 c	1541 c	1392 c	1589 d
Grass Urea	1899 b	2561 a	2744 a	2464 ab	1811 c	1860 b	1563 bc	2137 b
Saline No Urea	660 d	198 d	745 c	760 d	563 d	620 d	747 d	611 f
Saline Urea	762 d	560 cd	1352 bc	960 d	625 d	669 d	739 d	810 e
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil N_2O-N Flux (g ha^{-1} hr^{-1})							
	---g ha^{-1} hr^{-1}---							
Corn No Urea	0.00 d	0.03	0.03 c	0.02 c	0.01 d	0.00 c	0.01 c	0.01 c
Corn Urea	0.04 cd	0.16	0.25 c	0.31 bc	0.35 cd	0.59 b	0.41 b	0.29 bc
Grass No Urea	0.01 cd	0.80	1.63 bc	0.11 bc	0.04 d	0.02 c	0.02 c	0.38 bc
Grass Urea	0.08 c	0.83	0.78 c	0.82 b	0.68 c	0.78 b	0.61 b	0.64 b
Saline No Urea	0.20 b	2.32	7.19 a	2.45 a	1.35 b	0.66 b	0.54 b	2.09 a
Saline Urea	0.29 a	2.00	4.00 b	2.78 a	1.89 a	1.22 a	1.12 a	1.88 a
P-value	<0.001	0.16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CH_4-C Flux (g ha^{-1} hr^{-1})							
	---g ha^{-1} hr^{-1}---							
Corn No Urea	-0.395 d	-0.130 bc	-0.193 c	-0.257 c	-0.284 c	-0.329 c	-0.378 d	-0.284 c
Corn Urea	-0.471 e	-0.192 c	-0.233 d	-0.270 c	-0.304 c	-0.343 c	-0.357 d	-0.312 d
Grass No Urea	-0.183 b	-0.060 ab	-0.067 b	-0.099 b	-0.088 b	-0.110 b	-0.116 bc	-0.106 b
Grass Urea	-0.221 c	-0.121 bc	-0.076 b	-0.077 b	-0.092 b	-0.102 b	-0.155 c	-0.121 b
Saline No Urea	-0.023 a	-0.001 a	0.002 a	-0.005 a	-0.009 a	-0.014 a	-0.025 a	-0.011 a
Saline Urea	-0.037 a	-0.002 a	-0.006 a	-0.006 a	-0.008 a	-0.068 b	-0.069 ab	-0.027 a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 3.1 Time Series Carbon Dioxide Flux Data Collected in Field from July 17, 2018 to July 24, 2018 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrow Indicates 31 mm Precipitation Event.

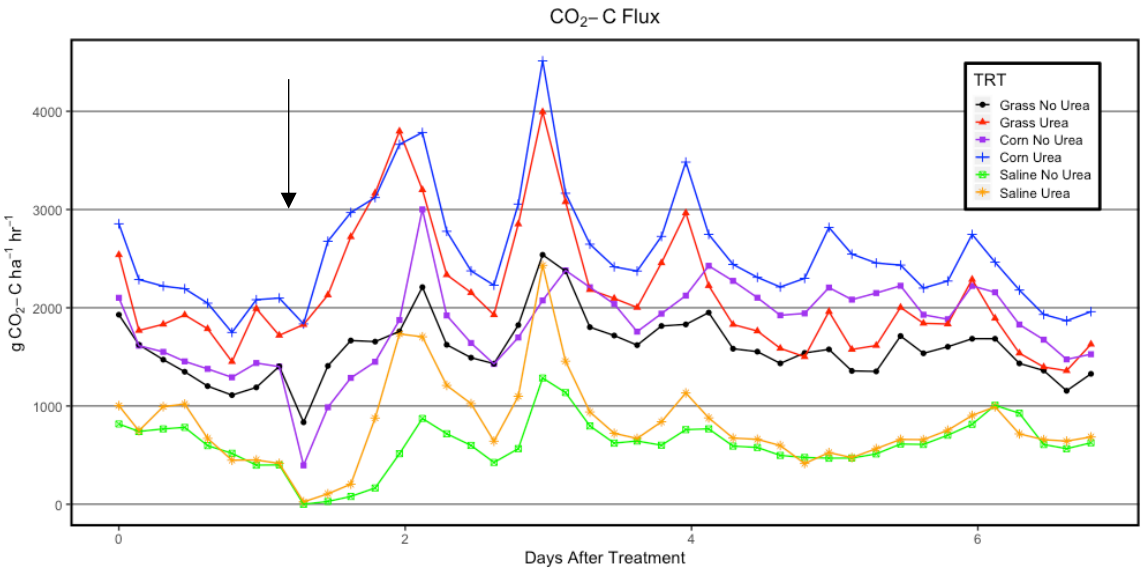
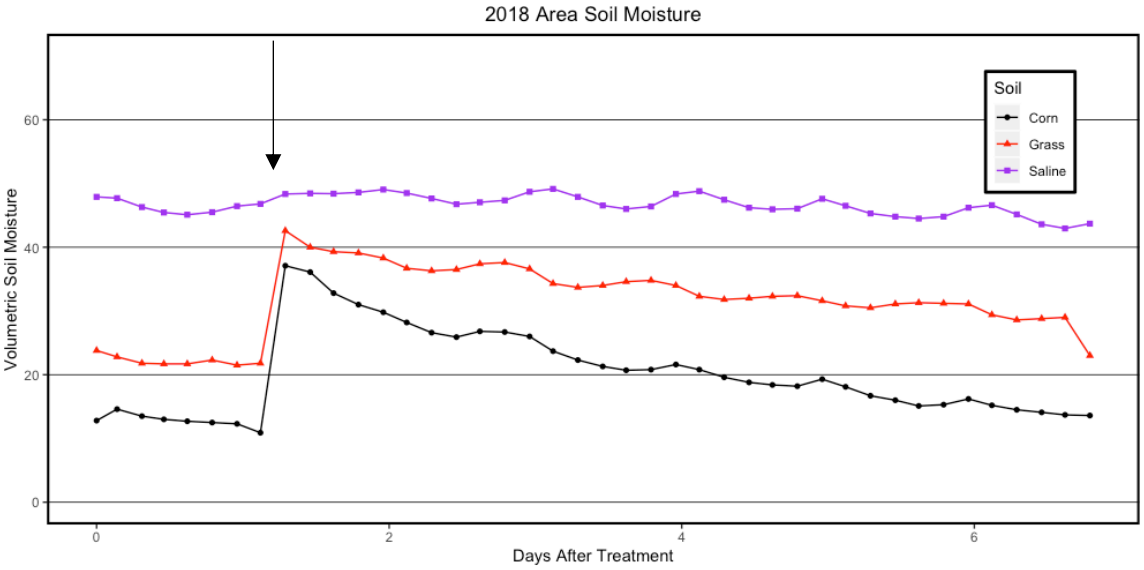


Figure 3.2 Time Series Soil Volumetric (%) Moisture Collected in Field from July 17, 2018 to July 24, 2018 in Clark County, SD from Saline, Grass, and Corn Zones. Arrow Indicates 31 mm Precipitation Event.



Nitrous Oxide

Nitrous oxide emissions are presented in a time series graph (Figure 3.3). The saline zone averaged the highest N_2O-N flux regardless of treatment over the 7 days with an average flux of 1.99 g N_2O-N ha^{-1} hr^{-1}. The average N_2O-N flux from the saline areas was higher than grass zone no urea, grass zone urea, corn zone no urea, and corn zone urea by 423%, 211%, 19800%, and 586%, respectively.

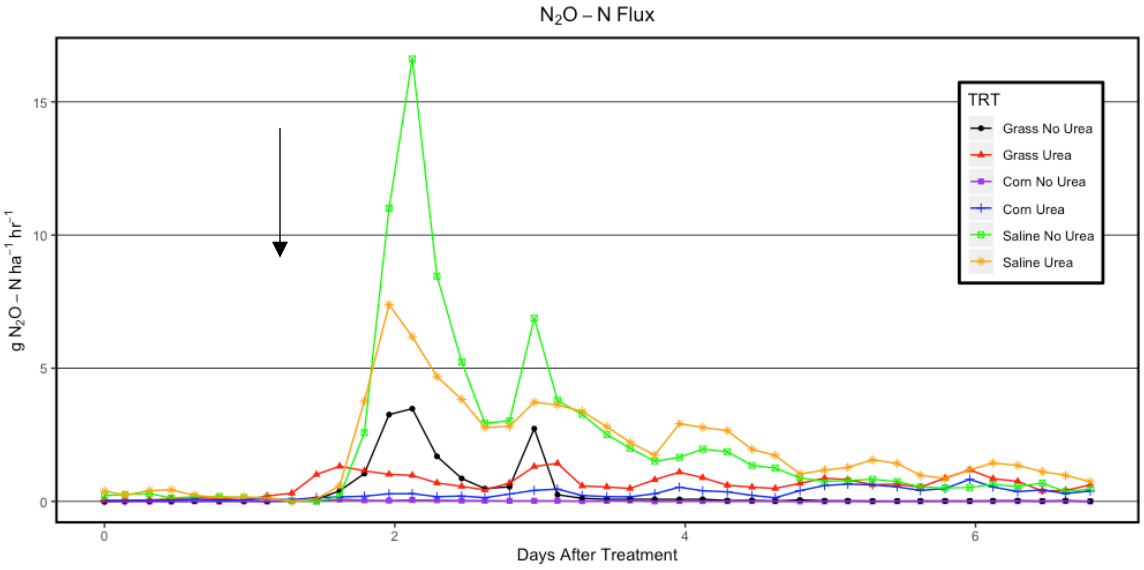
On day 1, before the rainfall, N_2O-N emissions were at the lowest averaging 0.1 g N_2O-N ha^{-1} hr^{-1} across treatments. After the precipitation on day 3, the saline zone had the peak observed N_2O-N emissions and averaged 7.19 g N_2O-N ha^{-1} hr^{-1} in no urea treatment and 2.78 g N_2O-N ha^{-1} hr^{-1} when treated with urea (Table 3.7).

Urea addition had mixed impacts on N_2O flux. In the saline area, urea increased N_2O-N emissions on days 1, 5, 6, and 7 of the study by 45%, 40%, 85%, and 107% (Table 3.7). However, averaged over the 7 days urea application did not influence N_2O emissions in saline soil. Urea increased N_2O flux in the grass zone on days 5-7 as well by an average of 2,783%, however again there was no difference with non-treated grass areas when averaged over the 7-day period (Table 3.7). Likewise, the corn area also was not influenced by urea when averaged over the entire study.

While urea addition may not influence N_2O emissions in this study as much as other conditions such as temperature, vegetation, or moisture; it is still seen that the saline area of the field released much larger amounts of N_2O-N when compared to soil under grass or corn vegetation. This suggests that N_2O emissions could be minimized by maintaining vegetative growth on the soil, whether it is corn or grass. This is likely due to plants shading the soil; which decreased mean soil temperature which is a driver in N_2O

emissions from soil. Plants also use water in the soil which can shorten the time the soil is at heightened moisture contents, which is also a driver of N_2O emissions (Figure 3.2). This effect was seen in Table 3.3 where the saline soil had higher soil temperature as well as moisture content as seen in Figure 3.2. Additionally, plants use nitrogen in the soil for growth. By transferring nitrogen from soil into the vegetative biomass, the reduced nitrogen content of the soil reduces the levels of nitrogen in the soil to be lost as N_2O. This could be the case, as the saline soil had higher NO_3^- content compared to the grass and corn zone soils (Table 3.5).

Figure 3.3 Time Series Nitrous Oxide Flux Data Collected in Field from July 17, 2018 to July 24, 2018 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrow Indicates 31 mm Precipitation Event.



Methane

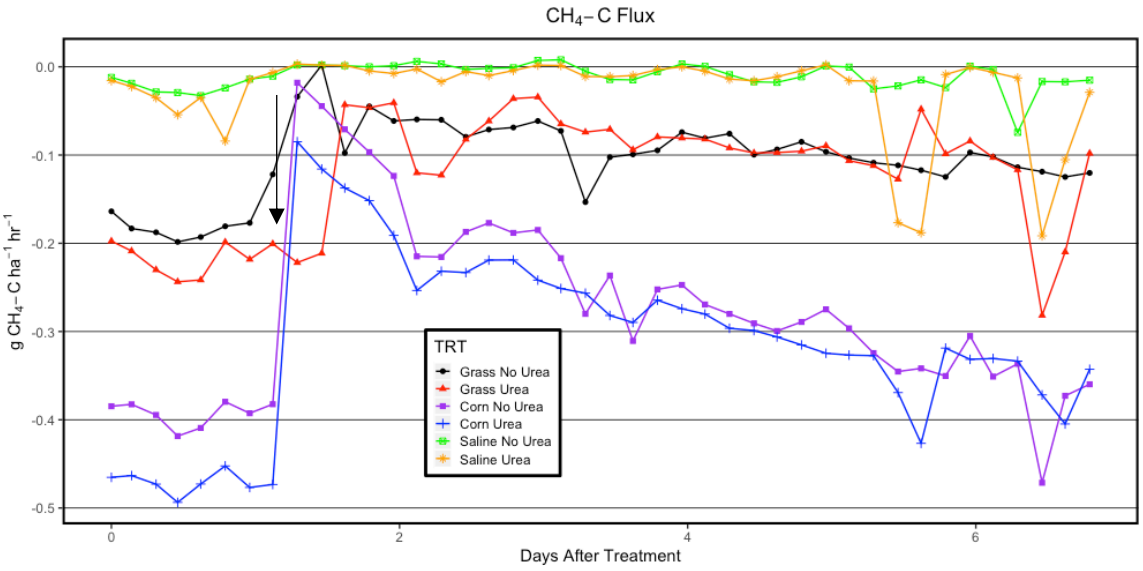
Methane emissions during the study were almost all negative values, meaning the soil was acting as a sink for CH_4, rather than a source. This allows analysis of the environmental benefits the different soil treatments are providing in terms of CH_4 removal from the atmosphere. When averaged over the length of the 7-day study, corn urea had the lowest CH_4-C flux of -0.312 g CH_4-C ha^{-1} hr^{-1}, whereas saline no urea had the highest flux of -0.0106 g CH_4-C ha^{-1} hr^{-1}, with grass treatment's flux rates falling between the two. Throughout the study, corn and grass treatments removed more CH_4 than the saline zone. Although the saline soils did not emit CH_4 in large amounts, they failed to remove CH_4 from the atmosphere as effectively as vegetated soils.

After the 31 mm rain event, the CH_4-C flux increased (Table 3.7, Figure 3.4). This increase was seen most drastically in the corn zone but still occurred in all treatments. This is because methane is produced by methanogenesis, carried out by soil bacteria in an anaerobic environment. Because the rain fell heavily, soil conditions became saturated for several hours. This may have allowed the rate of methanogenesis to increase temporarily as conditions became more reducing with a lack of oxygen and limited the rate of methane oxidation.

Urea additions did not impact CH_4-C flux as much as it did with CO_2-C emissions (Table 3.7). Although there was a difference in corn treatments with urea, this was not seen with grass or saline zones. Although the finding that urea increased the capacity of the soil to remove methane in the corn zone, the difference was small and not seen in grass or saline zones (Table 3.7).

The results show the saline soil took longer to recover from a rainfall event in terms of fixing CH_4 and has a limited ability to remove CH_4 from the atmosphere. When compared to corn and grass treatments, it appears that the corn vegetated soil functions better at fixing CH_4 than grass vegetated soil, however, this difference could be explained by the soil conditions. Table 3.5 shows that the grass zone selected for study had a higher $EC_{1:1}$ than the corn zone. This slightly elevated $EC_{1:1}$ may have influenced CH_4 emissions or fixation in the grass zone. Also, these values are very small and are relatively a small proportion of the total GHG emissions measured.

Figure 3.4 Time Series Methane Flux Data Collected in Field from July 17, 2018 to July 24, 2018 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrow Indicates 31 mm Precipitation Event.



2019

Carbon Dioxide

The second year of the study saw similar results to 2018. Grass and corn followed very similar flux patterns in 2019 in both urea and no urea treatments. Averaged over the 7 days, the grass and corn treatments with urea had the highest flux rate averaging 3089 g CO_2-C ha^{-1} hr^{-1} (Table 3.8). The grass no urea treatment was the next highest at 2,538 g CO_2-C ha^{-1} hr^{-1} which was 40% higher than the corn with no urea (Table 3.8). The saline zone had the lowest fluxes for CO_2 and was 324 g CO_2-C ha^{-1} hr^{-1} with no urea additions and 825 g CO_2-C ha^{-1} hr^{-1} when urea was added (Table 3.8). This is similar to 2018 where the saline soil had the lowest CO_2 emissions, however, grass and corn zone emissions increased compared to 2018 due to higher temperatures during the first four days (Table 3.1, Table 3.2).

After the 8.4 cm rainfall event at the start of day 5, CO_2 emissions decreased due to saturated soil conditions and a decrease in temperature (Figure 3.5). Emissions decreased in the grass zone by 27%, corn by 22%, and saline by 45% as a result.

Also similar to 2018, urea increased emissions in all vegetative zones as well. Urea increased CO_2 flux in grass chambers on days 3, 4, and 5 by 24%, 33%, and 23%, respectively, and increased CO_2 flux by an average of 19% over the week (Table 3.8). CO_2 flux from corn increased due to urea on every day measured by an average of 75% (Table 3.8). Urea increased CO_2 flux in saline soil by an average of 155% (Table 3.8). Less differences were seen between urea and non-urea treatments in all vegetation zones after day 4 when temperatures cooled, and the heavy precipitation may have leached some of the nitrogen away and minimized the effects of the added nitrogen.

The results from both 2018 and 2019 found similar trends and show that grass and corn vegetated soils produced similar levels of CO_2 emissions. Because CO_2 is used as an indicator of soil health, this is a positive outcome when compared to the saline soil (USDA-NRCS, 2019). Also, the grass vegetation treatment had potential to have higher CO_2 emissions than measured because it was located in a slightly more saline soil than the corn and salinity inhibits CO_2 evolution. This effect was seen in the saline soil which had the highest $EC_{1:1}$ of 4.95 dS m^{-1} and also the lowest CO_2 emissions. The saline soil had the lowest CO_2 fluxes both years due to limited plant and root growth and possibly reduced microbial activity. The measured soil emissions do not account for carbon sequestration by the plants themselves in the grass and corn zones, which would reduce net emissions. Thus, net carbon fluxes of the plant-soil systems cannot be computed for these zones. However, since there are no plants in the saline zone, the flux measured in the saline zones is also the net loss of carbon from the soil. Also, both years of experiments found urea to increase CO_2 emissions.

Table 3.8. 2019 Mean Flux of CO_2-C, N_2O-N, and CH_4-C as Observed for 7 Days from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N.

Treatment	Day 1	2	3	4	5	6	7	1-7
	Daily Mean Soil CO_2-C Flux (g ha^{-1} hr^{-1})							
	----------g ha^{-1} hr^{-1} ----------							
Corn No Urea	2239 b	2142 c	2263 bc	1673 c	1381 c	1396 b	1436 b	1812 c
Corn Urea	4801 a	4054 a	3610 a	2958 ab	2147 a	2022 a	2046 a	3162 a
Grass No Urea	3817 a	3070 b	2883 b	2294 bc	1746 b	1815 ab	1759 ab	2538 b
Grass Urea	4198 a	3488 ab	3564 a	3052 a	2156 a	1233 a	2186 a	3015 a
Saline No Urea	358 c	363 d	414 d	274 d	233 d	312 c	198 c	324 e
Saline Urea	729 c	1128 d	1777 c	825 d	202 d	524 c	564 c	825 d
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil N_2O-N Flux (g ha^{-1} hr^{-1})							
	----------g ha^{-1} hr^{-1} ----------							
Corn No Urea	1.25	0.72 c	0.48 c	0.76 cd	0.35 b	0.34 c	0.34 c	0.62 c
Corn Urea	4.26	5.25 bc	3.47 b	5.95 b	5.82 a	3.29 b	2.34 b	4.35 b
Grass No Urea	0.8	0.67 c	0.50 c	0.32 d	0.35 b	0.27 c	0.21 c	0.46 c
Grass Urea	1.31	3.53 c	3.77 b	5.03 bc	5.75 a	4.07 b	2.90 b	3.70 b
Saline No Urea	3.39	8.73 ab	4.96 b	3.14 bcd	5.52 a	3.12 b	2.94 b	4.89 b
Saline Urea	5.57	13.13 a	13.25 a	10.85 a	5.42 a	10.65 a	11.34 a	9.87 a
P-value	0.075	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CH_4-C Flux (g ha^{-1} hr^{-1})							
	----------g ha^{-1} hr^{-1} ----------							
Corn No Urea	-0.086 b	-0.052 c	-0.080 c	-0.048 bc	-0.042 c	-0.037 b	-0.054 d	-0.058 c
Corn Urea	-0.140 c	-0.054 c	-0.058 bc	-0.066 c	-0.024 b	-0.039 b	-0.045 cd	-0.064 c
Grass No Urea	-0.076 b	-0.027 b	-0.051 b	-0.048 bc	-0.018 b	-0.029 b	-0.036 bc	-0.041 b
Grass Urea	-0.056 b	-0.040 bc	-0.033 b	-0.026 b	-0.012 b	-0.027 b	-0.027 b	-0.033 b
Saline No Urea	0.012 a	0.014 a	0.011 a	0.007 a	0.005 a	0.007 a	0.005 a	0.009 a
Saline Urea	0.009 a	0.009 a	-0.004 a	0.007 a	0.005 a	0.007 a	0.002 a	0.005 a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 3.5 Time Series Carbon Dioxide Flux Data Collected in Field from July 16, 2019 to July 23, 2019 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrows Indicate Chronological 13, 3, and 84 mm Precipitation Events.

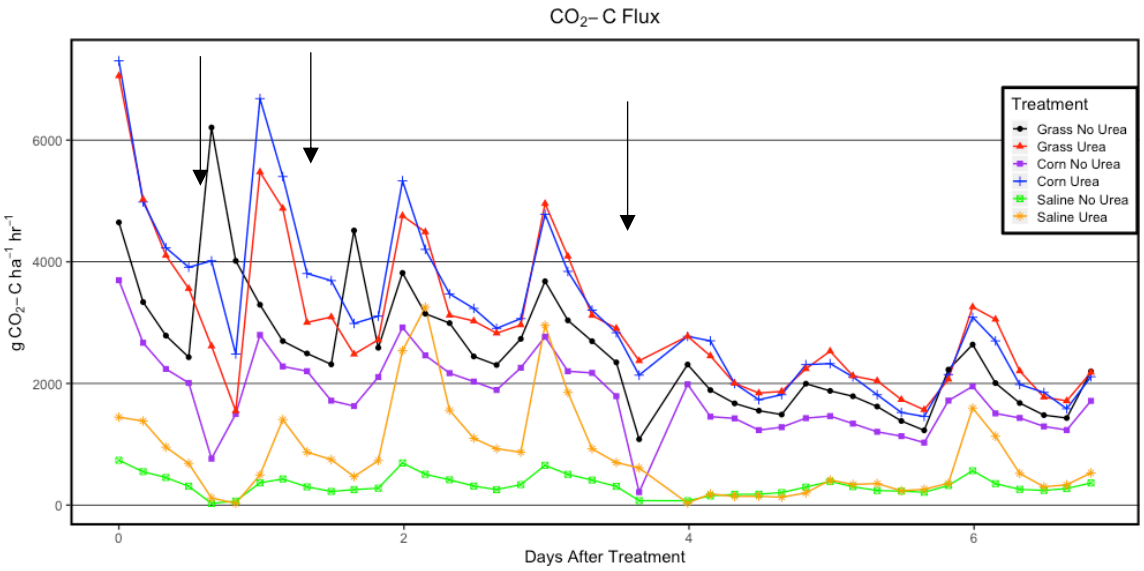
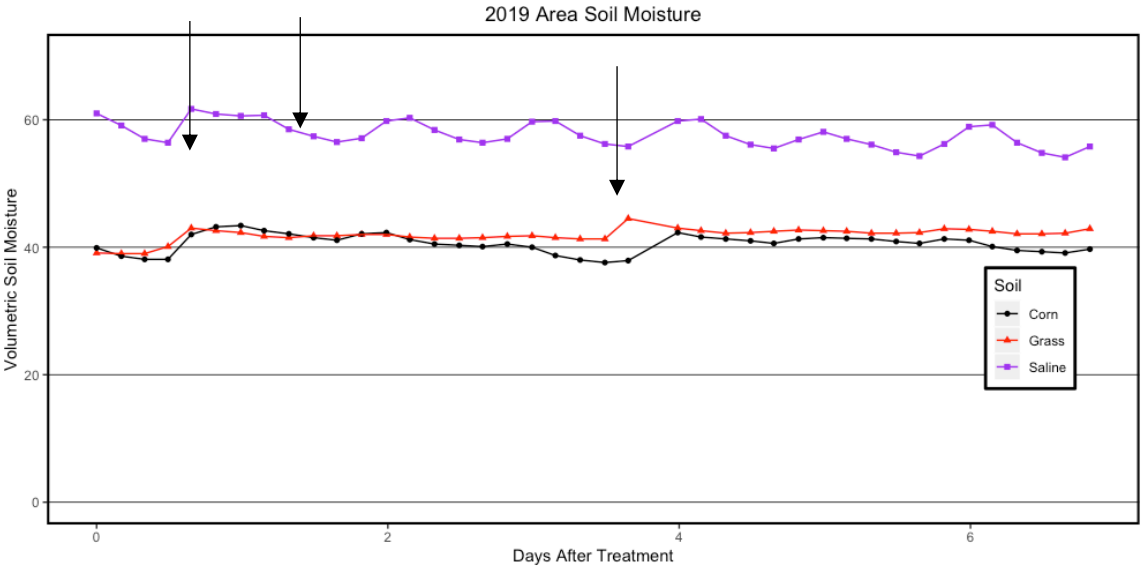


Figure 3.6 Time Series Soil Volumetric (%) Moisture Collected in Field from July 16, 2019 to July 23, 2019 in Clark County, SD from Saline, Grass, and Corn Zones.



Nitrous Oxide

Averaged over the seven day study in 2019 the saline urea treatment produced the highest N_2O-N flux of 9.87 g N_2O-N ha^{-1} hr^{-1} which was 2,046%, 167%, 1,492%, 127%, and 102% higher than grass zone no urea, grass zone urea, corn zone no urea, corn zone urea, and saline zone no urea, respectively (Table 3.8). N_2O-N fluxes were similar among treatments during day 1 (Figure 3.7).

The grass and corn treatments with no urea added had the lowest emissions throughout the study and remained below 1 g N_2O-N ha^{-1} hr^{-1} for most of the measured period, averaging 0.46 and 0.62 g N_2O-N ha^{-1} hr^{-1} for grass and corn, respectively (Table 3.8, Figure 3.7). In contrast, the saline zone with no urea added averaged 4.89 g N_2O-N ha^{-1} hr^{-1} over the 7 days and peaked at 21 g N_2O-N ha^{-1} hr^{-1} after the first rain event 0.75 days into the study (Table 3.8, Figure 3.7).

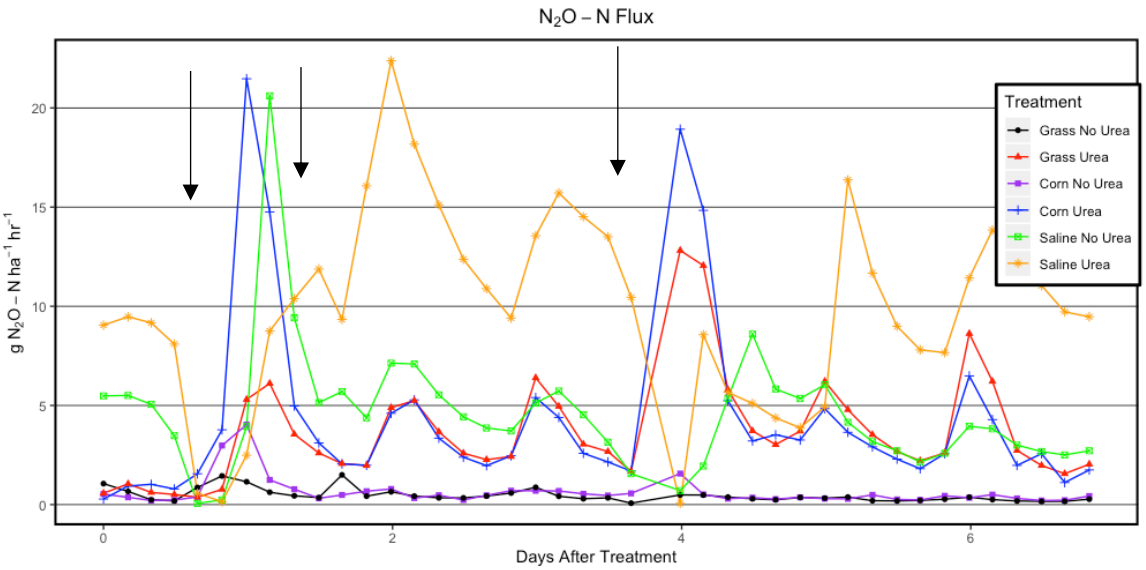
Urea increased N_2O emissions in all treatment zones. When urea was added, both grass and corn averaged higher emissions of 3.70 and 4.35 g N_2O-N ha^{-1} hr^{-1}, with higher peaks after rainfall events on 0.75 and 3.8 days after urea application (Table 3.8, Figure 3.7). This was a 704% and 602% increase for grass and corn, respectively. Urea addition to the saline soil increased N_2O-N emissions by an average of 102% (Table 3.8).

The results of the N_2O flux data reveals the lack of environmental services provided by saline soils. Figure 3.5 shows that saline soils without urea applied produced N_2O emissions similar to those found in the corn and grass zones which had urea applied. This demonstrated what may have occurred when these areas were managed separately. However, when treated with identical management and urea was applied to the saline soil as well, emissions more than doubled. The N_2O-N emissions from the saline soil with

urea were initially lower than the same treatment in corn and grass after a rainfall for a short time, however this was due to standing water in the chamber rings preventing gas evolution from the soil during that time. After the water infiltrated a few hours later or was manually removed, N_2O-N flux increased rapidly to levels ~300% higher than those in the corn and grass treatments with urea (Figure 3.7). N_2O is 298 times more potent of a GHG than CO_2 (EPA, 2018). Therefore, the emission of N_2O is not desirable and the saline soil produces a disproportionate level of N_2O when compared to corn or grass vegetated non-saline soils.

The high levels of N_2O produced in the saline soils could be the result of several factors. First, soil temperature was found to be higher in the saline soils compared to grass or corn soils by 2.6°C to 4.3°C, respectively, which were shaded by residue and plant growth (Table 3.4). Second, soil moisture was higher in the saline soil compared to grass or corn soils by 0.17 cm^3 cm^{-3} (Table 3.4, Figure 3.6). Both of these conditions favor increased N_2O emissions (Smith et al., 1998). Also, soil tests showed that soil NO_3^- was more than double in the saline soil compared to grass and corn, although values were similar due to low sample size and high variability (Table 3.6).

Figure 3.7. Time Series Nitrous Oxide Flux Data Collected in Field from July 16, 2019 to July 23, 2019 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrows Indicate Chronological 13, 3, and 84 mm Precipitation Events.



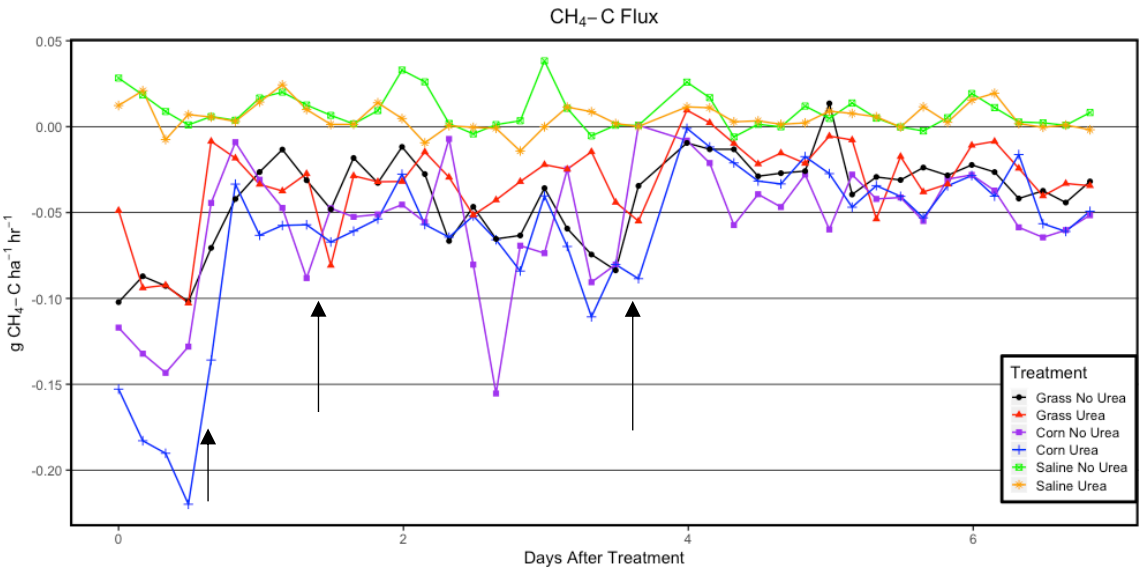
Methane

Similar to 2018, CH_4 flux was near zero for the majority of the study and urea did not impact CH_4 flux. Saline soil CH_4 flux was near zero for the entire study (Table 3.8). The grass vegetated soil initially had a slightly lower flux ~ -0.09 g CH_4-C ha^{-1} hr^{-1} but then increased to ~ -0.035 g CH_4-C ha^{-1} hr^{-1} (Figure 3.8). Likewise, the corn treatment initially had a lower flux ~ -0.15 g CH_4-C ha^{-1} hr^{-1} but then increased to identical levels as grass vegetated soil (Figure 3.8).

These results are similar to those of 2018 where when soil moisture was lower, such as day 1 in 2019, CH_4 fluxes were lower. However, after rainfall and the saturated soil conditions throughout the rest of the study in 2019, CH_4 flux stayed near zero and never decreased again as in 2018 due to constant high moisture in 2019 during the study.

Although the grass and corn vegetated soils fixed more CH_4 than saline soils when dry, the values are very small and not significant compared to N_2O and CO_2 emissions.

Figure 3.8. Time Series Methane Flux Data Collected in Field from July 16, 2019 to July 23, 2019 in Clark County, SD from Saline, Grass, and Corn Zones; with Urea Applied at 0 or 224 kg ha^{-1} N. Arrows Indicate Chronological 13, 3, and 84 mm Precipitation Events.



Emissions Discussion

The addition of urea increased the flux rate of CO_2 in all three sites in this experiment: non-saline corn, non-saline grass, and saline barren. This effect was seen during both years of the study and may partially be due urea hydrolysis by soil enzymes as CO_2 is released as a byproduct (Sigurdarson et al., 2018). Increases in CO_2 as a result of N additions in our study were comparable to those reported by others (Sainju et al.,

2008; Silva et al., 2008). Sainju et al. (2008) reported CO_2 emissions increases of 102% when 78 kg N ha^{-1} was applied as urea and mono ammonium phosphate to a field in Rasmussen, MT compared to a control where no nitrogen fertilizer was applied. Silva et al. (2008) reported an increase in CO_2 emissions by 170% in a saline soil when urea was applied at 200 mg N kg^{-1} dry soil. The results of our study found more modest increases in CO_2 emissions, which in 2018 were 35%, 40%, and 33% in grass, corn, and saline soils, respectively, and in 2019 by 19%, 75%, and 155%, respectively. However, Thies (2018) reported slightly reduced CO_2 emissions in response to urea applications on a non-saline Brandt silty clay loam soil with no crop.

The results reported by Thies (2018) on the impact of urea on N_2O emissions also contradict those found in our study. Thies (2018) reported a 57% reduction in N_2O emissions as a result of urea application at the same rate used in our study. However, this could be due to varying environmental conditions, as our study found N_2O emissions were highly dependent of rainfall and temperature and the study conducted by Thies (2018) was in June, whereas our study was in July. The dependency upon soil moisture and soil NO_3^- content as described by Bremner (1997) may also explain experimental differences. In 2018 our study found that urea application did not increase or decrease N_2O-N emissions when averaged over the 7-day study. However, in 2019 urea increased N_2O-N emissions by 704%, 602%, and 102% in grass, corn, and saline soils, respectively. Likewise, Schmer et al. (2012) conducted a study in North Dakota on switchgrass plots and reported a 213% increase in N_2O emissions in response to 67 kg ha^{-1} N additions. Dusenbury et al. (2008) reported a 354% increase in cumulative N_2O-N emissions in a wheat-wheat no till system when 245 kg N ha^{-1} was applied over the

course of the two years. Silva et al. (2008) found similar increases to our study, with a 765% increase in N_2O flux when urea was added to saline soil at 200 mg N kg^{-1} dry soil in a laboratory study.

Urea did not influence CH_4 emissions in our study which was in contrast to what was theoretically proposed by numerous authors that CH_4 emissions would decrease (Mosier et al., 1991; Bosse et al., 1993; Hansen et al., 1993; Hutsch et al., 1993; Bronson and Mosier, 1994; Dunfield et al., 1995; Dunfield and Knowles, 1995). However, CH_4 emissions in our study were very small, which could may be why differences due to urea were not detected. Our measured CH_4 emissions levels did agree with Topp and Pattey (1997) however, as they proposed that CH_4 flux in soil is typically negative unless under heavily reducing condition. In our study, the only soil to produce positive CH_4 flux was the saline soil which had dispersed soil structure and higher soil moisture, both of which encourage reducing soil conditions. Similarly, Le Mer and Roger (2001) reported that soil CH_4 flux in aerobic soils were rarely higher than 0.1 mg CH_4 m^{-2} h^{-1}. Our study agreed with this, as no CH_4 flux rates were detected higher that 0.007 mg CH_4 m^{-2} h^{-1}.

The average CO_2-C flux rates in our study for 2018 averaged 1589, 1821, and 611 g ha^{-1} hr^{-1} for non-urea treated grass, corn, and saline soils, respectively. In 2019 CO_2-C emissions averaged 2538, 1812, and 324 g ha^{-1} hr^{-1} for non-urea treated grass, corn, and saline soils, respectively. In comparison, Thies (2018) reported flux rates of 1318 g CO_2-C ha^{-1} h^{-1} in June. Frank et al. (2002) reported peak flux rates of 2417 g CO_2-C ha^{-1} hr^{-1} on a non-grazed mixed grass prairie Werner–Sen–Chama complex which was similar to our results in 2019 for the grass mixture with 2538 g ha^{-1} hr^{-1}. Setia et al. (2011) reported that saline soils in Australia had 50% lower CO_2 fluxes compared to non-saline soils. Our

study also found reductions in saline soil CO_2 flux, where saline soils averaged 13-38% of grass and 18-34% of corn CO_2 production. These results and past studies all indicate that the flux rates measured in our study are reasonable and that salt-affected soils have reduced CO_2 emissions. This implies reduced microbial activity, although not directly measured.

The average N_2O-N flux rates in our study for 2018 averaged 0.38, 0.01, and 2.09 g ha^{-1} hr^{-1} for non-urea treated grass, corn, and saline soils, respectively. In 2019 N_2O-N emissions averaged 0.46, 0.62, and 4.89 g ha^{-1} hr^{-1} for non-urea treated grass, corn, and saline soils, respectively. These ranges were similar to those reported by previous researcher's findings. Thies (2018) reported emissions of 0.81 g N_2O-N ha^{-1} hr^{-1} in mid-June and Lai (2017) reported average N_2O emissions of 0.20 g ha^{-1} hr^{-1} from a continuous wheat field in North Dakota. However, neither of these studies measured saline soil GHG emissions. Although our experimental findings in the corn and grass soils were similar to the aforementioned studies, the average N_2O-N emissions were much higher from saline areas. This indicates that barren saline soils likely produce disproportionately higher N_2O emissions, even though there may be reduced microbial activity as mentioned earlier. This could be the result of higher soil moisture, reduced soil oxygen, or chemical reactions within the soil (Hu et al., 2015; Braker and Conrad, 2011; Huang et al., 2015; Khalil et al., 2004; Snyder et al., 2009; Bremner et al., 1980). Revegetating these saline soils has potential to reduce unfavorable N_2O emissions while increasing CO_2 emissions, which while usually not favorable in most cases is desired in the case of soil health. Revegetation may reduce saline soil N_2O emissions by reducing soil nitrate, soil temperature, and soil moisture.

The CH_4-C emissions measured in this study were small in all treatments and ranged from -0.5 to 0.05 g CH_4-C ha^{-1} hr^{-1}. This agreed with Le Mer and Roger (2001) where no peak flux values for CH_4 were above 1 g CH_4 ha^{-1} hr^{-1}. Wang and Bettany (1995) reported a peak CH_4 flux of 0.27 g CH_4 ha^{-1} hr^{-1} after a 79 mm precipitation event. This gives us confidence in our flux values and allows us to examine our data even though values were small. Our results thus show that salt-affected soils, while not necessarily producers of CH_4, do not as effectively remove CH_4 from the atmosphere as they have average fluxes higher than those recorded in the grass and corn soils. However, this is not as large of a concern compared to N_2O which has a GWP of 298 compared to CH_4 GWP of 25 and CO_2 emissions which were produced in larger amounts (up to 6,000 g CO_2-C ha^{-1} hr^{-1}).

CHAPTER 4: IMPACTS OF INDIVIDUAL PLANT COMPONENTS ON GREENHOUSE GAS EMISSIONS AND CHEMICAL PROPERTIES OF A SALINE-SOIL IN SOUTH DAKOTA

4.1 ABSTRACT

Phytoremediation is one method of improving salt affected soils' physical and chemical properties. However, there has been minimal research on how phytoremediation may also influence greenhouse gas emissions (GHG) and their environmental services. This study quantified how individual plant components and whole plant systems influenced saline soil properties ($EC_{1:1}$, pH, Na, NO_3^-, NH_4^+) and GHG emissions. Simulated root exudates, root respiration (via CO_2 injection) and perennial grass mixture tissue residue (12.8 C:N ratio) additions [20% slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners), 35% AC saltlander green wheatgrass (*Elymus hoffmannnii*), 12.5% western wheatgrass (*Agropyron smithii* Rydb), 32.5% creeping meadow foxtail (*Alopecurus arundinaceus* Poir)] were utilized in laboratory experiments to examine the impacts to GHG emissions from a Cresbard-Cavour loam saline soil. Soil samples from saline and non-saline sites also were collected from the field where plants had, or had not, already established and GHG emissions were quantified. A single addition of simulated root exudates did not impact soil chemical properties tested, but increased CO_2-C flux by 91% to 1115 ug CO_2-C kg^{-1} hr^{-1} over the first 24 hrs with differences lasting 4 d. Exudates also increased N_2O-N flux by 3,167% to 1.57 ug N_2O-N kg^{-1} hr^{-1} for 1 d. The 20% CO_2 + 80% N_2 gas injection at 14.5 L hr^{-1} for 6.5 days increased pH from 6.26 to 6.34 and decreased NO_3^- by 25 ug g^{-1} to 64 ug g^{-1} compared to N_2 only. Gas injection had minimal direct changes on soil chemical tests and had no impacts lasting >1 d on CO_2-C

flux after injection ceased. However, CO_2 injection increased N_2O-N flux 198% for 1 d from a base level of 2.81 ug N_2O-N kg^{-1} hr^{-1} and by 88% on day 2 from 1.91 ug N_2O-N kg^{-1} hr^{-1}. Plant residue addition of 3,900 kg ha^{-1} increased N_2O-N flux for at least 8 d by an average of 119% compared to control levels of 0.522 ug N_2O-N kg^{-1} hr^{-1} and CO_2-C flux by 582% from 526 ug CO_2-C kg^{-1} hr^{-1} in the first 8 d and 59% after 10 weeks compared to non-treated control rates of 501 ug CO_2-C kg^{-1} hr^{-1}. Residue additions increased soil $EC_{1:1}$ by 0.12 dS m^{-1} from 2.079 dS m^{-1} and increased NO_3^- from 137 ug g^{-1} to 183 ug g^{-1} compared with non-treated control. After 7 weeks of barley growth soil $EC_{1:1}$ was reduced from 6.30 dS m^{-1} to 5.92 dS m^{-1} and NO_3^- from 509 ug g^{-1} to 428 ug g^{-1} compared to non-planted control and increased N_2O-N and CO_2-C flux by 224% and 244%, respectively, from control levels of 0.359 ug N_2O-N kg^{-1} hr^{-1} and 206 ug CO_2-C kg^{-1} hr^{-1}. Field collected samples in saline sites with AC Saltlander Green Wheatgrass, Creeping Meadow Foxtail, Western Wheatgrass, and Slender Wheatgrass present had an $EC_{1:1}$ of 3.61 dS m^{-1}, pH of 6.48, and sodium content of 1493 ug g^{-1} compared to barren saline soil which had an $EC_{1:1}$=8.03 dS m^{-1}, pH=5.92, and sodium= 2575 ug g^{-1}. CO_2-C flux from saline soil with plants was 124% greater compared to barren saline soil but was similar to the flux from non-saline soil from the same area. Overall, establishing plants on saline soils may increase soil CO_2 and N_2O emissions through substrate additions and residue decomposition, and decrease soil NO_3^- and EC; making conditions more favorable to further revegetation.

4.2 INTRODUCTION

Plant growth is needed for soil health, making it a priority to maintain or reestablish vegetation to areas affected by salinity or sodicity. Phytoremediation has

several benefits over chemical remediation through amendments such as gypsum or elemental sulfur. These include less input costs over time as well as a source of revenue through forage production (Qadir et al., 2006c). Additionally, phytoremediation can stimulate microbial activity through root-soil interactions. More research needs to be done in the Northern Great Plains on phytoremediation, as few publications have investigated the subject thoroughly. It is important to specifically measure not only how plant growth impacts chemical properties of salt affected soil, but also the greenhouse gas fluxes to fully understand what interactions are taking place in the soil. Plants interact with the soil in countless ways. For the purpose of this study, three specific individual interactions were investigated. These individual components included simulated root respiration, simulated root exudate excretion, and aboveground residue accumulation. These components were represented together by growing barley (*Hordeum vulgare*) in saline soil in a laboratory setting, as well as taking field samples where plants revegetated saline soil.

Objectives

To achieve the goal of understanding, analyzing, and quantifying how plants impact saline soils, individual plant system components were tested in separate, but related, experiments to quantify how different plant components play a role in interacting with saline soil. The objectives of these experiments were to measure how plants and their individual components influence saline soil chemical properties such as electrical conductivity (EC), pH, sodium (Na), nitrate (NO_3^-) and ammonium (NH_4^+) concentrations and GHG emissions.

4.3 MATERIALS AND METHODS

Soil Collection

The same Cresbard-Cavour loam saline soil from Clark, SD [44°42'10.99"N, 97°52'44.41"W] was used for the root exudate, plant residue, and root respiration (carbon dioxide) experiments. Soil was collected from the top 15-cm on October 24, 2018. There was no vegetative growth on the site, which was located at the backslope position, where a non-saline soil was transitioning into a saline footslope region of the field. The soil had a starting $EC_{1:1}$ of 2.17 dS m^{-1}, pH of 6.24, Na content of 720 ug g^{-1}, and NO_3^- content of 93 ug g^{-1} and NH_4^+ content of 3 ug g^{-1}.

Due to possible residual mesotrione herbicide present in the soil which was applied on June 27, 2018 at 0.105 kg ai ha^{-1}, a different saline soil was used for the whole plant experiment. The soil for the whole plant experiment was collected on May 3rd, 2018 from the top 15-cm and was on a similar slope position and transition zone located at 44°42'11.49"N, 97°52'44.26"W. However, this soil had a starting EC of 6.29 dS m^{-1}, pH of 6.49, Na content of 2189 ug g^{-1}, NO_3^- content of 519 ug g^{-1}, and NH_4^+ content of 9.5 ug g^{-1}. Soils were stored in a cooler at 2° C from the time of sample collection to experimental use.

A field validation experiment used soil collected from the same field on April 26th, 2019 and was collected with a coring device 5-cm in diameter and 10-cm deep with samples taken from a non-saline area with and without plant growth and also from a saline area with and without plant growth. Soil test values for the four soils can be found in Table 2.7. Plant growth associated with these samples consisted of 55% AC Saltlander green wheatgrass (*Elymus hoffmannnii*), 15% creeping meadow foxtail (*Alopecurus*

arundinaceus Poir), 7.5% western wheatgrass (*Agropyron smithii* Rydb), and 22.5% slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners).

Greenhouse Gas Flux Measurement

For all experiments GHG flux was measured 4-7 days. Measured gases included CO_2, N_2O, and CH_4. To take measurements, a LI-COR 8100A (LI-COR, Lincoln, NE) system which collects gas samples was connected to a Picarro G2508 (Picarro Inc., Santa Clara, CA) system for gas sample analyzation. The LI-COR system was configured according to LI-COR protocols to be used as a flask sampler setup. The flask sampling system uses sealed containers for analysis of small samples, rather than larger LI8100-104 chambers. For all of the conducted experiments, with the exception of the field validation experiment, 500 g of air-dried soil was placed in a 1-L glass jar and sealed with a lid which had inlet and outlet air ports for air circulation. During measurement, the jars used mixed air. The gas samples were collected automatically every hour. A subsample of the collected gas was analyzed with the Picarro G2508 cavity ringdown spectrometer for CO_2, N_2O, and CH_4 measurements. A LI-8150 multiplexer (LI-COR, Lincoln, NE) controlled flask sampling. During individual flask measurement, the system measured gas concentration every second for 10 minutes. While one jar is sampling in a closed system with the analyzer, all other jars have ambient air continuously circulated through them. Because of this, no pre-purge or post-purge time was needed. Every 24 hrs, flasks were weighed on a scale and water was added to keep each at the desired gravimetric soil moisture level between 30 and 35%. Flux measurements were corrected for air temperature. For the field validation experiment, samples were only measured on

the LI-COR system for CO_2, and were variable in sample weight and were measured for 5 minutes during each measurement cycle which was repeated over 3 days.

Flux Calculation

SoilFluxPro™ ver 4.0.1 software was used to calculate exponential flux of all gases. Flux was calculated using the following equations:

$$G' = G'_s + [G'_0 - G'_s]e^{-at} \qquad \text{(Equation 1)}$$

where G' is the instantaneous water vapor dilution-corrected chamber gas mole fraction, G'_s is the water vapor dilution-correction gas concentration in the soil surface layer under the chamber, and a is a rate constant. The flux is calculated using the initial slope ($\frac{\partial G'}{\partial t}$ at $t = 0$) of the function at the time of chamber closing when G' is close to the ambient level (G'_0). $\frac{\partial G'}{\partial t}$ is calculated by the equation

$$\frac{\partial G'}{\partial t} = a[G'_s - G'_0]e^{-at}. \qquad \text{(Equation 2)}$$

Flux is then calculated using the equation

$$F_c = \frac{VP_0(1-W_0)}{RS(T_0+273.15)} \frac{\partial G'}{\partial t} \qquad \text{(Equation 3)}$$

where F_c is the soil gas flux, V is the volume, P_0 is the initial pressure, W_0 is the initial water vapor mole fraction, S is soil surface area inside the chamber, T_0 is the initial air temperature, and $\frac{\partial G'}{\partial t}$ is the initial rate of change in water vapor dilution-corrected gas mole fraction (LI-COR, 2019). For all lab studies, 45 to 600 seconds after the start of sampling was used for flux measurements, with the field validation experiment using 45 to 300 seconds which was adequate for calculating CO_2 flux. All equations used are built in to the SoilFluxPro™ software as developed by LI-COR and adjusted for individual gases.

Carbon Dioxide Experiment

The hypothesis of this study was that CO_2 addition would lower the soil pH by formation of carbonic acid with soil water and was designed to simulate the effects of plant root respiration in saline soils. The objective of this experiment was to quantify how simulated plant root respiration may impact soil pH. If soil pH gets low enough, free lime and gypsum in the soil may be brought into solution and become a source of cations to replace sodium on clay exchange sites.

Eight 1-L jars were each filled with 500 g of air-dried saline soil. A mixture of 80% N_2 and 20% CO_2 gas was percolated through the soil medium over 6.5 days at a rate of 14.5 L hr^{-1}. A compressed gas cylinder was hooked to a 4-way splitting manifold with each of the four lines attached to a gas diffuser stone and regulated for fine adjustments. The diffuser stone was placed at the bottom of each jar before soil was added. An elbow joint was used to connect the gas diffuser to the line so the line would run up the side of the jar rather than through the middle of the soil medium to minimize gas escape along the line channel. An identical setup was used with the remaining four jars, except 100% N_2 gas was used as a control. Soil was wetted to 40% gravimetric moisture content in each jar. A total of 2.26 m^3 of gas was percolated through each jar of soil. Immediately after the tanks ran empty, the jars were rewetted to 35% gravimetric moisture content and placed on the LI-COR flask system for 7 days for GHG analysis. Water was added daily to maintain 35% moisture content. The experiment was run twice, with similar results; so results were combined.

Root Exudates Experiment

The hypothesis was that exudate addition to soil would stimulate microbial activity which would be observed as increased CO_2 and N_2O emissions. The objective of this experiment was to simulate the root exudate component of plant growth in saline soil to quantify soil GHG emissions which can be used as indicators of biological activity.

The amount of simulated root exudates added was comparable to the amount produced by plant roots over the course of a growing season, but addition was compressed into a one-week study. Composition of the simulated exudate solution was based on past studies that examined the exudates of a wheatgrass or barley species (Rovira, 1969; Henry et al., 2007; Vančura, 1964). The experiment consisted of eight-1 L glass jars, each of which was filled with 500 g air dried saline soil. Each jar was wetted to 40% gravimetric moisture content and placed on the LI-COR flask system for 3 days to allow emissions to settle to a baseline with no additional water added during this time. After 3 days, 20 mL of simulated root exudates was added to four of the jars from a stock solution and additional nano-pure water if needed to bring soil to 35% gravimetric moisture. This stock solution was composed of 317 mg glucose, 452.8 mg malic acid, 302.4 mg oxalic acid, 0.8 mg alanine, 19.2 mg proline, 2.56 mg valine, and 320 mL of nano-pure water (Table 4.1). The stock solution had an EC of 1.45 dS m^{-1}, pH of 2.79, and sodium concentration of 350 ug g^{-1}. This addition resulted in each treated jar having an addition of 19.8 mg glucose, 28.3 mg malic acid, 18.9 mg oxalic acid, 0.05 mg alanine, 1.2 mg proline, and 0.16 mg valine (Table 4.1). This equates to 7.92 mg C sourced from glucose, 10.2 mg C from malic acid, 5.1 mg C from oxalic acid, 0.02 mg C from alanine, 0.62 mg C from proline, and 0.08 mg C from valine for a total of 24 mg C

per treated jar (Table 4.1). The 4 non-treated jars were rewetted to 35% moisture using nano-pure water with no exudates solution as a control. All jars were then placed back on the LI-COR flask system for 6 days to monitor changes in GHG emissions. Water was added to all jars each day (10 to 15 mL) to maintain 35% moisture content. The experiment was run a second time with all factors being identical except the stock exudate solution was made with 240 mL of water instead of 320 mL, resulting in a slightly more concentrated solution with an $EC_{1:1}$ of 1.47 dS m^{-1}, a pH of 2.69, and a sodium content of 355 ug g^{-1}. This was done so that only 15 mL of this solution was added to maintain 35% moisture, although each chemical component was at the same total amount as run 1. This change was done due to less moisture loss in the second run compared to the first run and following the identical procedure as run 1 would have resulted in over 35% gravimetric moisture. Similar results were obtained from the runs and data were combined.

Table 4.1 Simulated Root Exudate Solution Compositional Components.

	Stock Solution	Individual Flask Addition	
	mg	mg	mg C
Glucose	317	19.8	7.92
Malic Acid	452.8	28.3	10.2
Oxalic Acid	302.4	18.9	5.1
Alanine	0.8	0.05	0.02
Proline	19.2	1.2	0.62
Valine	2.56	0.16	0.08

Plant Residue Experiment

The hypothesis was that plant residue would stimulate microbial activity and therefore increase CO_2 and N_2O emissions from the soil. The objectives of this experiment were to examine how the residue addition component of a plant system impacts saline soil chemical properties and GHG flux.

The plant residue experiment consisted of adding plant residue from vegetation grown in saline conditions to a saline soil. The experiment measured 8 jars in one experimental run with four jars filled with 500 g of air-dried soil with no residue to be used as a control. The other four jars were filled with a mixture of 500 g of air-dried soil and 2.15 g of plant residue, equivalent to a rate of 3,900 kg ha^{-1} of biomass production. Plant residue was obtained from the same field where the soil was collected and consisted of 35% AC Saltlander Green Wheatgrass (*Elymus hoffmannnii*), 32.5% creeping meadow foxtail (*Alopecurus arundinaceus* Poir), 12.5% western wheatgrass (*Agropyron smithii* Rydb), and 20% slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners). Plant residue was collected on September 10th, 2018, was oven dried at 60 °C and stored until use for the experiment. Before the residue was added to the soil it was cut into ~2-3 cm pieces. Plant residue used was 3.5% N and 44.6% C and had a C:N ratio of 12.8 as determined by mass spectrometry (Sercon Limited, Gateway Crewe, UK) and had a sodium content of 1407 ug g^{-1} as measured with flame photometry. Water was added to bring soil water to 30% gravimetric moisture using nano-pure water. GHG emissions from soils were analyzed for 6 days after residue addition. Water was added each day to maintain 30% gravimetric moisture content.

The experiment was run two times, with each run showing similar results. After removal from gas analyzer, the jars were allowed to incubate at ambient temperatures (~22 °C) in the laboratory for 10 weeks before the experiment was concluded to allow decomposition of the residue; with additional water added weekly to maintain desired moisture content of 30%. These jars were placed on the LI-COR flask system after the 10-week period to determine if differences in emissions were still sustained. During this measurement, moisture was maintained at 30% for the first 3 days. The remaining 4 days, the soil was allowed to dry to determine if emission differences would be maintained over different moisture contents. Jar weights were measured daily to determine moisture content.

Whole Plant Experiment

The hypothesis of this experiment was that CO_2-C flux would increase in soils with plants present due to shoot and root respiration and exudate secretion, N_2O-N flux would decrease due to nitrogen removal from the soil, pH would decrease due to root respiration and exudate secretion, Na and NO_3^- would decrease through plant uptake, and $EC_{1:1}$ would decrease due to plant ion uptake. The objectives of this experiment were to quantify and observe how growing plants would impact the soil GHG flux as well as chemical properties of a saline soil.

The whole plant experiment consisted of growing barley (*Hordeum vulgare*) plants in a saline soil for 7 weeks. This experiment represented all combined components of the three prior experiments. The experimental setup again consisted of 8 jars, 1-L in size each. In four jars, 500 g of air-dried saline-sodic soil was added. In the other four jars, 500 g of air-dried saline-sodic soil was also added and 12 barley seeds were planted

per jar; 2 cm below the surface. All eight jars were then wetted to 30% gravimetric moisture content. Once plants emerged after 7 days, plants were thinned to 7 plants per jar. Soil was rewetted every other day to 30% moisture content (~20 ml).

Plants grew in the greenhouse for 7 weeks at 23°C and a day length of 13 hr light and 11 hrs dark. After 7 weeks, an average of 1.26 g of biomass present over the rim of the jar was removed. Jars were then attached to the LI-COR to measure GHG for 4 days. Water was added daily (~10-15 ml) to maintain desired moisture content at 30% w/w. Once gas sampling ended, all remaining aboveground biomass was removed for tissue analysis. Root biomass also was measured by sieving the damp soil, washing the roots, air-drying for 24 hours, and weighed. This experiment was performed twice, and all data were analyzed together.

Field Collected Samples Experiment

The objective of this experiment was to quantify CO_2-C emissions from non-saline and saline soils, with and without plants, to determine the impact revegetation can have on saline soils and also to quantify the difference in soil chemical tests as a direct result of plant growth, as the samples were taken only 1 m from each other.

The field collected samples experiment consisted of soil samples taken from the field where plant growth had already established in a Cresbard-Cavour loam saline soil to support the results of the previous four experiments as one system. Samples were also taken from a non-saline region for comparison. Four soil cores (replications) were taken from each of the four treatments: good soil no plants, good soil plants, saline soil no plants, and saline soil plants. These cores were allowed to air dry for 10 days, ground with a mortar and pestle, and passed through a 6mm sieve, with larger pieces of plant and

root debris removed. The soil was then weighed for each sample and wetted to 50% water filled pore space following procedures described by Solvita and Woods End Laboratories (Mt. Vernon, ME). This was equivalent to a gravimetric moisture content of 30% w/w. Samples were measured for CO_2-C emissions for 3 days. No additional water was added during these 3 days and when the experiment was concluded, soil moisture had decreased to 15.5% w/w. One replication was removed from saline plants treatment data analysis due to an error in data recording. Following emission data collection, soil was sampled for chemical analysis.

Soil Analysis

For all experiments, at the conclusion of the experimental run, a soil sample was taken and was air-dried at 37.8° C, ground and sieved to 2-mm, and tested for $EC_{1:1}$, pH, Na, NO_3^- and NH_4^+. $EC_{1:1}$ was measured using an Orion Star A215 (Thermo Scientific, Waltham, MA) and pH was measured using a 1:1 soil to water ratio using an Accumet Excel XL60 pH meter with the same soil slurry, with EC measured first (Fisher Scientific, Hampton, NH) following methods described by Grafton (2015). This was performed with 10 g oven dried soil and 10 mL nano-pure filtered water which was stirred immediately and after 15 min. Measurements were taken 30 min after water addition. NO_3^- and NH_4^+ was measured using an Astoria Nutrient Analyzer (Astoria-Pacific, Inc. Clackamas, OR) following methods described in Maynard and Karla (1993) in which extraction was performed with 10 g soil using 100 ml 1.0 *M* KCl, shaken for 1 hr, and filtered through fine porosity Ahlstrom filter paper. Na was extracted using 1 M ammonium acetate at pH 7.0 following methods in (Grafton, 2015) of a 1:10 soil to extract ratio which is shaken for 5 min at 200 epm and filtered through Whatman No. 2

filter paper and measured on a Jenway PFP7 flame photometer (Cole Parmer, Staffordshire, UK).

Tissue Analysis

Tissue analysis was done on the barley above ground biomass in the whole plant experiment. Tissue samples were dried in a forced air oven at 60° C for 72 hours, ground with a mortar and pestle and tested for sodium concentration. Sodium in plant tissue was performed by weighing 0.25 g of ground plant tissue and adding 1 mL of 30% H_2O_2 and 3 mL of nitric acid and microwaved for 45 min. The dissolved solution was then diluted to 25 mL total volume with nano-pure water and tested using atomic absorption flame photometry on a Jenway PFP7 flame photometer (Cole Parmer, Staffordshire, UK). Carbon and total nitrogen was tested using mass spectrometry (Sercon Limited, Gateway Crewe, UK).

Statistical Analysis

All statistics were conducted in R (ver 1.1.383) (R Core Team, 2017). Statistical analysis was performed on flux data collected as well as all soil and tissue test data collected. Two sample t-tests were performed for comparison between treated samples and non-treated samples for each experiment to determine p-value significance. Two sample t-tests were run for both emissions and soil test differences. Before analysis was done, any outliers were removed from the dataset using DFFITS in R (Hebbali, 2017). Outliers that were removed were the result of gas samples taken directly after or during water additions, machine default error readings, or poor placement of sampling ports near obstructions in the flask (plants, CO_2 stone tube). For field validation experiment an ANOVA analysis was performed among the four treatments with the following model:

$$y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

Where y_{ij}= ith observed sample value from the ith population, μ= the overall mean, τ_i= soil and plant treatment effect in the ith treatment level which is the difference between the mean of the ith treatment level and the overall mean, and ϵ_{ij} is the random error. If Pr(>F) was found to be less than a=0.05 significance level, a Fisher Least Significant Difference (LSD) post-hoc test was conducted in R to determine differences between treatments using library agricolae (de Mendiburu,2009). Graph figures were generated using the ggplot2 package (Wickham and Winston, 2016).

4.4 RESULTS AND DISCUSSION

Greenhouse Gas Emissions

Carbon Dioxide Experiment

The N_2+CO_2 treatment initially had a very high CO_2-C flux rate as residual gases in the soil from injection were lost (Figure 4.2). The CO_2-C flux rates were near 20,000 ug CO_2-C kg soil^{-1} hr^{-1} for the first 3 hours but then dropped to about 500 ug CO_2-C kg soil^{-1} hr^{-1} for the remainder of the experiment. The N_2 only treatment had the reverse results; initially CO_2-C flux was near zero as there was no CO_2 in the injection treatment and thus little CO_2 in the soil atmosphere (Figure 4.2). After the first day of removal from gas injection and the soil reached equilibrium with the atmosphere, CO_2-C fluxes were similar between the N_2+CO_2 treatment and the N_2 only treatment for days 2-4 (Table 4.2). From days 5-7 there were differences, but they were relatively small; with the CO_2 treatment producing higher CO_2-C fluxes by an average of 6%.

Similar to the CO_2-C fluxes, in the early stages of the experiment within a couple hours after removal from gas injection, N_2O-N flux was higher in the CO_2 treated soil at

10 ug N_2O-N kg $soil^{-1}$ hr^{-1} compared to N_2 treated soil at 3.7 ug N_2O-N kg $soil^{-1}$ hr^{-1} (Figure 4.1). However, unlike CO_2-C flux, the N_2O-N flux difference between treatments was maintained from days 1-4 and then flux was similar between treatments for the remainder of the experiment (Table 4.2). The differences in N_2O-N flux were significant as seen in Figure 4.8 which also shows an initial increase in N_2O-N flux over 3-4 hours and then a gradual decline until day 2 in CO_2 treatment. The N_2 only treatment followed a very similar trend, with an initial increase in N_2O-N flux and then a gradual decline until about day 3. The initial increase of N_2O-N flux in both treatments and subsequent decrease is likely the result of denitrification, as neither gassing treatment contained oxygen. This could have resulted in anaerobic conditions which would favor denitrification and thus N_2O production. It is unclear however, why the CO_2 only treatment produced higher N_2O-N fluxes and could be the result of complex microbe-soil atmosphere interactions.

Methane production was unaffected by CO_2 gas injection when compared to N_2 only gassing treatments and over the course of the 7 day measurement period, there were no differences. Averaged across treatments over the study, CH_4-C flux was -0.027 ug CH_4-C kg $soil^{-1}$ hr^{-1} (Table 4.2).

Carbon Dioxide Experiment Discussion

The injection of CO_2 increased N_2O emissions; however, this may not be the case in a field setting. The methods used in the lab likely resulted in anaerobic conditions in the soil due to no oxygen in the gases used, which likely caused denitrification. Whereas in the field some oxygen is present in the soil atmosphere and may reduce this effect, unless this experiment is used to represent the very interface of soil roots and the soil

surface which is in contact with the root hairs. This study indicated that when CO_2 injection ceases, the built-up CO_2 in the soil wa lost within several hours. This means unless constant plant growth and root respiration is taking place, the CO_2 is quickly released to the atmosphere unless soil structure limits the diffusion of gases through the soil profile. To our knowledge the monitoring of GHG in response to simulated root respiration has not been studied in past research, as most of the focus is on the impact to soil pH.

Root Exudates Experiment

CO_2-C and N_2O-N flux increased within 1-3 hours after exudate addition (end of day 3) (Figures 3.4 and 3.5). The increase in the N_2O-N flux lasted for 24 hours, whereas the CO_2-C increase lasted for 4 days (Table 4.3). One day after exudate additions, (day 4) N_2O-N fluxes in exudate treated soil had an average flux rate of 1.57 ug N_2O-N kg $soil^{-1}$ hr^{-1} whereas non-treated soil had an average flux of 0.02 ug N_2O-N kg $soil^{-1}$ hr^{-1} (Table 4.3). During the entirety of the experiment after exudate addition there were no differences in CH_4-C flux between treatments with the exception of day 7 where exudate addition reduced CH_4-C flux by 0.004 ug CH_4-C kg $soil^{-1}$ hr^{-1} compared to untreated soil (Table 4.3). This small difference is not environmentally significant. Averaged over the 6 days after exudate addition, the exudate treated soils had N_2O-N and CO_2-C flux rates that were 513% and 37% higher than untreated soil, respectively (Table 4.3).

Root Exudate Experiment Discussion

The results of this experiment show that root exudates could increase N_2O emissions from saline soils immediately after they are released from plant roots and increase soil CO_2 emissions for a slightly longer period of time. Soil microbes most likely

utilized substrates with a lower C:N ratios such as the amino acids proline, valine, and alanine. Thus, freeing more nitrogen in the soil which can result in the short N_2O-N burst seen in the experiment (USDA-NRCS, 2011). In contrast, the higher C:N ratio substrates would be utilized by microbes over a longer period of time, resulting in longer increases in CO_2-C emissions. Even so, the results suggest that immediately after roots exude carbon substrates soil microbes begin to utilize them, as seen by the increase in CO_2-C respiration in Figure 4.2. This relatively quick use of added carbon in the form of simulated exudates also was found in similar studies (Traoré et al., 2000; Kunc and Macura, 1966). Traoré et al. (2000) added simulated root exudates at 2 g C kg^{-1} soil and reported that the 87% of the added carbon was mineralized in the form of CO_2 in the first 75 hours. Kunc and Macura (1966) also conducted a similar study and reported 85% of added C mineralized as CO_2 in the first 76 hours. In our study 47% of the added C was mineralized as CO_2-C in the 75 hours. The lower rate of mineralization compared to that reported by Traoré et al. (2000) is likely due to inhibited microbial activity or lower microbial populations in the saline soil when compared to that of a non-saline soil used by Traoré et al. (2000), however this was not directly measured in this experiment. The addition of these root exudates did not impact CH_4-C emissions. CH_4-C emission is driven more by soil moisture as seen in flux data from the first day of the experiment which had higher moisture (35% vs 30%) and was a CH_4 source (slightly positive CH_4-C flux of 0.037 ug CH_4-C kg $soil^{-1}$ hr^{-1}) compared to being a sink (-0.025 ug CH_4-C kg $soil^{-1}$ hr^{-1}) when soil was at 30% moisture content.

This study demonstrated that soil GHG emissions respond quickly to the addition of simulated root exudates which was similar to results found in other studies (Traoré et

al, 2000; Kunc and Macura, 1996). Although the increases in GHG were short lived in the lab experiment, these effects may be sustained in a field setting over the growing season as plants release exudates slowly over time, unlike the addition of all at once approach used in this study. The increase in N_2O emissions is a pitfall, as N_2O is 298 times more potent of a GHG than CO_2. However, the increase in CO_2-C flux is an indicator of microbial activity in the soil. Plant roots and soil microbes form strong relationships with each other through root exudates and this increased microbial activity could be utilized by plants to form symbiotic relationships for nutrient uptake and to help reduce water stress (Gargallo-Garriga et al., 2018). Thus, if the goal is to increase and restore microbial activity in saline soils, it appears that the introduction of plants could accomplish this in part through root exudation (Rovira and Brisbane, 1967; Baudoin et al., 2003). Additionally, the aboveground portion of plants not accounted for in this experiment would fix CO_2 and could help offset the increases in soil CO_2 as a result of exudate release.

Plant Residue Experiment

The addition of plant residues to saline soils induced changes in GHG fluxes throughout the 8-day study period. As the plant residue broke down by microbial degradation, N_2O gas was released and was on average 641% higher than the non-treated soil during days 2-8 after residue addition (Table 4.4). No differences were seen in N_2O-N during the first day likely because the addition of water to the air-dried soil also induced a burst in N_2O-N which masked differences due to the residue addition. However, the water-induced N_2O-N burst decreased after 2 days and the influence of the residue could be seen (Figure 4.7). N_2O-N flux over the measured period in non-treated

saline soil averaged 0.522 ug N_2O-N kg $soil^{-1}$ hr^{-1} and during days 3-8 flux averaged closer to 0.1 ug N_2O-N kg $soil^{-1}$ hr^{-1} (Table 4.4). This was in contrast to the residue treatment which averaged 1.145 ug N_2O-N kg $soil^{-1}$ hr^{-1} throughout the study, an increase of 119% (Table 4.4).

Changes in CO_2 were also observed as a result of plant residue additions. Like N_2O, a water-induced burst of CO_2-C was also seen in the non-treated saline soil which also decreased in less than a day (Figure 4.8). However, the differences in treated vs non-treated soils were apparent with CO_2-C flux in saline soil treated with residue higher than that of the non-treated soils (Figure 4.8). Averaged over the 8 days of the study, saline soil with residue had a CO_2-C flux of 3587 ug CO_2-C kg $soil^{-1}$ hr^{-1} compared to the non-treated soil at 526 ug CO_2-C kg $soil^{-1}$ hr^{-1}, a 582% increase (Table 4.4). The addition of plant residue to the soil also maintained this increase over non-treated soil in the long term as well. When the soil was measured for CO_2 again 10 weeks after treatment, there were sustained differences (Figure 4.10); during the first 3 days after placing on the LICOR again (when moisture was kept at 30%) the residue treated soils maintained a CO_2-C flux near 1100 ug CO_2-C kg $soil^{-1}$ hr^{-1} which was 34-67% higher over the non-treated soil (Table 4.5). During the next 4 days when soil moisture was allowed to decline, the residue treated soil still maintained 64% higher CO_2-C flux rates. Averaged over the 7-day measured period, residue treated soil had a flux rate of 795.9 ug CO_2-C kg $soil^{-1}$ hr^{-1} and non-treated soils averaged 500.8 ug CO_2-C kg $soil^{-1}$ hr^{-1} (Table 4.5). Residue additions did not influence CH_4 emissions and were near 0 during the course of the experiment.

Plant Residue Experiment Discussion

The addition of plant residue to saline soil resulted in increased N_2O and CO_2 emissions, similar to the effects of root exudates. The increase in N_2O emissions as a result of plant residue additions can be explained by the residue itself. Plant material contains nitrogen compounds within its tissues. As soil microbes decompose the residue, these nitrogen containing substrates are released, increasing soil nitrate and ammonium. An increase in soil NO_3^- inhibits the formation of N_2 in the nitrogen cycle and can increase N_2O emissions as a result. This could explain the increase seen in this study as well as direct releases of N_2O from the decaying residue. The differences in CO_2-C fluxes between treated and non-treated saline soils are a likely the result of available substrate for microbes. The changes in CO_2-C during the experiment were likely driven by microbial processes and activity and showed results similar to those found by Wong et al. (2009). The addition of plant matter would provide both a short and long-term source of carbon for soil microbes to utilize as an energy source. This increase in available carbon would allow for higher microbial activity and thus the resulting increase in CO_2 fluxes as seen in several other studies (Wong et al., 2009; Rao and Pathak, 1996). Wong et al. (2009) reported a 186% increase in 12-week cumulative CO_2-C emissions when kangaroo grass was added to a saline soil at 10 t ha^{-1}. Our study found a 582% increase in CO_2-C flux for the first 8 days and a 34-67% increase after 10 weeks when measured for a 4-day period. Thus, for restoring microbial activity in saline soil, plant residue additions are a viable method by which plants can be used to achieve this goal. This study also showed that the increase in CO_2 emissions from microbial activity as a result of organic matter additions are sustained over long periods of time when compared to those

resulting from root exudates (>10 weeks from residue compared to 4 days from exudates). Thus, a buffer is created so that during times when plants cease growth or reduce exudation rates, the plant residues present can provide a more stable supply of carbon for soil microbial consumption. Some research has found differing results such as Al-Kaisi and Yin (2005) where a field study in a 3 year corn-soybean rotation with residues left in the field had 24% lower CO_2 emissions. However, this difference is likely because Al-Kaisi and Yin (2005) left the residues on the surface whereas our study incorporated the residue in the soil and temperatures were fairly consistent. Al-Kaisi and Yin (2005) made the point that the residue likely shaded the soil and reduced temperatures as well as created a barrier for soil-atmosphere gas exchange and that there was minimal contact of the residue with the soil surface.

Whole Plant Experiment

During the measured period of four days, emissions from soil planted with barley averaged 1.163 ug N_2O-N kg $soil^{-1}$ hr^{-1} whereas control soil without plants averaged 0.359 ug N_2O-N kg $soil^{-1}$ hr^{-} (Table 4.6). The barley planted soil also had consistently higher CO_2-C emissions, averaging 709.1 ug CO_2-C kg $soil^{-1}$ hr^{-1} compared to the non-planted control soil of 206.3 ug CO_2-C kg $soil^{-1}$ hr^{-1} (Table 4.6) This 244% increase in CO_2-C respiration in barley soil treatment is likely the result of two factors: 1) the presence of roots which respire CO_2 as a result of metabolism and 2) increased microbial respiration driven by plant root exudates and plant-root associations.

The presence of plants in the soil did not impact CH_4-C emissions during this study, with both treatments following identical trends (Table 4.6, Figure 4.13). CH_4-C emissions remained near zero during the study for both treatments, with barley planted

soil averaging 0.006 ug CH_4-C kg $soil^{-1}$ hr^{-1} and non-planted soil averaging 0.005 ug CH_4-C kg $soil^{-1}$ hr^{-1} (Table 4.6).

Whole Plant Experiment Discussion

Even though the barley plants were grown in a more saline soil than those of the previous experiments, the same trends were seen in GHG emissions. When plants were established in the saline soil the fluxes of N_2O-N and CO_2-C both increased, while there was no impact on CH_4-C flux. This supports the findings from the exudates, residue, and root respiration experiments.

It is likely that the increase in N_2O emissions associated with barley growth was a result of decaying lower leaves on the plants, resulting in a similar phenomenon seen in the residue experiment as well as the secretion of root exudates which was also shown to increase N_2O emissions as seen in the simulated exudates experiment (Table 4.2; Table 4.3). The decaying leaves occurred because barley accumulates salts in the leaf tissue which results in premature lower leaf death and decay (Munns et al., 1995).

The increase in CO_2 emissions from the barley planted soil were similar to results observed by Rastogi et al. (2002) where it was reported that soils with established plants typically have CO_2 fluxes of 2-3 fold higher than barren soils. This is likely again due to similar reasons found in the root exudates and residues addition experiments. Our study found that saline soil with barley had 244% higher CO_2 respiration which is very similar to what Rastogi et al. (2002) reported.

From a global warming perspective, the increase in GHG emissions is not a desirable outcome. However, from a soil health perspective the increase in CO_2-C indicates increased biological activity in the soil, from both plant roots and soil microbial

communities, although not directly measured. Also, these emissions were only measured from soil sources, and did not factor plants into the equation. Although plants were present during measurements in the barley experiment, they did not result in decreased CO_2-C emissions, likely due to low rates of photosynthesis due to lack of sunlight in the lab where measurements were taken and the small amount of aboveground biomass allowed in the jars during measurements due to limitations of the equipment. Therefore, in a field setting were plants are actively photosynthesizing and fixing carbon, the net CO_2-C flux could be reduced or even negative which would be beneficial from a global warming viewpoint. Additionally, in the long-term N_2O emissions could be expected to decline as plants utilize the excess NO_3^- in the saline soils and reduce soil N_2O emissions.

Field Collected Samples Experiment

The samples taken from the field showed similar results to those found in the lab setting in which barley plants where grown. Samples from the field were only measured for CO_2, with a non-saline soil also measured for comparison purposes. As expected, all soils had a burst of CO_2-C immediately after wetting which then quickly decreased to a baseline respiration level (Figure 4.14). However, the saline soil which had no plants established, had an initial burst in CO_2-C four times lower, peaking at ~5000 ug CO_2-C kg $soil^{-1}$ hr^{-1} compared to the other saline soil with plants and non-saline soil with and without plants at ~20000 ug CO_2-C kg $soil^{-1}$ hr^{-1} (Figure 4.14). Throughout the measurement period, it was observed that both the non-saline soil and saline soil which had plants present emitted higher rates of CO_2-C than those without plant growth (Figure 4.14). After the initial CO_2 burst, on days 2-3 flux rates ranked non-saline plants > saline plants > non-saline no plants > saline no plants. This same ranking was found when the

CO_2-C flux was averaged over the entire measured period as well, with flux values of 3517, 3119, 2015, and 1395 ug CO_2-C kg $soil^{-1}$ hr^{-1} for non-saline plants, saline plants, non-saline no plants, saline no plants treatments, respectively (Table 4.7).

Field Collected Samples Experiment Discussion

Since soil samples from locations with and without plants were taken from only 1 meter apart, it can be assumed that differences in CO_2 respiration were a direct result of whether plants were present or not; either by plants revegetating the saline site or by preventing the soil from becoming further degraded by increases in salt concentration. The saline soil without plants had very low respiration levels compared to the good soil without plants which averaged 44% higher CO_2-C flux. Not only plants impacted CO_2 soil respiration, but elevated soil EC values ~6.3 dS m^{-1} also impacted CO_2-C evolution. This phenomenon of EC impact on soil CO_2 evolution was also reported by several other studies (Adviento-Borbe et al., 2006; Pathak and Rao, 1998; Setia et al., 2011). Adviento-Borbe et al. (2006) reported that saline soil under corn had CO_2 emissions decrease from 5.06 g CO_2-C m^{-2} to 3.66 g CO_2-C m^{-2} as EC increased from 0.5 dS m^{-1} to 2.0 dS m^{-1}. This difference was attributed to inhibited microbial activity and respiration (Adviento-Borbe et al., 2006). Pathak and Rao (1998) reported reductions in cumulative CO_2 emissions from 2.1 g kg-1 to 0.89 g kg-1 with increasing EC_e values from 1.1 dS m^{-1} to 96.7 dS m^{-1}. Setia et al. (2011) reported CO_2-C emissions of 0.6 mg g^{-1} soil at an $EC_{1:5}$ of 0.5 dS m^{-1} but only 0.3 mg g^{-1} soil at an $EC_{1:5}$ of 2.5 dS m^{-1}. The authors again attributed the reduced soil CO_2 emissions in saline soil to inhibited microbial activity due to osmotic stresses.

In our study, however, saline soil CO_2 respiration increased by 124% when plants were present and was near levels found in the non-saline soil with plants. Therefore, even though salinity inhibits CO_2 respiration through inhibited microbial activity, restoring plants on saline soils has potential to increase CO_2 evolution to rates of soils not affected by salt.

Tissue and Soil Chemical Tests

Carbon Dioxide Experiment

The injection of carbon dioxide did not change soil Na or NH_4^+ levels and was expected to decrease soil pH through formation of carbonic acid with soil water. However, this study showed an increase in soil pH to 6.34 compared to 6.26 in N_2 treated soil as well as decrease in $EC_{1:1}$ to 1.86 dS m^{-1} compared to 2.09 dS m^{-1} in N_2 control (Table 4.8).

Carbon Dioxide Experiment Discussion

The lack of a decrease in pH could be the result of the injected carbon dioxide not forming carbonic acid due to the already acidic starting pH of the soil (Hinsinger et al., 2013; Lindsay, 1979). It could also be that it would take a longer duration of CO_2 injection to induce pH changes in the bulk soil than performed in this study length of a few days. However, longer studies have been conducted and also had mixed results (Biose et al., 2016; Patil et al., 2010; He et al., 2019). Biose et al. (2016) had reported a soil pH increase from 6.31 to 6.7 at the 15-30 cm depth and from 5.89 to 6.39 at the 45-60 cm depth when CO_2 was injected 60 cm below the soil surface of field plots for 8 weeks. However, a very similar field design study reported decreases in pH from ~6.4 to ~6.0 after 21 weeks compared to controls at the 0-30 cm depth (Patil et al., 2010).

Additionally, He et al. (2019) percolated CO_2 through a soil for 36 days and saw an increase in pH from 6.8 to 7.0. Our study found increases in soil pH from 6.26 to 6.34, and thus was within the range of results reported by Biose et al., (2016) and He et al. (2019).

It is important to note that the CO_2 treatment in our study had significant decreases in soil NO_3^- from 93 ug g^{-1} down to 64 ug g^{-1} compared to 89 ug g^{-1} found in the N_2 only treatment (Table 4.8). This indicates that higher levels of denitrification could have occurred in the CO_2 treatment, which would explain the higher N_2O-N fluxes. This increase in denitrification could also explain the lack of expected pH change because denitrification causes an increase in soil pH which would counter the effects of a pH decrease expected from the CO_2 injection. Further research must be done on this subject to confidently claim that increases in pCO_2 as a result of root respiration will reduce soil pH.

Root Exudates Experiment

The addition of simulated root exudates to a saline soil had no significant impact on soil $EC_{1:1}$, pH, Na, NO_3^-, or NH_4^+ (Table 4.8). A change in pH was expected due to the low pH of the simulated root exudate solution, however this was not seen.

Root Exudates Experiment Discussion

The lack of differences in soil tests could be due to the buffering capacity of the soil and the relatively small amount of exudate solution which was added to the bulk soil. Likewise, very little nitrogen was contained in the simulated root exudate solution, so no change occurred as a result of the exudate addition. Both treatments increased NO_3^- levels to ~123 ug g^{-1} compared to the starting level of 93 ug g^{-1}, indicating that mineralization

occurred during the study; but was not influenced by exudate additions as indicated by the near identical NO_3^- levels found between exudate addition and control treatments. It could be possible root exudates can influence the pH close to the root surface of plants which has been documented in previous studies where 8 weeks of soybean or barley growth reduced soil pH from 8.5 to 7.0 near the root interface (Youssef and Chino, 1989).

The root exudate experiment indicates that a year's worth of simulated root exudates are not likely to be a source of soil chemical parameter changes, at least in the short term. It is possible root exudates can induce changes near the root surface in the short term. However, changes to bulk soil may take years and was not observed in the 7-day experiment.

Plant Residue Experiment

The plant residue addition to saline soil increased soil $EC_{1:1}$ and NO_3^- but did not impact pH, Na or NH_4^+ content (Table 4.8). The addition of residues increased $EC_{1:1}$ from 2.08 dS m^{-1} to 2.20 dS m^{-1} and increased NO_3^- from 93 ug g^{-1} to 183 ug g^{-1} (Table 4.8). The non-treated soil also had NO_3^- increases from 93 ug g^{-1} to 137 ug g^{-1} which may have occurred due to mineralization.

Plant Residue Experiment Discussion

The increase in $EC_{1:1}$ in residue treated soil compared to non-treated soil due is similar to the findings by Wong et al. (2009) and is likely the result of stored salts in the plant tissue being released into the bulk soil. Wong et al. (2009) reported that the addition of kangaroo grass in an acidic saline soil increased $EC_{1:5}$ at the 0- to 5-cm depth from ~1.7 dS m^{-1} to ~3.4 dS $m^{-}1$. As plants grow in saline soils, they can accumulate the salts

in a variety of methods in their tissues. When these residues are added to the soil, the stored salts and ions are released as plant cells break down (Wong et al., 2009).

However, the increase seen in this study may not represent what would happen in a field setting. This is because the plants would first have to establish in the saline soil and then accumulate salts in the tissues, thus actually lowering the $EC_{1:1}$ of the soil. The $EC_{1:1}$ could then be increased as that residue breaks down. In this study though, the residue was collected from the field and then added to a saline soil which did not have the $EC_{1:1}$ reduced first by plant growth. Therefore, this experiment is only representing the latter half of the cycle; where the plant has already been established and then is added to the soil. The whole plant experiment discussed later, represents the first half of the cycle; where the plant establishes and removes salts from the soil.

Sodium behaves in a similar fashion, however an increase in sodium as a result of residue addition was not detected in our study. Similar to the exudates experiment, nitrification occurred in the residue experiment as seen by the increase in NO_3^- in the control treatment. The addition of residues further increased NO_3^- as a result of nitrogen being released from decaying plant residues. This increase in NO_3^- could also explain some of the increases in N_2O emissions mentioned earlier since higher soil NO_3^- levels can promote higher N_2O fluxes.

Previous studies found pH changes with the addition of residues, but it is dependent on starting soil pH, type of residue, and various processes which take place in the soil. Wong et al. (2009) found in alkaline saline soil, residue addition decreased pH from 10.1 to 9.2 at the 10- to 20-cm depth but no change was detected in an acidic saline soil. Yan et al. (1996) reported a pH increase after 45 days from 6.00 to 6.29 after the

addition of field bean residue. Yan et al. (1996) also mentioned that pH can also decrease if nitrification takes place. Yan and Schubert (2000) also reported pH increases due to residue additions from 6.2 to 6.6 when faba bean was added and 6.4 when wheat was added. However, our study did not detect any change in pH where the treatment and control pH was 6.41 and 6.42, respectively.

The implications of this experiment show that when residues are added back to the soil in saline sites, EC and NO_3^- levels may increase. Although these increases are unfavorable from an environmental standpoint, they do not take into account the first half of the cycle of plants actually growing in the soil or the potential improvements in physical characteristics of the soil. This experiment was done in a closed system, if leaching were allowed the addition of residues could actually decrease $EC_{1:1}$. This effect was reported by Mahmoodabadi et al. (2013) where the addition of pistachio residue at a rate of 50 g kg^- reduced EC to 3.6 dS m^{-1} compared to control soil of 4.0 dS m^{-1} after 5 months and 4 pore volumes of leaching.

Whole Plant Experiment

Barley growth in saline soil reduced the $EC_{1:1}$ and NO_3^- of the soil but did not impact pH, Na, or NH_4^+ content. There was a slight increase in pH and decrease in Na, but neither were significant at the 0.05 level. From the time of sowing barley seeds to the end of the experiment 7 weeks later, barley treatment reduced soil $EC_{1:1}$ from 6.30 dS m^{-1} to 5.92 dS m^{-1} (Table 4.8). Barley plants also reduced NO_3^- from a starting level of 519 ug g^{-1} to 428 ug g^{-1} and was less than the ending NO_3^- level of 509 ug g^{-1} for the non-planted control (Table 4.8).

Tissue analysis found that barley plants harvested after the 7-week experiment contained 5.0% Na, 4.8% N, and 38.1% C. This gave the tissue a C:N ratio of 7.8. These results showed that barley took up large amounts of sodium in the tissue of the young plants while also removing nitrogen from the soil. Both of which are good first steps in remediating saline sodic soils.

Whole Plant Experiment Discussion

The results of this experiment were promising for restoring saline soils in the field. The reduction in $EC_{1:1}$ as a result of plant uptake of ions (Wong et al., 2009; Yanai et al., 1995) is beneficial for further promoting plant growth and may condition the soil for less salt tolerant plant species to eventually be planted. The reduction of soil $EC_{1:1}$ due to plant growth has been reported by other studies as well. Qadir et al. (1997) grew sesbania, sordan, and kallar grass in the field and found EC reductions of 3.1, 1.8, and 2.5 dS m^{-1} compared to 1.0 dS m^{-1} in the control plot. Qadir et al. (1996a) also found that combined with leaching, kalar grass reduced soil EC from 9.8 dS m^{-1} to 2.9 dS m^{-1} compared to non-planted control of 4.3 dS m^{-1}.

The reduction in NO_3^- likely also resulted from plant uptake of the nutrient. This NO_3^- removal may be responsible for the slight increase in soil pH (Walker, 1960; Kirkby, 1968; Grinsted et al., 1982; Nye, 1981) even though it was not different from the control (p value=0.07).

Field Collected Samples Experiment

The samples taken from the field under non-saline and saline soils with and without plant growth supported the findings of the previous experiments. In non-saline soils tested, there were no differences in $EC_{1:1}$, pH, or Na due to plant establishment

(Table 4.8). However, saline soils did have differences in soil chemical tests as a result of plant growth. Non-saline soils had lower $EC_{1:1}$ (<0.4 dS m^{-1}) and Na (<50 ug g^{-1}) regardless of plant growth compared to saline soils (Table 4.8). In non-saline soils without plants, pH levels were lower (pH=6.24) than those in saline sites with plants (pH=6.48). However, non-saline soil with or without plants both had higher pH values than those found in saline sites without plants (pH=5.92) (Table 4.8). Additionally, NO_3^- levels were lower in non-saline soils with (20 ug g^{-1}) or without plants (32 ug g^{-1}) compared to saline soil without plants (232 ug g^{-1}) (Table 4.8).

In saline soils, plants were able to influence $EC_{1:1}$, pH, Na, and NO_3^- (Table 4.8). When plants were not present in saline soils, the $EC_{1:1}$ was higher, pH was lower, and Na was higher. Saline soils with plants present had a soil $EC_{1:1}$ of 3.61 dS m^{-1} compared to 8.03 dS m^{-1} when plants were not present (Table 4.8). Similarly, Na levels were lower when plants were present at 1493 ug g^{-1} compared to 2575 ug g^{-1} when plants were not present, a 42% reduction (Table 4.8).

Field Collected Samples Experiment Discussion

Similar to what the barley experiment showed and what past studies have found, the field collected samples had an increase in pH when plants were present (Yanai et al., 1995; Marschner and Römheld, 1983). Saline sites with plant growth had a pH of 6.48 compared to 5.92 under saline conditions without plants (Table 4.8). This difference in pH could be the result of plant uptake of NO_3^- in the soil; because to remain electrically balanced, plants release either OH^- or bicarbonate when they remove NO_3^- from the soil (Walker, 1960; Kirkby, 1968; Grinsted et al., 1982; Nye, 1981). This may be the case because NO_3^- levels were higher in saline soils without plants by 625% compared to

vegetated saline soils (Table 4.8). This indicates that plant growth reduced the high NO_3^- levels in saline soils, which otherwise remain high in NO_3^- without plants to utilize it.

The large differences in $EC_{1:1}$ and Na levels between saline samples with and without plants is likely due to increased leaching through root channels and plant uptake (Qadir et al., 2006b). This is supported by other studies which found similar results through phytoremediation. Ashraf et al. (2010) reported that kallar grass reduced soil EC from 22.0 dS m^{-1} to 12.6 dS m^{-1} in one year and reduced EC by 87% after 5 years. Sesbania and kallar grass have also been found to reduce soil EC by 47.4% and 38.5%, respectively, compared to controls (Ahmad et al., 1990). These large differences due to plant growth suggest that given enough time, phytoremediation has the potential to reduce soil $EC_{1:1}$ enough to encourage further plant growth and can help with the first steps to reclaiming severely salt-affected soils, or at least preventing increases in salinity.

Table 4.2. Greenhouse Gas Flux Rates of a South Dakota Saline Soil with and without CO_2 Injection

	Day							
Treatment	1	2	3	4	5	6	7	Mean
	Daily Mean Soil N_2O Flux (ug N_2O-N kg $soil^{-1}$ hr^{-1})							
80% N_2 + 20% CO_2	8.369	3.591	0.309	0.164	0.178	0.126	0.085	1.852
100% N_2	2.812	1.913	0.989	0.472	0.356	0.213	0.186	0.931
Standard Error	0.281	0.139	0.059	0.042	0.070	0.049	0.040	0.066
p-value	<0.001	<0.001	<0.001	0.002	0.160	0.363	0.169	<0.001
	Daily Mean Soil CO_2 Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})							
80% N_2 + 20% CO_2	2725	556	475	444	454	426	381	781
100% N_2	489	572	501	444	431	401	356	454
Standard Error	282	9.64	9.18	5.75	5.99	6.53	5.23	39.0
p-value	0.087	0.088	<0.001	0.717	<0.001	<0.001	<0.001	0.083
	Daily Mean Soil CH_4 Flux (ug CH_4-C kg $soil^{-1}$ hr^{-1})							
80% N_2 + 20% CO_2	-0.019	-0.025	-0.017	-0.022	-0.032	-0.035	-0.047	-0.028
100% N_2	-0.013	-0.030	-0.019	-0.019	0.024	-0.041	-0.031	-0.026
Standard Error	0.009	0.012	0.003	0.002	0.003	0.006	0.005	0.002
p-value	0.712	0.880	0.711	0.522	0.210	0.600	0.101	0.581

Table 4.3. Greenhouse Gas Flux Rates of a South Dakota Saline Soil with Addition of Simulated Root Exudates Between Days 3 and 4 of Experimental Run Lasting 10 Days.

	Day										
Treatment	1	2	3	4	5	6	7	8	9	10	4-10 Mean
	Daily Mean Soil N_2O Flux (ug N_2O-N kg $soil^{-1}$ hr^{-1})										
Exudates	4.201	3.039	0.235	1.565	0.041	0.036	0.178	0.138	0.036	0.034	0.343
No Exudates	4.276	4.067	0.422	0.024	0.049	0.039	0.104	0.052	0.069	0.019	0.056
Standard Error	0.155	0.167	0.030	0.104	0.005	0.004	0.051	0.045	0.014	0.009	0.023
p-value	0.541	<0.001	0.008	<0.001	0.376	0.675	0.496	0.309	0.372	0.240	0.001
	Daily Mean Soil CO_2 Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})										
Exudates	1350	1246	688	1115	667	651	427	359	315	285	590
No Exudates	1355	1321	731	583	505	454	379	365	316	285	432
Standard Error	29.5	24.0	10.0	30.2	9.1	10.1	4.5	3.3	2.6	4.2	7.4
p-value	0.623	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.570	0.690	<0.001
	Daily Mean Soil CH_4 Flux (ug CH_4-C kg $soil^{-1}$ hr^{-1})										
Exudates	0.009	0.003	-0.014	-0.022	-0.025	-0.026	-0.029	-0.026	-0.030	-0.032	-0.026
No Exudates	0.012	0.003	-0.013	-0.022	-0.024	-0.026	-0.025	-0.025	-0.031	-0.030	-0.025
Standard Error	0.002	0.001	0.001	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p-value	0.172	0.796	0.326	0.959	0.719	0.561	0.040	0.493	0.812	0.420	0.087

Table 4.4. Greenhouse Gas Flux Rates of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions Over 8 Day Experimental Run.

	Day								
Treatment	1	2	3	4	5	6	7	8	Mean
	Daily Mean Soil N_2O Flux (ug N_2O-N kg $soil^{-1}$ hr^{-1})								
Residue	2.132	1.708	1.132	0.903	0.773	0.692	0.434	0.370	1.145
No Residue	2.134	0.741	0.110	0.067	0.075	0.118	0.103	0.068	0.522
Standard Error	0.047	0.040	0.036	0.030	0.027	0.029	0.028	0.031	0.021
p-value	0.982	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CO_2 Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})								
Residue	4851	4990	4325	3514	2793	2241	1813	2050	3587
No Residue	1253	715	366	336	294	345	242	298	526
Standard Error	131	143	126	104	84	59	68	156	45
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CH_4 Flux (ug CH_4-C kg $soil^{-1}$ hr^{-1})								
Residue	0.008	0.007	0.006	0.005	0.001	-0.005	-0.007	-0.012	0.002
No Residue	0.006	0.001	-0.010	-0.020	-0.020	-0.017	-0.020	-0.030	-0.011
Standard Error	0.001	<0.001	0.001	0.001	<0.001	0.001	0.002	0.004	<0.001
p-value	0.308	0.005	<0.001	<0.001	<0.001	0.002	0.005	0.090	<0.001

Table 4.5. Greenhouse Gas Flux Rates of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions After Resting for 10 Weeks and Measured Over 7 Days.

	Day							
Treatment	1	2	3	4	5	6	7	Mean
	Daily Mean Soil CO_2 Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})							
Residue	1093	1109	1104	761	577	470	464	796
No Residue	813	664	698	464	349	289	285	501
Standard Error	31.1	30.5	28.7	21.4	13.0	10.0	10.8	11.8
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4.6. Greenhouse Gas Flux Rates of a South Dakota Saline Soil with and without Barley Vegetation Present after 7 Weeks Growth

	Day				
Treatment	1	2	3	4	Mean
	Daily Mean Soil N_2O Flux (ug N_2O-N kg $soil^{-1}$ hr^{-1})				
Barley	1.459	1.991	1.824	1.147	1.163
No Plants	0.298	0.385	0.430	0.319	0.359
Standard Error	0.071	0.093	0.088	0.055	0.041
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CO_2 Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})				
Barley	638	751	731	717	709
No Plants	132	214	237	248	206
Standard Error	18.4	19.5	18.7	19.0	9.6
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
	Daily Mean Soil CH_4 Flux (ug CH_4-C kg $soil^{-1}$ hr^{-1})				
Barley	0.007	0.008	0.004	0.004	0.006
No Plants	0.006	0.005	0.005	0.004	0.005
Standard Error	<0.001	<0.001	<0.001	<0.001	<0.001
p-value	0.289	<0.001	0.394	0.366	0.006

Table 4.7. Soil Carbon Dioxide Burst Test Flux Rates of a South Dakota Soil from Salt Affected and Non-Salt Affected Regions Where Plants Did and Did Not Establish

	Day			
Treatment	1	2	3	Mean
	Daily Mean Soil CO_2Flux (ug CO_2-C kg $soil^{-1}$ hr^{-1})			
Non-Saline Plants	5409 a	3014 a	2128 a	3517 a
Non-Saline No Plants	3216 b	1583 c	1245 c	2015 c
Saline Plants	5168 a	2405 b	1785 b	3119 b
Saline No Plants	2089 c	1270 d	834 d	1395 d
Standard Error	139	40	28	52
P-Value	<0.001	<0.001	<0.001	<0.001

Table 4.8. Soil Chemical Test Parameter Results after Treatments for Soils Used in Simulated Root Exudate, Plant Residue, Root Respiration, Whole Plant, and Field Validation Experiments

	Soil Parameter				
	EC	pH	Na	NO_3^--N	NH_4^+-N
Treatment	dS m^{-1}	-----	ug g^{-1}	ug g^{-1}	ug g^{-1}
Exudates	2.152	6.31	730	123	3.4
No Exudates	2.119	6.25	719	124	3.8
p-value	0.716	0.37	0.727	0.529	0.684
Residue	2.200	6.41	649	183	0.8
No Residue	2.079	6.42	654	137	0.5
p-value	0.045	0.70	0.719	<0.001	0.461
80% N_2 + 20% CO_2	1.860	6.34	678	64	0.8
100% N_2	2.090	6.26	677	89	0.6
p-value	0.003	0.006	0.972	<0.001	0.552
Barley	5.920	6.60	2028	428	3.1
No Plants	6.300	6.49	2086	509	2.8
p-value	0.041	0.07	0.151	<0.001	0.430
Non-Saline Plants	0.227 c	6.37 ab	45 c	20 b	9 b
Non-Saline No Plants	0.351 c	6.24 b	49 c	32 b	6 b
Saline Plants	3.605 b	6.48 a	1493 b	33 b	19 a
Saline No Plants	8.032 a	5.92 c	2575 a	232 a	22 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4.9. Whole Plant Barley, Residue, and Field Validation Experiment's Plant Tissue Test Results.

	Mass $Flask^{-1}$	N	C	C:N Ratio	Na
	g	----------%	----------	-	ug g^{-1}
Barley Shoots	0.64	4.8	38.1	7.8	500
Barley Roots	0.34	-	-	-	-
Residue Experiment	2.15	3.5	44.6	12.8	1407
Field Validation Experiment Saline Plants	-	3.0	36.9	12.4	-

Figure 4.1. Nitrous Oxide Flux Time Series of a South Dakota Saline Soil with and without CO_2 Injection. Error Bars= 1 Standard Error.

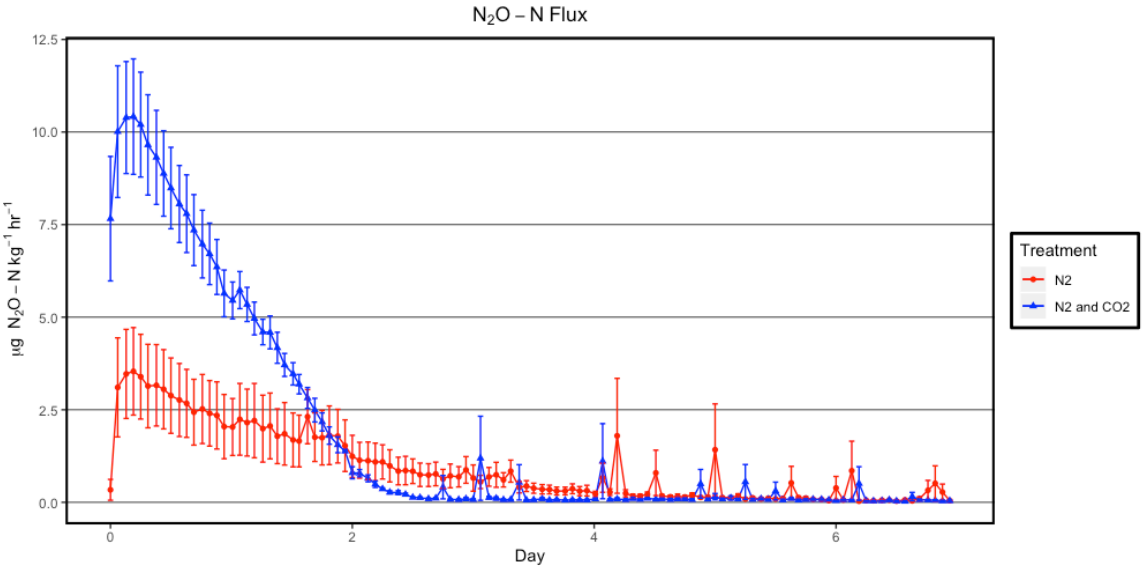


Figure 4.2. Carbon Dioxide Flux Time Series of a South Dakota Saline Soil with and without CO_2 Injection. Error Bars= 1 Standard Error.

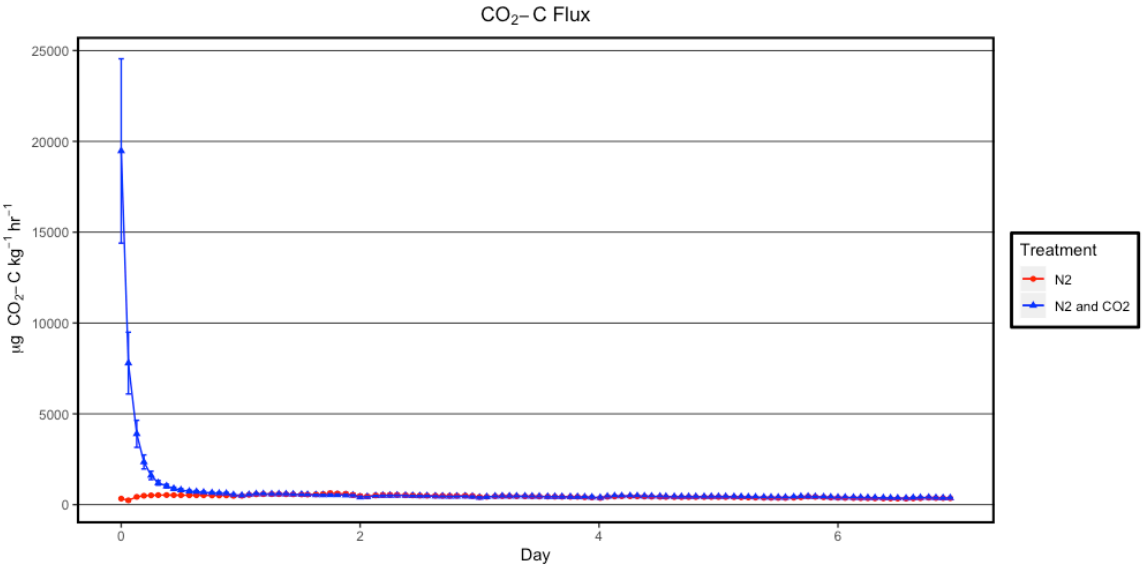


Figure 4.3. Methane Flux Time Series of a South Dakota Saline Soil with and without CO_2 Injection. Error Bars= 1 Standard Error.

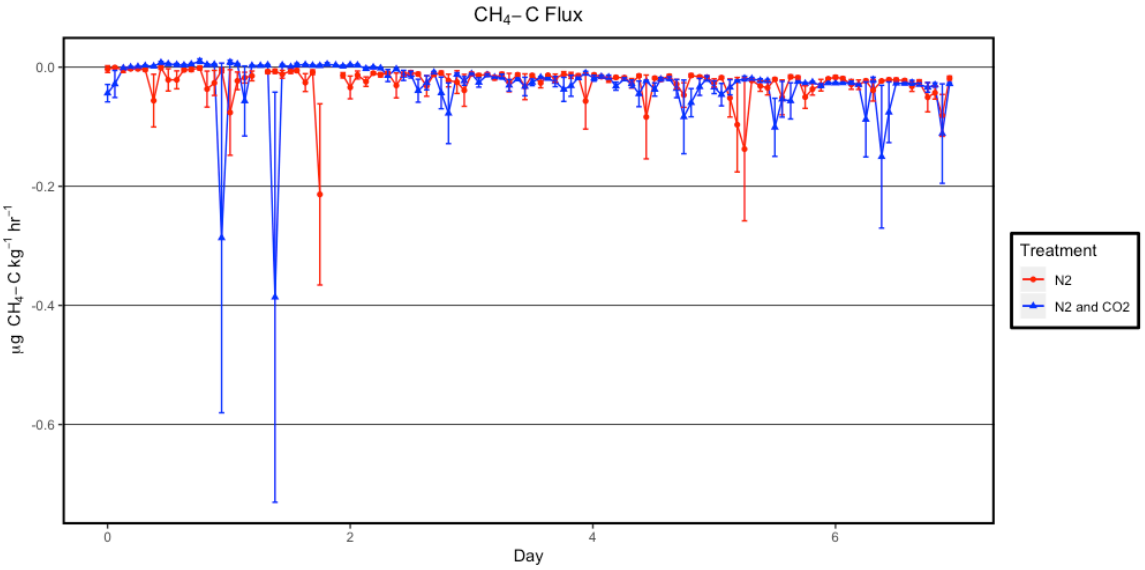


Figure 4.4. Nitrous Oxide Flux Time Series of a South Dakota Saline Soil with Addition of Simulated Root Exudates on Day 3 of Experimental Run Lasting 10 Days. Error Bars= 1 Standard Error. Arrow Indicates Time of Exudate Addition.

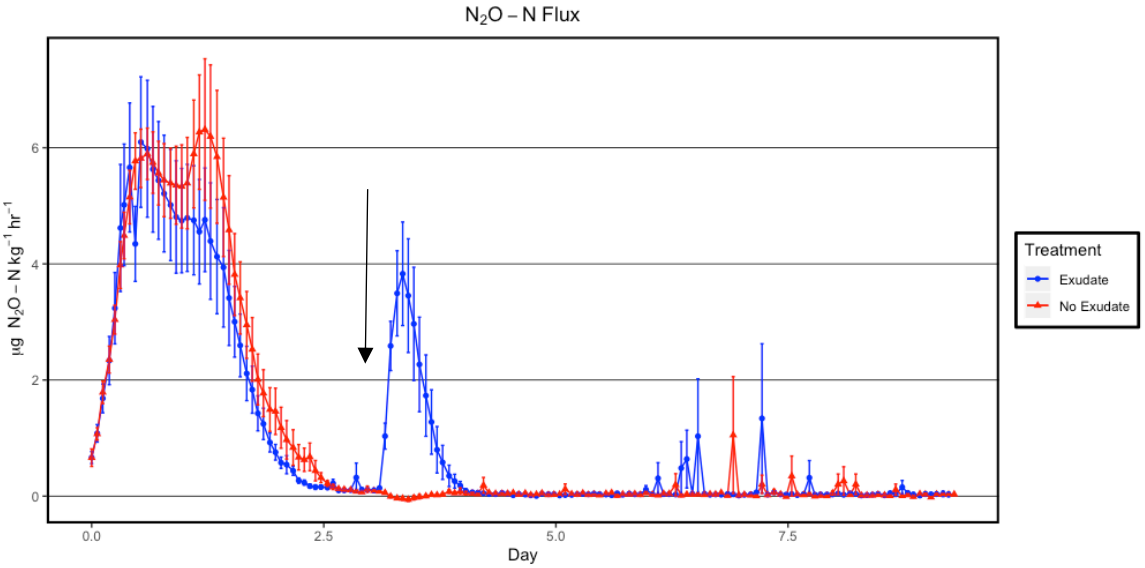


Figure 4.5. Carbon Dioxide Flux Time Series of a South Dakota Saline Soil with Addition of Simulated Root Exudates on Day 3 of Experimental Run Lasting 10 Days. Error Bars= 1 Standard Error. Arrow Indicates Time of Exudate Addition.

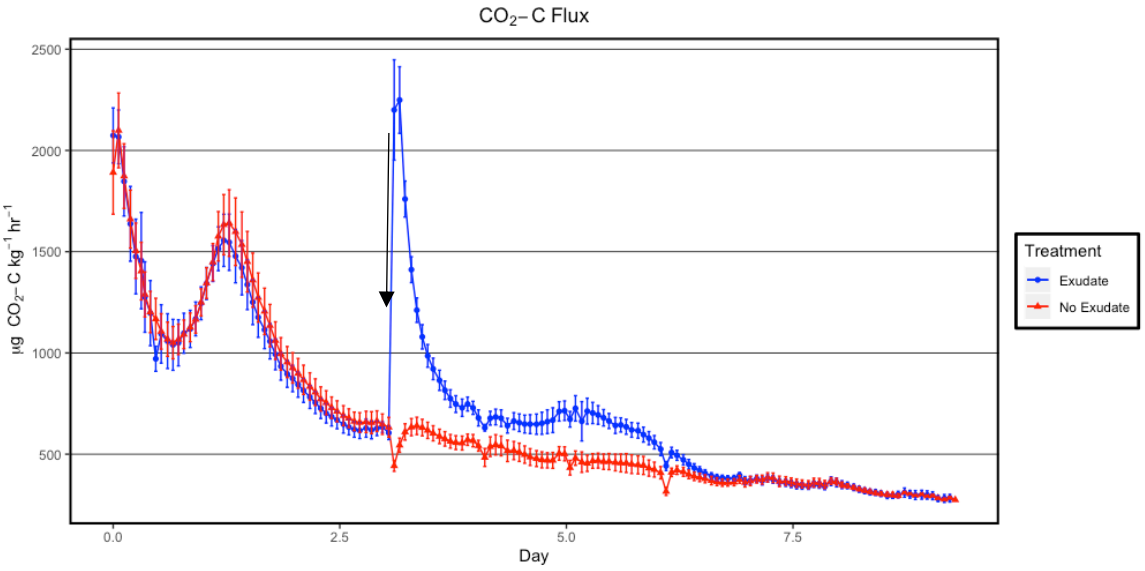


Figure 4.6. Methane Flux Time Series of a South Dakota Saline Soil with Addition of Simulated Root Exudates on Day 3 of Experimental Run Lasting 10 Days. Error Bars= 1 Standard Error. Arrow Indicates Time of Exudate Addition.

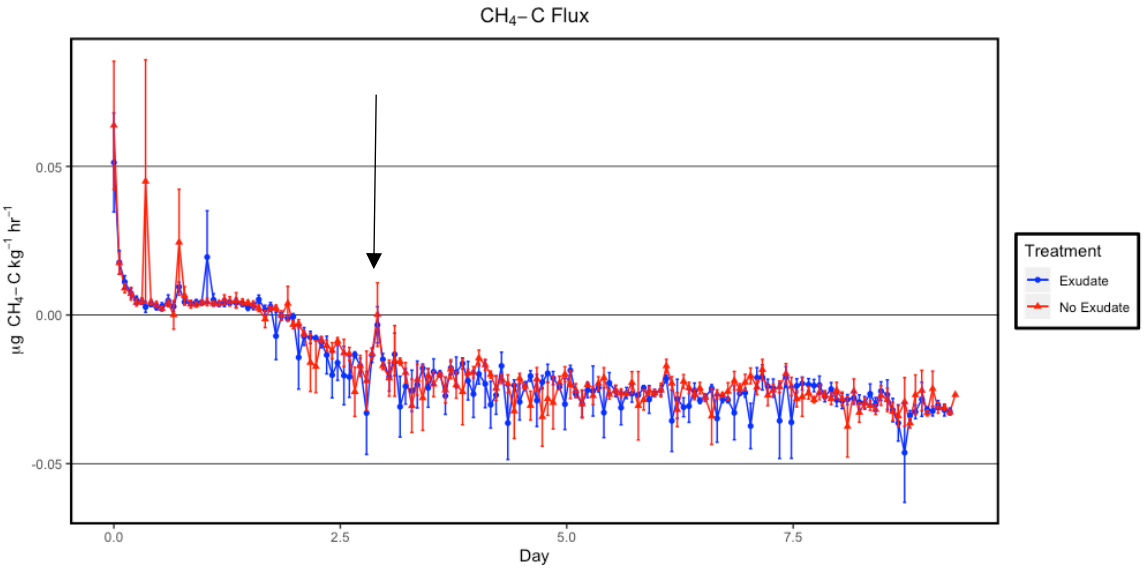


Figure 4.7. Nitrous Oxide Flux Time Series of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions Over 7 Day Experimental Run. Error Bars= 1 Standard Error.

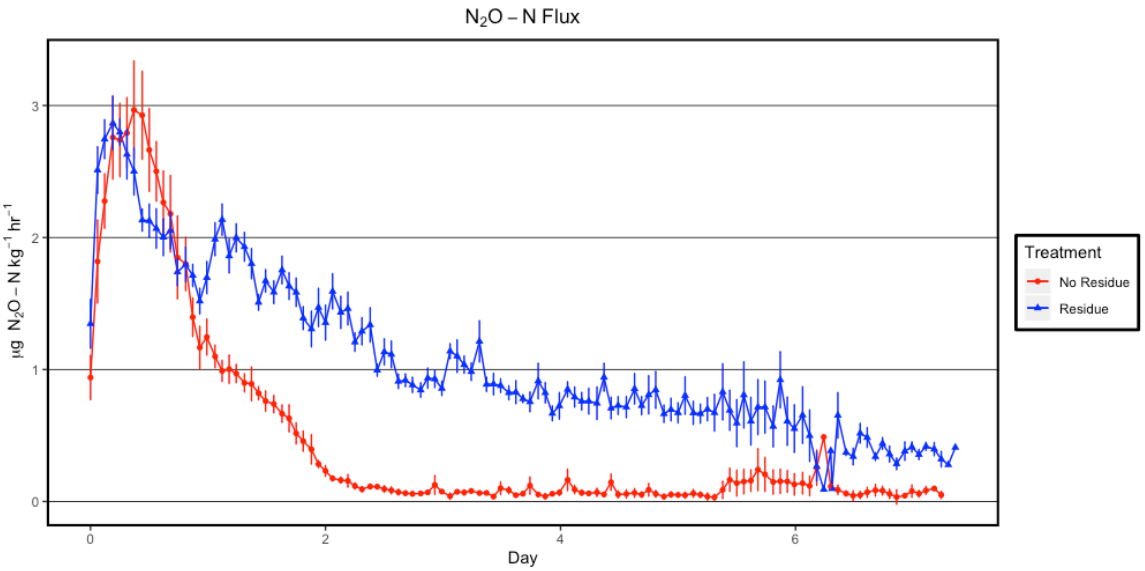


Figure 4.8. Carbon Dioxide Flux Time Series of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions Over 7 Day Experimental Run. Error Bars= 1 Standard Error.

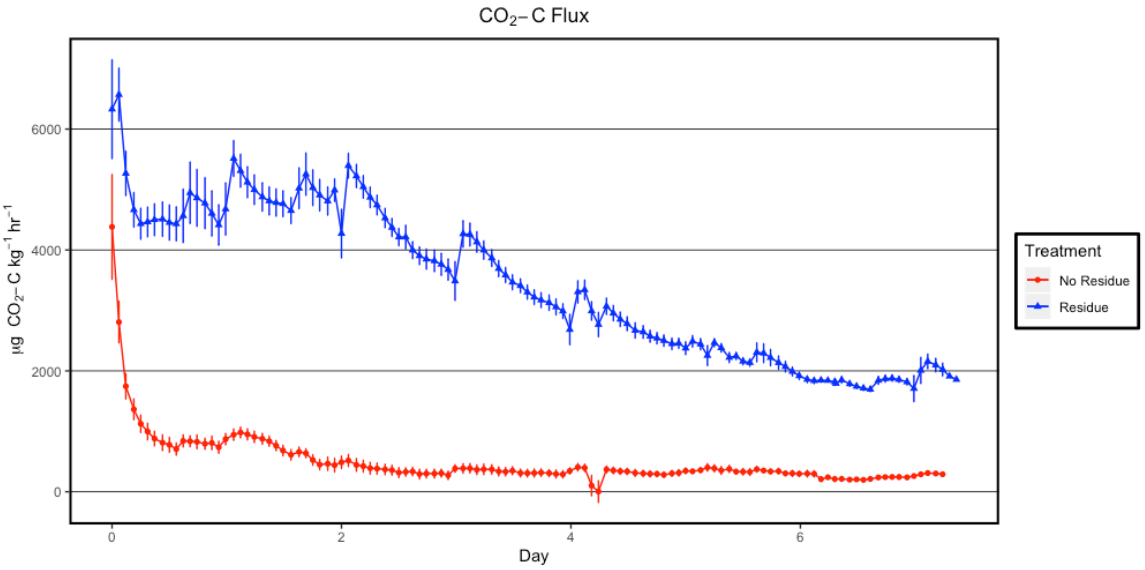


Figure 4.9. Methane Flux Time Series of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions Over 7 Day Experimental Run. Error Bars= 1 Standard Error.

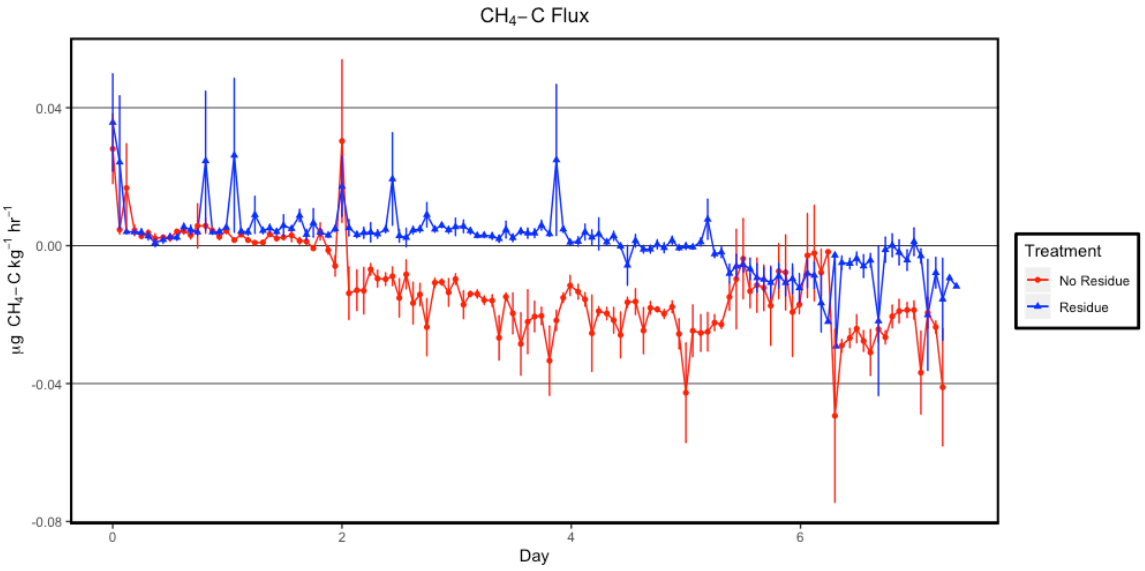


Figure 4.10. Carbon Dioxide Flux Time Series of a South Dakota Saline Soil with and without Perennial Grass Mixture Residue Additions after Resting for 10 Weeks. Error Bars= 1 Standard Error.

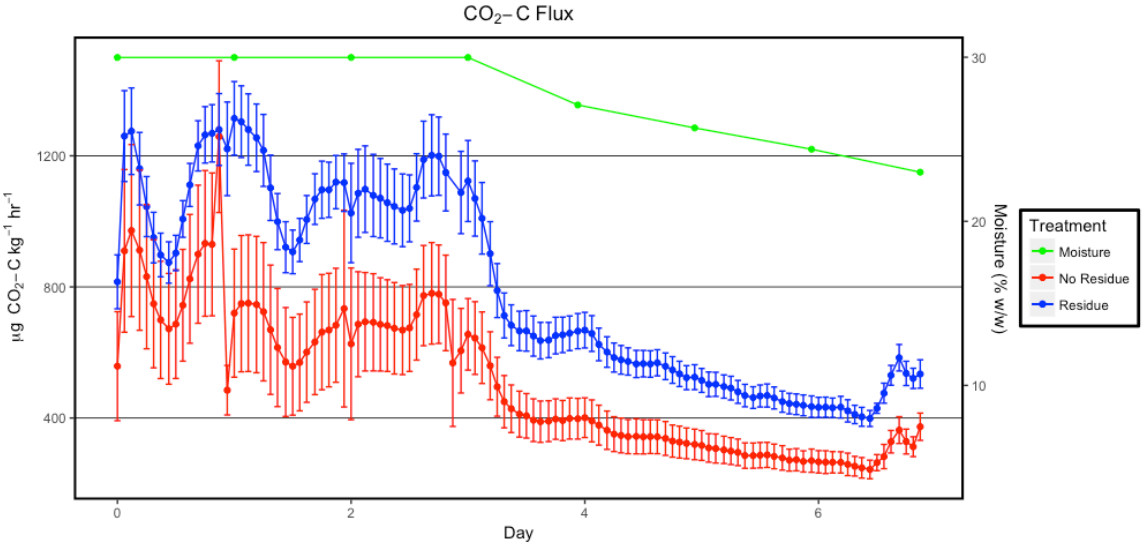


Figure 4.11. Nitrous Oxide Flux Time Series of a South Dakota Saline Soil with and without Barley Vegetation Present after 7 Weeks Growth. Error Bars= 1 Standard Error.

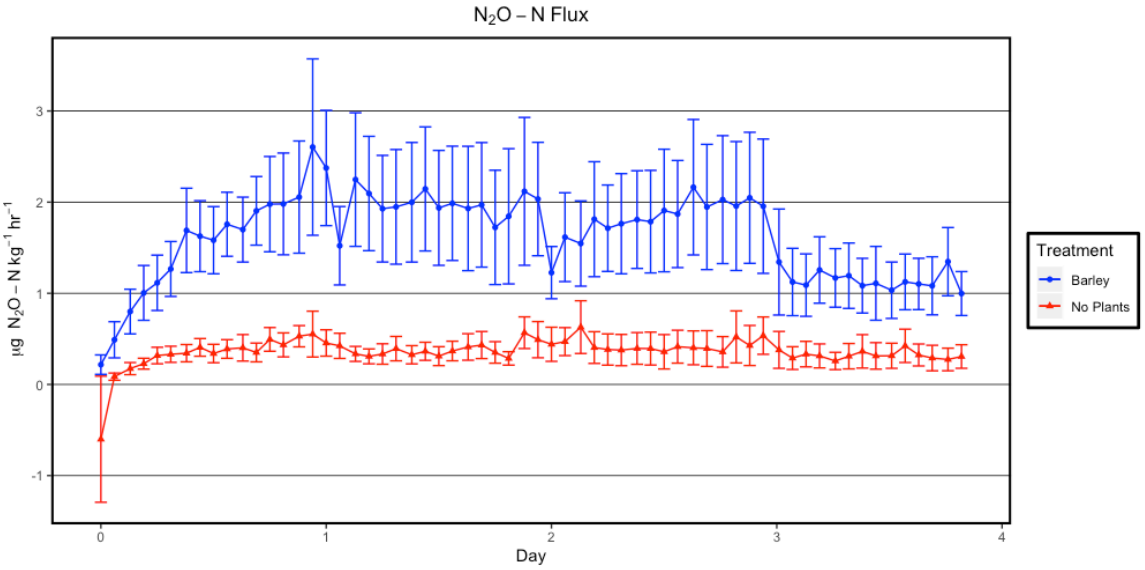


Figure 4.12. Carbon Dioxide Flux Time Series of a South Dakota Saline Soil with and without Barley Vegetation Present after 7 Weeks Growth. Error Bars= 1 Standard Error.

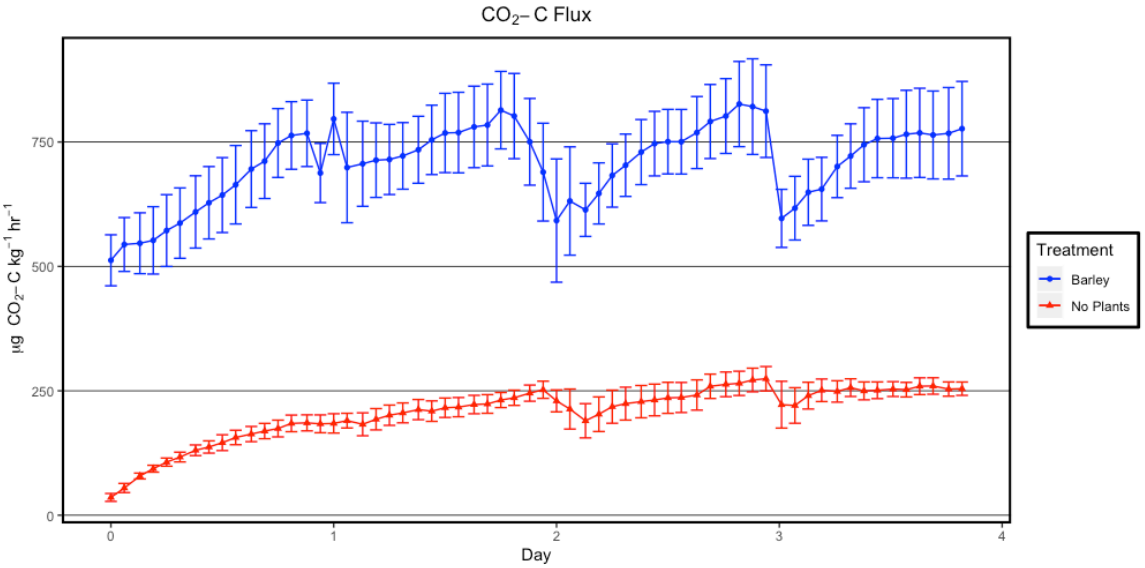


Figure 4.13. Methane Flux Time Series of a South Dakota Saline Soil with and without Barley Vegetation Present after 7 Weeks Growth. Error Bars= 1 Standard Error.

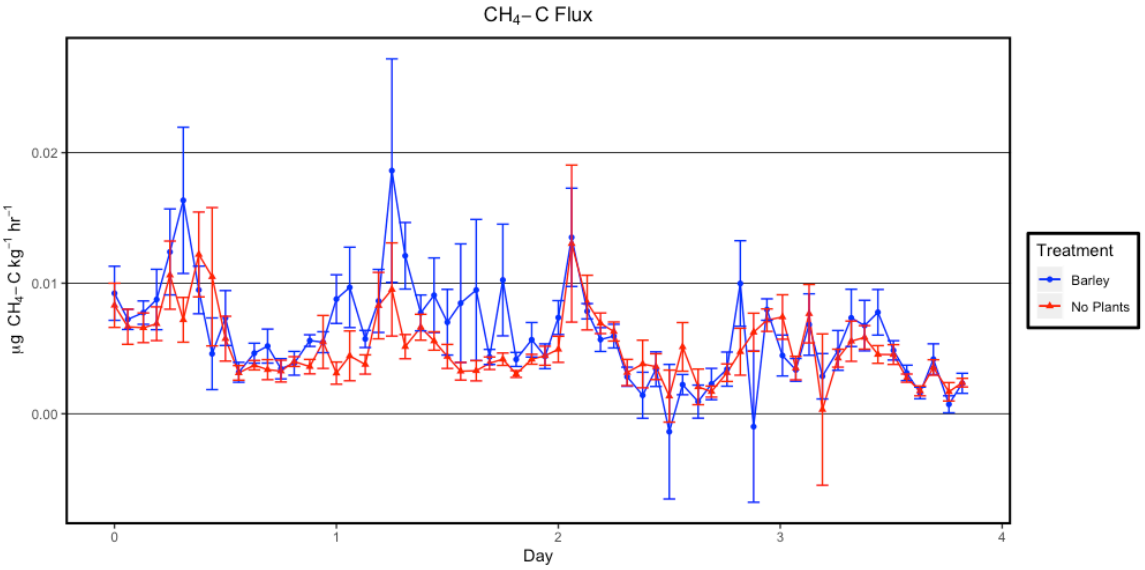
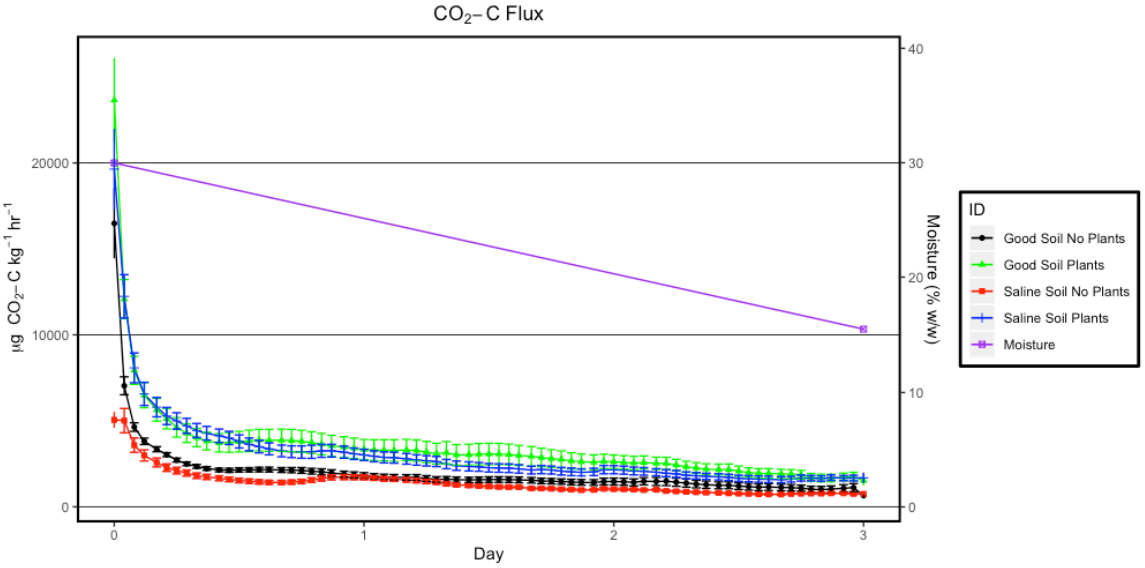


Figure 4.14 Soil Carbon Dioxide Burst Test Flux Time Series of a South Dakota Soil from Salt Affected and Non-Salt Affected Regions where Plants did and did not Establish. Error Bars= 1 Standard Error.



CHAPTER 5: CONCLUSIONS AND FUTURE RESEARCH

Conclusions

Phytoremediation can be a viable method to restore productivity to salt-affected soils in Eastern South Dakota. However, results in the field may take multiple years to become apparent. Over two growing seasons, two perennial grass mixtures were able to successfully establish on a salt-affected site. The first year of establishment faced heavy weed pressure and required herbicide intervention to aid establishment. However, by the second growing season perennial grass mixtures only required one herbicide application early in the season, and future years may not require any additional input costs. Although perennial grass mixtures did not influence changes in soil electrical conductivity or sodium compared to a corn and fallow control, they did outproduce corn in biomass production when no additional fertilizers were applied and also suppressed weeds more than corn in both saline and non-saline plots. Species such as AC Saltlander, slender wheatgrass, and creeping meadow foxtail were the dominant species of the mixtures used and appear well adapted for saline conditions.

The GHG emissions were quantified in the field sites and it was discovered salt-affected soils have drastically reduced CO_2 respiration which is indicative of inhibited microbial activity. Soils which were vegetated with either corn or a mix of slender wheatgrass and beardless wildrye had higher CO_2 respiration and lower N_2O production as well. Saline soil with no vegetation had more N_2O emissions which is a potent greenhouse gas and is likely the result of constantly high soil moisture, higher soil temperature, and elevated soil nitrate levels. Plant establishment on these sites have the potential to reduce N_2O emissions by providing shade which would reduce soil

temperature, introduce root systems for water use to reduce moisture levels, and nutrient uptake to remove excess nitrate which can threaten ground water sources and increase soil N_2O emissions. Repeated urea application to these barren saline sites is likely the cause of the high soil nitrate levels and the research performed in the field shows that urea application to these soils also increases N_2O emissions, primarily after rain events.

While remediation in the field may require years to become effective, lab experiments showed that some impacts can be detected relatively quickly. Root respiration creates anaerobic zones near the root-soil interface and can increase N_2O emissions. Likewise, root exudation provides substrates for microbial decomposition which increases N_2O as well as CO_2 emissions. The breakdown of plant residue on the soil surface also increases these GHG emissions through substrate additions. The effects of root respiration, exudation, and residue decomposition were detectable the same day of addition, indicating rapid microbial, enzyme, or chemical responses. However, unless constantly supplied by living plants, only residue additions provided long term substrates to provide measurable differences from controls for greater than one week. The presence of plant systems as measured in the lab by either barley or perennial grass mixtures both increased GHG emissions, as found in individual component experiments, but also reduced soil electrical conductivity and sodium.

The findings of this research were that phytoremediation's effects can be measurable immediately in a laboratory setting where all other variables are isolated and held constant. However, field experiments were influenced by high precipitation, weed competition, and continuous supply of salts from subsoil deposits. Therefore, the overall conclusions of this research are that phytoremediation can likely reclaim salt-affected

soils over multiple years but can promptly begin the process of restoring soil health through carbon substrate additions which stimulate soil microbial communities during the same year of implementation. The larger challenge in these sites however is the constant replenishing of salts from shale deposits in the soil parent material, which is why multiple years are required for reclamation.

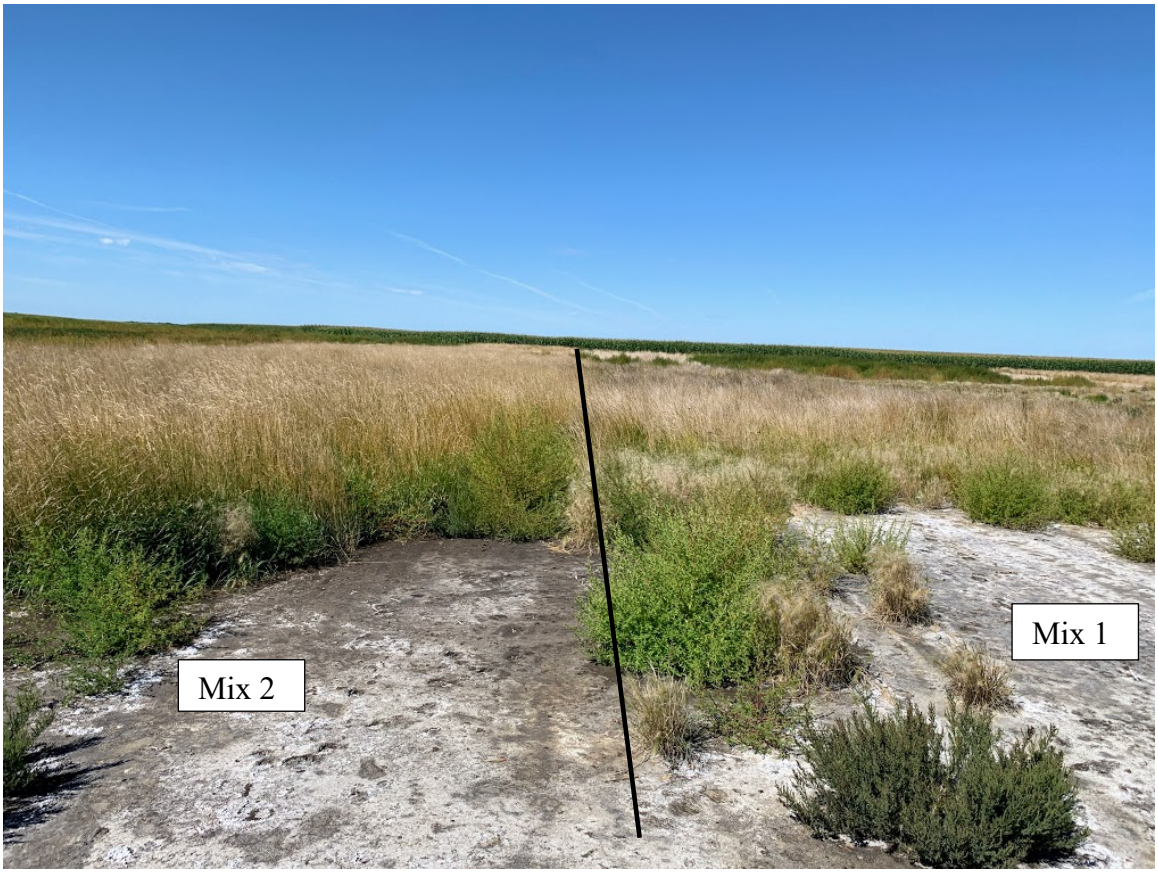
Future Research

Data collection should continue to take place on the field site where perennial mixtures are established to monitor progress. Soil samples should be taken at the same time and same points as previous years to monitor soil parameters such as electrical conductivity and sodium to determine if, over time, perennial grass mixtures can reduce these parameters to more favorable levels. Likewise, biomass and weed pressure levels should continue to be measured to determine long term weed suppression and forage production rates of the different mixtures. Mixture species composition could also be monitored to determine if changes occur within mixtures due to a superior species outcompeting other species and if future mixes should have different species components. Other future research could integrate livestock on these sites to quantify how an integrated livestock system could impact soil conditions and vegetative performance as well as economic influences. Long term studies should be conducted to determine if perennial grass mixtures influence soil bulk density, structure, and porosity over time compared to non-vegetated regions and corn treatments.

APPENDIX

Appendix 1. Mix 1 and Mix 2 On May 13, 2019.

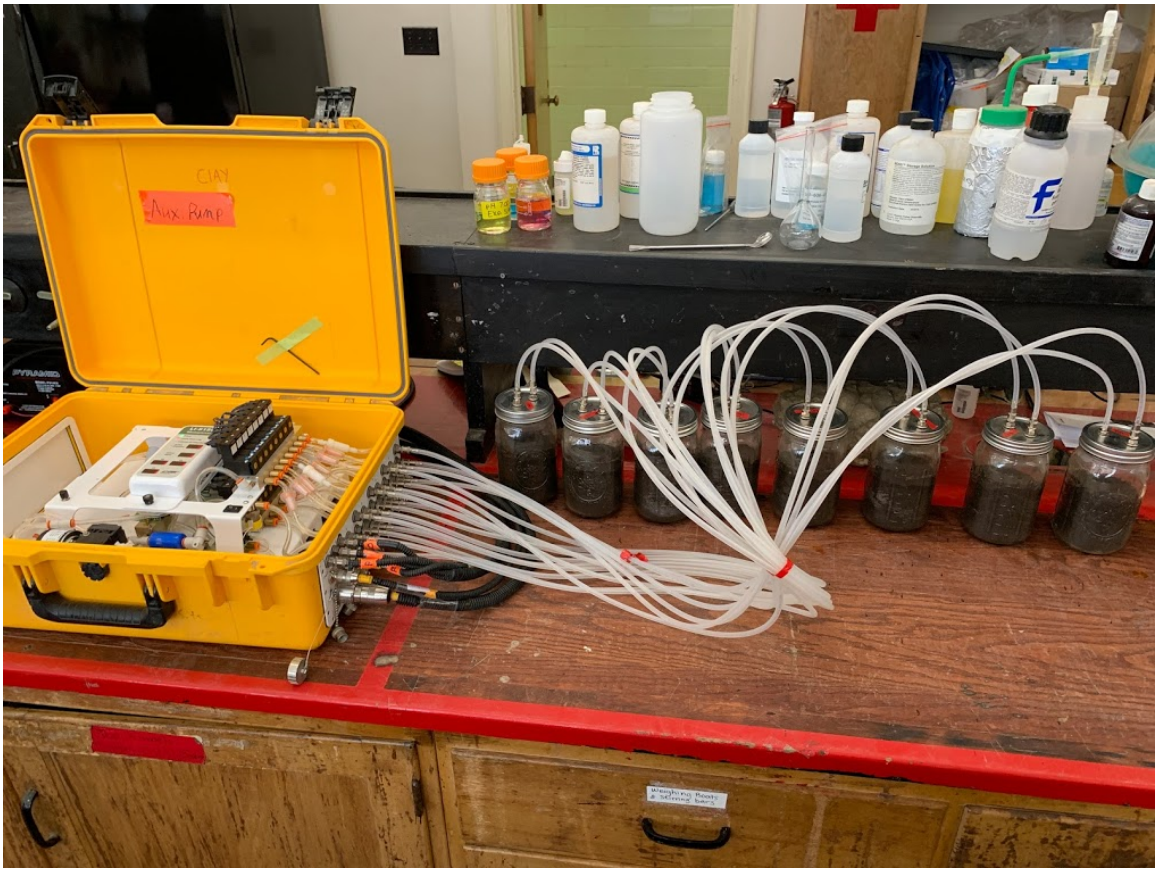
Appendix 2. Mix 1 and Mix 2 at Maturity on August 29, 2019 Transitioning into a Saline Zone.



Appendix 3. Greenhouse Gas Measurement Chamber Placement in Saline Zone 2019.

Appendix 4. Greenhouse Gas Measurement Chamber Placement in Grass Zone 2019.

Appendix 5. Greenhouse Gas Measurements Utilizing Flask System Design on Root Exudate Experiment.



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