

BIOLOGICAL RESPONSE TO SULFATE SALINITY IN RICHLAND COUNTY,
NORTH DAKOTA

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Biological Response to Sulfate Salinity in Richland County, North Dakota

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ABSTRACT

Soil salinization is a major threat to agricultural ecosystems. Consequently, an understanding of agro-ecosystem functions affected by increasing levels of soil salinity is critical for land management. The purpose of this research was to determine the effects of soil salinity on corn and soybean growth and soil microbial activity in southeastern North Dakota soils with naturally occurring soil salinity. A field study assessed corn and soybean yield, and a laboratory study assessed microbial respiration in response to increasing salinization. Both corn and soybean yield were significantly impacted by salinity in sandy loam soils, declining after a threshold of 4.57 dS m^{-1} and 2.98 dS m^{-1} , respectively. These thresholds are higher and lower than previously established threshold tolerances for corn and soybean, respectively. Microbial respiration patterns in sandy loam soils could not be significantly explained by EC_e , potentially indicating a community composition that has acclimated to sulfate-based salinity in these soils.

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GENERAL INTRODUCTION

Soil Salinity and Agriculture

Soil salinity is the accumulation of excess salts, such as calcium-, magnesium-, and sodium sulfates and chlorides, in the soil that inhibit plant function and growth (Keller et al., 1986). Soil salinity interferes with biological uptake of nutrients and water, thus disturbing necessary physiological functions required for growth and development of plants and soil organisms (Munns, 2002). As a consequence, salinization is a substantial contributor to land degradation and, consequently, a major threat to soil health. Globally, approximately 831 million ha of land are affected by soil salinity (Martinez-Beltrán and Manzur, 2005), and salinization is predicted to impact 50% of all arable land by 2050 (Wang et al., 2003). The global extent of salinization has both social and economic implications. Given the propensity of soil salinity to impede agricultural productivity, its impacts threaten both the global food supply and agricultural profits, resulting in an estimated US \$27.3 billion annually in crop losses (Qadir et al., 2014).

Limitations to Previous Literature on Crop Response to Soil Salinity

There are two main types of salinization: 1) primary salinity and 2) secondary salinity. Primary or dryland salinity is caused by the natural accumulation of soluble salts in soil from saline parent material or capillary rise from saline groundwater (Rengasamy, 2010). Secondary or irrigation salinity is anthropogenic and can be caused by the accumulation of salts from use of poor quality irrigation water (Rengasamy, 2010). Previous literature establishing crop tolerance thresholds to salinity primarily address crop responses to secondary salinity. In these studies, crops are irrigated with saline solutions typically composed of dissolved sodium chloride (NaCl) and calcium chloride (CaCl₂) under greenhouse or controlled plot conditions (Maas, 1993). Crop

tolerance is determined by assessing crop biomass or yield against the electrical conductivity (EC) of the soil water extract (EC_e) in the root zone as determined by a saturated paste extract (Maas, 1993; Katerji et al., 2005). Typically, studies assessing crop tolerance to salinity employ these methods. However, these procedures limit the applicability of results for several reasons. The assessment of salinity under greenhouse or controlled plot conditions limits the ability to extrapolate results to real-world conditions (Maas, 1993). As a result, conclusions from the studies are difficult for producers managing saline soils to apply. Additionally, the use of one or two salts to establish thresholds also limits the application of these results to field conditions, as salinity is not always attributed to excess chloride salts and often contains heterogeneous mixtures of salts in natural systems (Bernstein, 1975). Furthermore, in regions dominated by sulfate salinity, osmotic stress and specific ion toxicities associated with salinity may vary because of differences in solubility and anionic constituents (Tölgyessy et al., 1993; Curtin et al., 1993). Another limitation to previous research is the use of irrigation water to induce salinity. Irrigation-induced salinity simulates secondary salinization which may not necessarily be indicative of the impacts of primary salinization on crops. For example, crops experiencing secondary salinity experience excess salts at germination and vegetative stages, whereas primary salinity could potentially impact crops at later reproductive growth stages, as salts typically originate with depth in the soil profile (Richards, 1954). Because the effects of salinity vary based on the life stage of the crop (Maas, 1993), it is possible that differences in crop response could occur with primary salinization.

Soil Salinity in Richland County, North Dakota

Richland County, North Dakota is impacted by primary, sulfate-dominated salinity (Keller et al., 1986). Weathered parent material from repeated glaciations of the Lake Agassiz

plains and sediment transported by the Sheyenne River delta has contributed to naturally saline groundwater (Table 1; Benz et al., 1961; Clayton and Moran, 1982). A recent wet cycle beginning in 1993 (Franzen and Richardson, 2000) caused the depth to this saline pore-filled water to decrease (Maianu et al., 1985). Naturally high rates of evapotranspiration have exacerbated capillary rise of dissolved sulfate and carbonate salts from the groundwater (Abrol et al., 1998). As a result, of the 6.2 million ha of arable land within the Red River Valley of the North, 597,000 ha are classified as moderately saline and 1.2 million ha are classified slightly saline (Hadrich, 2012). Salinity in this region contributes to an estimated US \$150 million in crop losses (Hadrich, 2012). Given the extent of agriculture in this region (Baker, 1967), it is immensely important to understand the impacts of primary, sulfate-dominated salinity under field conditions on agro-ecosystem function so producers can effectively manage these problem soils and reduce financial losses attributed to salinization.

Purpose and Importance of Study

The purpose of this study was to ascertain the effects of natural variation in sulfate-dominated soil salinity on corn and soybean growth and development and on microbial activity.

Table 1. Selected chemical analysis of groundwater in Richland County.

Ionic Constituent	Concentration mg L⁻¹
Ca ²⁺	63.6
K ⁺	2.20
Mg ²⁺	21.9
Na ⁺	4.3
Cl ⁻	<3.0
CO ₃ ²⁻	<1.0
HCO ₃ ⁻	287.0
NO ₃ ⁻	<0.09
SO ₄ ²⁻	26.5

† Groundwater chemistry from the Sheyenne Delta aquifer observation well (Well Index: 126334) sampled on 19th of June, 2014 (North Dakota State Water Commission, 2014).

Research was designed to complement greenhouse and experimental plot studies previously published, as well as establish more meaningful relationships between soil salinity and agricultural productivity that are more pertinent to producers who are impacted by salinization in the Red River Valley. The field component of this research is of fundamental importance because, unlike previous studies, crop response was assessed under natural, field conditions and thus provides the potential to formulate real-world results that can be used for crop yield prediction curves and the establishment of salinity thresholds for corn and soybean indicative to the Red River Valley. The main question addressed in this study was how natural salinity gradients affect crop yields and soil metabolic activity in two soil types: 1) silty clay loam soils and 2) sandy loam soils.

LITERATURE REVIEW

Introduction

Soil salinity under field conditions is an interactive soil chemical property influenced by several environmental conditions in the soil system (Maas, 1993; Volkmar et al., 1997; Rengasamy, 2010). Consequently, it is important to understand how the magnitude of salinity can change under field conditions. The proceeding sections address how soil physical and chemical properties, as well as geology and topography, impact the accumulation and transport of soluble salts in the soil. Understanding how salinity fluctuates in the soil may provide greater insight into why biological response changes across natural gradients in soil salinity.

Measurement of Soil Salinity (Electrical Conductivity)

There are multiple methods for quantifying soil salinity. Typically, salinity is indirectly measured by electrical conductivity (EC). Electrical conductivity is the ability of a solution to conduct an electrical current (Richards, 1954). Increases in dissolved salt constituents effectively increase the ability of a solution to conduct an electric current resulting in higher measured EC values (Suarez, 2005). The magnitude of conductivity also varies based on the composition of ionic species in the solution (Table 2; Tölgyessy et al., 1993). Electrical conductivity can be estimated by remote sensing using electromagnetic meters (EC_a), such as the Geonics EM 38, or through laboratory methods, such as 1:1 soil to water slurries ($EC_{1:1}$) and saturated paste extracts (EC_e). Electrical conductivity can also be mathematically calculated. Conductivity can be estimated by the summation of converted concentrations of individual ions in solution (Table 2; Tölgyessy et al., 1993). Additionally, linear relationships exist between EC and osmotic potential (Ψ_s ; Suarez, 2005), total dissolved solids (TDS; Suarez, 2005) and ionic strength of the solution (μ ; Griffin and Jurinak, 1973).

Table 2. Individual ionic conductivity factors contributing to electrical conductivity.

Ionic Constituent	Conductivity Factor (f_i) [†] $\mu\text{S cm}^{-1}$ per mg L^{-1}
Ca^{2+}	2.60
K^+	1.84
Mg^{2+}	3.82
Na^+	2.13
Cl^-	2.14
CO_3^{2-}	2.82
HCO_3^-	0.715
NO_3^-	1.15
SO_4^{2-}	1.54

† Conductivity factors determined by Tölgyessy et al. (1993).

Factors Affecting Soluble Salt Accumulation in Soils

Solubility of Soil Salts

The accumulation of salts in the soil is a function of both the water content of the soil and the solubilities of the salts (Rengasamy, 2010). In effect, the distribution of soluble salts in the soil is dependent on the amount of water and its movement through the soil (Maianu et al., 1985). Consequently, management of soil salinity is inherently linked to soil water and entails incorporation of field-scale management of soil water (Qureshi et al., 2007).

In order for salts to impair plant and microbial life, they must be solvated into their constituent ions (Volkmar et al., 1997). In effect, factors affecting solubility are important to consider given that these parameters dictate whether or not salts will dissolve in soil water and affect biota. Solubility is the amount of solute (salt) that dissolves in a given amount of solvent (water). The major salts contributing to soil salinity in the southeastern portion of North Dakota's Red River Valley are hydrated and anhydrous forms of sodium sulfate (Na_2SO_4) and magnesium sulfate (MgSO_4 ; Keller et al., 1986). The solubilities of Na_2SO_4 and MgSO_4 at 20 °C are 252 g L^{-1} and 161 g L^{-1} , respectively (FAO, 1973). Based on these solubilities, larger

quantities of Na_2SO_4 dissolve in the same amount of water when compared to the same mass of MgSO_4 . The solubilities of salts impact the magnitude of the EC value given that a salt must be dissolved into its constituent ions in order to influence how well the solution conducts an electric current (Rhoades et al., 1999). In effect, the more salt that dissolves, the higher the value of the EC (Suarez, 2005). The degree of solubility of salts is affected by physical and chemical properties of the environment, such as the amount of water available for solvation, pH, temperature, ion activity of the constituent ions, and soil texture (Richards, 1954; Suarez, 2005; Rengasamy, 2010).

Soil Physical and Chemical Properties Affecting Solubility

Environmental conditions must favor dissolution of the salt, as movement or accumulation of salts only occurs if salts are dissolved in the soil solution (Maianu et al., 1985; Li et al., 2013). In general, increased temperature facilitates increased solvation because there is a greater amount of energy available to break the ionic bonds between the salt constituents (Black and Muller, 2010). However, in some instances, salts have retrograde solubility, in which solvation increases as temperature decreases. For example, solubility of calcium carbonate or calcite (CaCO_3) decreases nonlinearly between temperatures of 20 and 95 °C (Coto et al., 2012).

The pH of the soil solution will also impact the solubility of salts (Lindsay, 1979) because of its influence on the formation of strong complexes (Jackman and Black, 1951). For example, at pH values of 6.0, more than 80% of phosphorus from magnesium and calcium is in a soluble form available for plant uptake (Jackman and Black, 1951). However, as pH increases above 7.0, the presence of soluble phosphorus in the same solution decreases exponentially to values below 10% (Jackman and Black, 1951). Because complexes are precipitated, neutral

minerals, salt constituents that complex with phosphorus compounds are not in an aqueous form that could contribute to soil salinity (Suarez, 2005).

Ion activity can also influence the solubility of salts through formation of ion pairs of sulfate or carbonate anions with multivalent cations (Naidu and Rengasamy, 1993). Ion pair formations result in salts that are not completely dissociated (Kester and Pytkowicz, 1969). The incomplete dissociation results in a weak electrolyte in solution (Kester and Pytkowicz, 1969). Because ion pairs have neutral charges, they do not contribute to the EC of the solution (Suarez, 2005). Consequently, a solution saturated with respect to gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) can reach a maximum EC of 2.2 dS m^{-1} (Bernstein, 1975; He et al., 2015), after which the ion pairs of gypsum (CaSO_4°) inhibit further increases in EC. The impact of ion pairing in solution can be enhanced in the presence of common ions (Bennet and Adams, 1972). For example, excess $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ in 0.005 M MgSO_4 forms $1.40 \text{ mM MgSO}_4^\circ$ (Tanji, 1969). However, as ionic strength of the solution increases with the addition of 0.05 M MgSO_4 , ion pairing of MgSO_4° increases to 19.28 mM (Tanji, 1969).

Soil texture has immense impacts on soil salinity given that soil water retention and movement are influenced by this physical property (Li et al., 2013). In general, finer textured soils or soils high in clay content retard water movement within a soil (Setia et al., 2011). The higher retention of water is, in part, attributed to the high specific surface area of clay particles (Peterson et al., 1996). Sand and clay have specific surface areas of $12.2 \text{ m}^2 \text{ g}^{-1}$ and $421.8 \text{ m}^2 \text{ g}^{-1}$, respectively (Banin and Amiel, 1970). The high specific surface areas of clay particles facilitate higher retention and imbibition of soil water because of increased absorption of water on the soil particle surface (Banin and Amiel, 1970). The subsequent increase in water holding capacity could result in decreased drainage and decreased leaching of soluble salts from the soil profile

because of lower hydraulic conductivities associated with increasing specific surface areas of clay particles (Peterson et al., 1996). Typical leaching fractions for coarse and fine textured soils are 0.15 and 0.10, respectively (Martinez-Beltrán, 1999). In effect, clay has higher water retention and thus higher retention of soluble salts because less water is leached from these soils. However, while more salts are retained in the profile, the increase in water retained in the soil effectively dilutes the soil solution, producing lower EC values than would be observed for the same salt content on a dry soil basis in a coarser textured soil (Bernstein, 1975; Setia et al., 2011). Thus, with increased water retention, a higher EC would be necessary to impair biological growth and development in finer textured soils (Richards, 1954).

Capillary rise of salts in the soil profile is also influenced by texture. In general, finer textured soils can transport salts farther than coarser textured soils due to the smaller pore spaces between particles. The higher distance of capillary rise of water in finer textured soils is best explained mathematically (Zhmud et al., 2000; Eq. 1)

$$h_c = \frac{2\gamma \cdot \cos(\alpha)}{g(p_l - p_g)r} \quad (1)$$

where h_c is the distance of capillary rise (cm), γ is the surface tension between liquid and air (N cm⁻¹), α is the contact angle, g is the acceleration due to gravity in (cm² s⁻¹), p is the density of the liquid (l) and gas (g), respectively (g cm⁻³), and r is the capillary radius (cm; Zhmud et al., 2000). The distance travelled by water as a result of capillarity is higher in finer textured soils because of the smaller pore spaces that reduce the capillary radius (Li et al., 2013). However, the rate of capillary rise is generally higher in coarser textured soils because of the increased hydraulic conductivity (Li et al., 2013).

Topography and Slope

Some geomorphic features of particular landscapes are conducive for soluble salt accumulation. Ditch-effect salinity is a common secondary source in the flatter regions of Richland County and across eastern North Dakota (Skarie et al., 1986). Ditch-effect salinity is caused by deposition of salts from laterally-flowing water originating from ditches or drains (Skarie et al., 1986). As the salts are transported across the adjacent landscape, evaporation transports salts closer to the soil surface, where they are deposited once the water evaporates (Skarie et al., 1986). Water movement in soil is controlled by the total potential of soil water (ψ_T ; Philip, 1960; Eq. 2).

$$\psi_T = \psi_g + \psi_m + \psi_o \quad (2)$$

where ψ_g is the gravitational potential, ψ_m is the capillary or matric potential, and ψ_o is the osmotic potential. Consequently, when a drainage ditch lies adjacent to uncovered, arable land, the resulting pressure potentials between the two landforms result in a gradient that pulls water towards the landform with lower potential: the uncovered land (Farahani and DeCoursey, 2000). In effect, the bare, uncovered field has a higher evaporative demand. As a result of the lower water potential, water is pulled from the ditch into the field, moving dissolved salts with it (Skarie et al., 1986).

The Phreatic Zone

Groundwater has an immense influence on the accumulation of soluble soil salts. In instances where the depth to pore-filled water, or phreatic zone, is shallower than a critical depth in the soil profile, evapotranspiration can substantially increase the amount of capillary rise from the phreatic zone occurring within the profile (Shah et al., 2007). This effect is enhanced in soils containing higher sand fractions, which result in capillary rise of 0.14 cm d^{-1} compared to 0.10

cm d⁻¹ in clayey soils at the same critical depth (Kovda, 1964). The onset of above normal precipitation in North Dakota beginning in 1993 (Franzen and Richardson, 2000) decreased the depth to pore-filled water (Maianu et al., 1985), which contributed to the accumulation of soluble salts in the root zone (Li et al., 2013). The accumulation of salts was attributed to increased capillary rise from naturally saline water from the phreatic zone, which was significantly enhanced due to the shallower depth to pore-filled water (Li et al., 2013). Groundwater chemistry also affects salt accumulation in soils as the chemical composition contributes to the ions that accumulate within the soil solution (Maianu et al., 1985).

Impact of Soil Salinity on Biota

Once salts are dissolved into the soil solution, biological growth and activity are inhibited by two main mechanisms: 1) osmotic or drought stress and 2) specific ion effects (Munns, 2002). Declines in both crop productivity and microbial activity are attributed to these stressors, which can cause both direct impacts on biological functions (Munns, 2002; Rath and Rousk, 2015), as well as indirect effects on soil physical and chemical conditions (Bronick and Lal, 2005; Horie et al., 2012).

Osmotic or Drought Stress

Drought stress is attributed to the alteration of the osmotic potential of the soil solution surrounding the root zone due to excess soluble salts (Maas and Nieman, 1978). While the osmotic potential generally does not influence soil water movement, it plays a significant role in the interaction between the membranes or diffusive barriers of roots, microbial populations, and the surrounding soil (Cowan, 1965). Consequently, root water uptake for plants becomes increasingly difficult given that the roots must exert more energy to remove water from the surrounding soil solution across the root membrane and into the plant (Volkmar et al., 1997). For

soil microorganisms, decreasing osmotic potentials force microbial populations to reallocate resources, potentially slowing the rates of biogeochemical processes in the soil and increasing energy expenditures to acclimate to lower osmotic potentials (Schimel et al., 2007).

The inability to uptake water from the soil solution causes the physiological drought stress commonly observed in plants affected by excess soluble salts (de Oliveira et al., 2013). Osmotic stress induces two physiological impacts: 1) cellular dehydration and 2) ion cytotoxicity (Munns, 2002). Cellular dehydration often results in cessation of growth and inhibition of metabolic processes (Gupta and Huang, 2014) because of the replacement of potassium (K^+) ions with Na^+ in necessary biochemical reactions (Horie et al., 2012). Potassium is essential for cell turgor maintenance, and the replacement of K^+ with Na^+ in metabolic processes during salinity stress inhibits K^+ uptake by the cell (Gupta and Huang, 2014). In an effort to maintain ionic balance within cells, some plants exhibit salt extrusion mechanisms that transport toxic ions, like Na^+ , to cell vacuoles for sequestration (Munns and Tester, 2008). Plants can also use organic solutes to osmoregulate and increase water intake (Empadinhas and da Costa, 2008; Gupta and Huang, 2014). For plants, osmoregulation maintains turgor pressure potential of cells by increasing the accumulation of organic solutes within the cell cytosol (Munns and Tester, 2008) that do not interfere with normal physiological function (Gupta and Huang, 2014). The increase in osmolytes in cells and membranes can facilitate water movement back into the cell, reducing the impact of osmotic stress on cellular dehydration (Horie et al., 2012).

Salt extrusion and accumulation of osmolytes are highly energetic processes that require both exportation and importation of extracellular ions against concentration gradients of membranes (Rath and Rousk, 2015). For salt exclusion mechanisms, the rate of salt exclusion must exceed the rate of salt uptake by plant roots for organisms to effectively compartmentalize

salts into cell vacuoles (Munns, 2002). If rates of uptake exceed rates of exclusion, salt constituents can be distributed to above-ground portions of the plant or throughout the cells (Munns, 2002; Wong et al., 2010). As non-sequestered salt constituents accumulate in the cells and tissues of organisms, specific ion effects begin to impede physiological function (Munns, 2002).

Specific Ion Effects

Specific ion effects caused by soil salinity are physiological effects of the individual salt constituents accumulating in organic tissue (Läuchi and Epstein, 1984). While most salt constituents are necessary for growth and development (Table 3; Epstein, 1972; Epstein, 1999), they can become lethal to cells and tissues of organisms in excess. The effects of ions vary based on the species and concentration of the ion contributing to the toxicity. However, a common symptom of most excessive ion concentrations is membrane damage (Volkmar, et al., 1997). Membrane damage results in several secondary effects including, but not limited to, reduced cell and leaf expansion, stomatal closure, photosynthetic inhibition, protein destabilization, and cell death (Aslam et al., 2011). The secondary effects are attributed to the replacement of K^+ with Na^+ in biochemical reactions, as well as alterations in protein structure as a result of excessive Na^+ and Cl^- (Shrivastava and Kumar, 2015). For example, salinity stress triggers stomatal closure in response to decreases in leaf turgor (Chaves et al., 2009). Stomatal closure results in a reduction in ambient carbon dioxide (CO_2) assimilated by the plant from inhibition of photosynthesis (Brugnoli and Lauteri, 1991). Because of the reduction in photosynthesis, chloroplasts within the cell become excited and produce reactive oxygen species (ROS; Aslam et al., 2011). Reactive oxygen species damage biomolecules required for normal physiological function and ultimately result in cell death (Das and Roychoudhury, 2014).

Table 3. Selected mineral nutrient concentrations in tissues of most plants required for growth and development (Epstein, 1972; Epstein, 1999).

Mineral	Concentration (% or mg L ⁻¹)
Calcium [†]	0.5
Magnesium [†]	0.2
Sulfur [†]	0.1
Sodium [‡]	10
Chlorine [‡]	100

† Macronutrient measured as a percentage.

‡ Micronutrient measured in mg L⁻¹.

Sodium and Calcium

Sodium has the most negative impact on plant growth and development when compared with other cation constituents of salts because of its ability to induce calcium (Ca²⁺) and K⁺ deficiencies (Bernstein, 1975). For example, Na⁺ displaces Ca²⁺ on cell walls of plant membranes (Cramer et al., 1985; Kinraide, 1998). Displacement of Ca²⁺ from the plant membrane causes protein denaturation and destabilization (Cramer et al., 1985). Calcium ions enable mechanisms of cell detoxification to counter the negative effects of Na⁺ (Lahaye and Epstein, 1969; Kinraide, 1998; Tas and Basar, 2009) and restore K⁺ levels for biochemical function (Cramer et al., 1985; Tuna et al., 2007), but the ameliorative effect is reduced when Na⁺ concentrations in an external solution exceed 250 mM (Cramer et al., 1985). Despite its potential ameliorative effects, prolonged exposure of excessive Ca²⁺ in the soil solution can still induce stressful conditions on plants (Parida and Das, 2005). Higher concentrations of both Na⁺ and Ca²⁺ reduce the osmotic potential of the soil solution and contribute to drought stress (Kinraide, 1998; Tölgyessy et al., 1993).

Magnesium

While magnesium (Mg^{2+}) is often associated with salinity, little is known about its toxic ion effects on plants. Similar to Ca^{2+} , Mg^{2+} is also recognized as an essential macronutrient for plants, as it plays a role in enzyme activation, chlorophyll structure, and stomatal maintenance and photosynthesis (Shaul, 2002). Additions of dissolved $MgSO_4$ to nutrient solutions have been linked to improved photosynthetic capacity in maize plants grown under Mg-deficient conditions (Jezek et al., 2015). Magnesium can also reduce the impacts of Na^+ -induced salinity using mechanisms similar to Ca^{2+} detoxification of Na^+ , but its effects are reduced in comparison to the beneficial effects of Ca^{2+} (Kinraide, 1998).

Alternatively, substantially larger declines in above-ground biomass of germinating corn was observed when corn seedlings were irrigated with dissolved $MgSO_4$ compared to $NaCl$, $CaCl_2$, $MgCl_2$, and Na_2SO_4 (Kaddah and Ghowail, 1964). At isosmotic concentrations of these salts, percent weights declined to 15.6, 41.3, 42.1, 40.2, and 31.7% relative to the non-saline control, respectively (Kaddah and Ghowail, 1964). Furthermore, indirect effects of excessive Mg^{2+} can induce environmental stressors. For example, high levels of Mg^{2+} in the soil solution contribute to the alteration of the osmotic potential, facilitating drought stress (Tölgyessy et al, 1993). Some studies have also observed an indirect effect of Mg^{2+} on soil structure and, consequently, soil water movement. Magnesium-rich soils can be structurally degraded (Zhang and Norton, 2002). The degradation is similar to Na^+ -induced dispersion of soil particles in sodic soils (Bronick and Lal, 2005). As soil particles disperse, water infiltration and hydraulic conductivity are reduced, which could exacerbate osmotic stress experienced by the soil organisms (Qadir et al., 2013). However, the dispersive effects of Mg^{2+} on soil structure are still controversial. Other studies reported that Mg^{2+} did not significantly increase dispersion in pure

montmorillonite clays when compared to excess Ca^{2+} (He et al., 2014) or reduce hydraulic conductivity in soils dominated by montmorillonite or kaolinite clays (Rowell and Shainberg, 1979).

Chloride and Sulfate

Both chloride (Cl^-) and sulfate (SO_4^{2-}) have detrimental effects on plant growth and development (Bernstein, 1975; Läuchi and Epstein, 1984). Chloride is an essential micronutrient for enzyme regulation and photosynthesis, but in excess of $800 \text{ mg soil kg}^{-1}$ (Jing et al., 1992) or $15 \text{ mmol Cl}^- \text{ L}^{-1}$ in the extract from a saturated paste (Maas, 1986), it becomes toxic to salt sensitive species like corn and reduces yield to 95% relative to the non-saline control (Jing et al., 1992). Chloride toxicity is attributed to interference with nitrate (NO_3^-) uptake (Grattan and Grieve, 1999) and chlorophyll degradation (Tavakkoli et al., 2010). Chlorophyll degradation reduces photosynthetic capacity in plants (Tavakkoli et al., 2010), and declines in photosynthesis ultimately diminish the plant's supply of carbohydrates that can be used for growth (Munns and Tester, 2008).

Sulfate (SO_4^{2-}) is considered a macronutrient and is a major component in biochemical compounds, like cysteine and glutathione, which regulate physiological function in plants (Leustek and Saito, 1999). Studies addressing the impacts of excess SO_4^{2-} salts on plant growth are rare (Curtin et al., 1993) and results vary among species. For example, the weight of seedling tops from germinating corn (G.H. 67 cultivar) irrigated with a 2 atm solution of NaCl ($\text{EC} \approx 5.1 \text{ dS m}^{-1}$) was reduced to 62% relative to the non-saline control (Kaddah and Ghowail, 1964). The weight was reduced to 52% relative to the control when the same variety was irrigated with a 2 MPa solution of Na_2SO_4 (Kaddah and Ghowail, 1964). Alternatively, soybeans were found to be more tolerant to SO_4^{2-} salinity when compared to Cl^- -dominated salinity (Gupta and Gupta,

1984). Dry matter yield of soybeans (variety Black Tur) remained relatively constant up to 88 meq $\text{SO}_4^{2-} \text{L}^{-1}$ ($\text{EC} \approx 6.50 \text{ dS m}^{-1}$; Gupta and Gupta, 1984).

Despite the reduction in vegetative corn biomass during germination, most studies typically attribute more detrimental specific ion toxicities to Cl^- ions because biochemical compounds containing SO_4^{2-} , like glutathione, are often considered defense compounds that can alleviate effects of abiotic stressors on plants (Leustek and Saito, 1999). Additionally, other biochemical compounds, like glutamine, can reduce excess SO_4^{2-} accumulation in the plant which can reduce detrimental impacts of SO_4^{2-} (Kowalska, 2005). However, similar to cations in the soil solution, increases in concentrations of either anion can still contribute to the reduction in the osmotic potential of the soil solution associated with drought stress (Tölgyessy et al., 1993).

It should also be noted that soils dominated by SO_4^{2-} salts may exhibit structural degradation. Ion pairing of SO_4^{2-} with Ca^{2+} and Mg^{2+} can exacerbate Na^+ -induced dispersion in sulfate dominated soils compared to Cl^- -dominated soils (Springer et al., 1999). Removal of Ca^{2+} and Mg^{2+} ions from solution facilitates adsorption of Na^+ on the exchange sites of soil particles (Springer et al., 1999). Because dispersion ultimately impacts soil water movement (Wong et al., 2010), osmotic stress could become more pronounced in SO_4^{2-} -dominated soils. Furthermore, in addition to ion pairing, the relatively low solubility of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) could also induce Ca-deficiencies in SO_4^{2-} -dominated soils (Curtin et al., 1993). Consequently, it is possible that declines in plants grown in soils with excessive SO_4^{2-} concentrations are partially attributed to deficiencies in Ca^{2+} (Janzen and Chang, 1987).

Soil Salinity and Agricultural Productivity

While research has been performed assessing the effects of most salt constituents on plant growth and development, it is important to acknowledge the influence of the inherent genetic

tolerances of species and their varieties to osmotic stress and specific ion toxicities (Munns, 2002). The combined effects of drought stress and specific ion effects manifest themselves as distinctive phenotypic effects on plant growth and development (Munns, 2002) and microbial activity (Rath and Rousk, 2015). The physiological impacts on plants and microbial populations vary not only with the composition and concentration of dissolved salts contributing to salinity (Maas and Neiman, 1978; Curtin et al., 1993), but also on the species of interest and the growth stage of the crop (Bernstein, 1975; Maas, 1993; Rath and Rousk, 2015).

Corn

Above-ground Biomass

In general, corn (*Zea mays*) is most susceptible to salinity during the vegetative stages of its life cycle, but the impact of salinity varies among vegetative growth stages (Table 4; Maas et al., 1983). In general, corn is less susceptible to salinity stress during germination compared to later vegetative growth stages (Maas et al., 1983). In a study assessing germination of hybrid G.H. 67 corn grown in coarse sand to Na-, Ca-, and Mg- chloride and sulfate salts, Kaddah and Ghowail (1964) reported that almost all corn plants successfully emerged despite a reduction in germination rate. However, sulfate salts (Na_2SO_4 and MgSO_4) induced greater reductions in germination rate of seedlings compared to Cl^- salts (Kaddah and Ghowail, 1964). Percent germination of Funk G4141, Pioneer 3369A, and Northrup King PX32 cultivars was also delayed, but percent emergence was not significantly reduced until an EC_e of 8.0 dS m^{-1} (1:1 NaCl:CaCl₂; Maas et al., 1983). Even more, some cultivars tested could germinate under salinities reaching 15.0 dS m^{-1} (Maas et al., 1983). Similarly, percent emergence of corn hybrid AG-6690 grown in sandy loam soils was not affected by EC of irrigation water (EC_i) up to 5.9 dS m^{-1} (1:1 NaCl:CaCl₂; Blanco et al., 2007).

Table 4. Corn growth responses to salinity from selected publications.

Authors	Life Stage [†]	Parameter [‡]	Medium [§]	EC _x [¶]	Salt [#]	EC _T ^{††} dS m ⁻¹	Slope ^{‡‡} % per dS m ⁻¹
Maas and Hoffman, 1977	Vegetative	Germination	Topsoil, Peat	EC _e	NaCl:CaCl ₂	8.0	--
Blanco et al., 2007	Vegetative	Germination	Sandy Loam	EC _i	NaCl:CaCl ₂	>5.9	--
Kaddah and Ghowail, 1964	Maturity	Yield	Sandy Clay Loam-Sandy Cay	EC _e	NaCl:CaCl ₂	2.0	20
Maas and Hoffman, 1977	Maturity	Yield	Topsoil, Peat	EC _e	NaCl:CaCl ₂	1.7	12
Katerji et al., 2000	Maturity	Yield	Loam	EC _{sw}	NaCl:CaCl ₂	1.3	10.5
Blanco et al., 2008	Maturity	Yield	Sandy Loam	EC _i	NaCl:CaCl ₂	1.7	21
Maas et al., 1983	Vegetative	Height	Unknown	EC _e	NaCl:CaCl ₂	0.7	4.9
Shalhevet et al., 1995	Vegetative	Height	Peat, Silt Loam, Sand	EC _e	NaCl:CaCl ₂	4.02	6.9
Blanco et al., 2008	Reproductive	Height	Sandy Loam	EC _i	NaCl:CaCl ₂	--	8
Blanco et al., 2007	Vegetative	Leaf Weight	Sandy Loam	EC _i	NaCl:CaCl ₂	1.9	14
Katerji et al., 1996	Reproductive	Leaf Area	Loam	EC _{sw}	NaCl:CaCl ₂	--	9.7
Amer et al., 2010	Reproductive	Leaf Area	Clay Loam	EC _i	Mixture	1.92	8.2
Shalhevet et al., 1995	Vegetative	Root Length	Peat, Silt Loam, Sand	EC _e	NaCl:CaCl ₂	4.09	9

† Life stage of corn measurements were collected from

‡ Crop parameter measured.

§ Medium used to grow corn.

¶ Type of salinity measured. EC_e is the electrical conductivity (EC) of a saturated paste extract, EC_i is the EC of the irrigation water applied to corn, and EC_{sw} is the EC of the soil solution within the pores.

Species of salt used to induce salinity. Mixture of salt composed of Ca²⁺, Na⁺, Mg²⁺, Na⁺, K⁺, CO₃²⁻, HCO₃⁻, Cl⁻, and SO₄²⁻.

†† Threshold salinity tolerance reported by the study.

‡‡ Slope of decline observed after threshold salinity tolerance.

After germination, corn seedlings become increasingly more susceptible to salinity stress (Kaddah and Ghowail, 1964; Maas et al., 1983). At later vegetative growth stages, significant declines in shoot growth of Funk G4141, Pioneer 3369A, and Northrup King PX32 cultivar seedlings occurred at a threshold EC_e (EC_T) above 0.7 dS m⁻¹ by 5% per unit increase in EC_e (Maas et al., 1983). Dry matter of these varieties at the seedling stage was reduced between 44 and 59% relative to a non-saline control (Maas et al., 1983).

The same corn varieties at mature growth stages (tasseling or grain-filling) were substantially more tolerant to increasing EC_e and maintained 90 to 100% relative biomass up to 9.3 dS m⁻¹ (Maas et al., 1983). Relative grain yield of these varieties at mature growth stages only dropped below those of the non-saline control when EC_e at the vegetative stages exceeded 3.0 dS m⁻¹ (Maas et al., 1983). Despite an increased tolerance of corn at germination and mature growth stages, greenhouse studies on the effects of soil salinity on corn yield indicate a lower tolerance threshold to excess soluble salts in the soil when corn was subjected to salinity stress

throughout the growing season. For example, irrigation water with a 2:1 NaCl and CaCl₂ concentration applied at seeding significantly decreased corn yield after a threshold EC_e (EC_T) of approximately 2.0 dS m⁻¹ by 7.0% per dS m⁻¹ increase (Kaddah and Ghowail, 1964). If salinity was initiated 21 days after seeding (during the seedling stage), yields declined by 20% per dS m⁻¹ after 2.0 dS m⁻¹ (Kaddah and Ghowail, 1964). Salinity induced during reproductive or tasseling stages resulted in declines of 10% per unit increase after an EC_T of 2.0 dS m⁻¹ (Kaddah and Ghowail, 1964).

In addition to the dependence of tolerance on life stage, both the threshold and slope of decline in corn yield seem to vary depending on the soil texture and nitrogen (N) application rate (Khalil et al., 1967; Beltrão and Asher, 1997). For example, in modeled simulations for corn grown in non-leached soils, corn yield declined after EC_e values of 1 dS m⁻¹ in sandy soils and 2 dS m⁻¹ in clay and loam soils (Beltrão and Asher, 1997). Regardless of texture, tolerance thresholds of corn yield to salinity increased with increasing nitrogen applications (Khalil et al., 1967; Beltrão and Asher, 1997; Azizian and Sepaskhah, 2013). However, yields of salinity-stressed corn at the highest rate applications of N were still lower than the non-saline control at the same application rate (Khalil et al., 1967). A review of the effects of soil salinity on crop development and yield by Katerji et al. (2000) reported results congruent with the studies by Kaddah and Ghowail (1964) and Beltrão and Asher (1997): declines in hybrid Asgrow 88 yield began at a threshold EC_e of 1.3 dS m⁻¹ and decreased by 10.5% per unit increase in pore water salinity (EC_{sw}) for a lysimeter experiment. Salt tolerance data compiled by Maas and Hoffman (1977) also substantiated these values with a threshold salinity tolerance of 1.70 dS m⁻¹ and a 12% decrease in relative corn yield for every dS m⁻¹ increase in salinity.

Previous literature has also reported decreases in corn height, leaf area index (LAI), and leaf nitrogen content in response to increasing soil salinity. Declines in growth are potentially a result of the reallocation of energy for growth processes to osmotic maintenance of cells in the plant (Läuchi and Epstein, 1984). Alternatively, declines in growth could be attributed to hormone signals from the root (Termaat et al., 1995). Growth hormones, like cytokinin and abscisic acid (ABA), are produced in roots under salinity stress and could thus be used as a signal to regulate shoot growth in water-stressed plants (Termaat et al., 1995). Height of corn hybrid AG 6690 exposed to salinity at vegetative life stages decreased by 13% per unit increase in EC_e above a threshold of 1.9 dS m^{-1} in sandy loam soils (Blanco et al., 2008). The threshold before decline increased to 4.02 dS m^{-1} and the slope of decline decreased to 6.9% per unit increase in EC_e when vegetative corn was grown in a mixture of peat, silt loam, and sand (Shalhevet et al., 1995). Height of hybrid cv SC704 mature corn decreased by 3.5% per unit increase in EC_e with no thresholds before decline in silty loam soils (Azizian and Sepaskhah, 2014). Similarly, mature AG 6690 decreased linearly by 8% per unit increase in EC_e with no reported threshold (Blanco et al., 2008). While no exact overlaps of data appear to occur among studies on corn height in response to salinity, the general pattern of decreased height were reported between all studies examined for both vegetative and reproductive life stages of corn.

General declines in LAI have also been observed for corn grown under saline conditions. Similar to declines in height, declines in leaf area can be attributed to decreases in cell and membrane turgor (Curtis and Läuchli, 1987). Another study demonstrated that declines in LAI may be the result of the reductions in photosynthesis during stressful conditions (Aslam et al., 2011). Reduction in photosynthesis contributes to a reduction in plant growth (Brugnoli and Lauteri, 1991) which would ultimately result in reduced leaf area (Munns and Tester, 2008).

Alternatively, the decline in leaf area could be an adaptation mechanism, which suggests that reduced cell size in response to decreased turgor facilitates more efficient maintenance of turgor at low water potentials (Cutler et al., 1977). In a silty loam soil, LAI of cultivar SC704 declined by 3.4% per unit increase in EC_e (Azizian and Sepaskhah, 2014), but the slope of decline in LAI nearly tripled per unit increase in EC_e when corn was grown in a clay loam soil (Amer, 2010). Similarly, canopy dry matter of hybrid Asgrow 88 grown in a loam declined by 9.7% per unit increase in EC_e , and the slope of decline increased to 11.4% when grown in clay soil (Katerji et al., 1996). Again, while slopes of decline vary among studies, a general decline in leaf area was reported for all research examined.

Plant uptake of nutrients is generally diminished by increasing levels of salinity because of the reduced osmotic potential of the soil solution surrounding the root zone of plants (Fageria et al., 2011). Nutrient uptake is both a function of transpiration rate and the ability of roots to absorb nutrients under water stress conditions (Tanguilig et al., 1987). However, studies examining the response of plant N-content have reported contradictory results. For example, total soluble N within a pure strain of corn grown in a 2:1 clay and sand mixture contained 150%, 200%, and 204% N relative to the control when irrigated with 50, 100, and 200 mM of NaCl, respectively ($EC \approx 6.2, 12.5, \text{ and } 25.0 \text{ dS m}^{-1}$, respectively; Bassuony et al., 2008). However, total N-content decreased by 1.4% per unit increase in EC_i (Bassuony et al., 2008). A lack of significant differences in N-content of both leaves and roots of an RX 770 hybrid was demonstrated by Tas and Basar (2009) who reported that regardless of the salts contributing to salinity, corn grown in a mixture of peat and silt maintained 1.44 to 2.26% N in leaves and 1.08 to 1.85% N in roots. While not significant, higher N contents in leaves and roots were observed when salts contributing to salinity contained nitrate (NO_3^- ; Tas and Basar, 2009). Similarly, Giza

310 corn grown in quartz sand had no significant changes in N-content in shoot dry mass up to an osmotic potential of -0.9 MPa ($EC \approx 22.5 \text{ dS m}^{-1}$; Hamdia and El-Komy, 1997). However, N assimilation from the air and uptake from fertilizers by corn was significantly decreased at -1.2 MPa ($EC \approx 30.0 \text{ dS m}^{-1}$) to 41.3% relative to the non-saline control (Hamdia and El-Komy, 1997). The discrepancies among results could potentially be attributed to the types of N compounds analyzed. For example, N-containing amino acids, proteins, and ammonium compounds accumulate in plants in response to increasing levels of salinity (Mansour, 2000), whereas NO_3^- decreases because of decreased photosynthetic activity and Cl^- inhibition (Tas and Basar, 2009).

Below-ground Biomass

Generally, below-ground biomass is less susceptible to salinity stress than the above-ground portion of plants (Bernstein and Hayward, 1958; Munns and Termaat, 1986). The increased tolerance of roots to salt stress is likely attributed to an ability to rapidly adjust the osmotic gradient of the root membrane when salt stress occurs (Hsiao and Xu, 2000). However, both corn root length and biomass have declined in response to increasing levels of dissolved NaCl. Corn (B73 variety) root length at the seedling life stage was significantly different between three levels of salinity, and biomass decreased by approximately 69 and 87% in 100 mM (12.5 dS m^{-1}) and 200 mM (25.0 dS m^{-1}) of NaCl, respectively (Hoque et al., 2015). Khatoun et al. (2010) corroborated these results at the germination stage of EV-1098 and Agaiti varieties, but found that the impact of salinity on root length was alleviated at later growth stages of the vegetative cycle. Similarly, root weight of a mature corn grown in a sandy loam soil declined by 11% per unit increase in depth weighted average EC_e (EC_{DWA}) in moist soils and 13% per unit increase in EC_{DWA} in dry soils (Al-Khateef et al., 1999). Root growth in this study

was completely inhibited in layers of the soil that exceeded an EC_e of 12.0 dS m^{-1} (Al-Khafef et al., 1999).

Despite declines in both root biomass and length being previously observed, it is important to reiterate that roots can recover from osmotic stress induced by salinity (Munns, 2002). In a study assessing the rate of root extension in corn seedlings in a nutrient solution, root extension of Pioneer 3906 in solutions up to 150 mM of NaCl (18.7 dS m^{-1}) were not significantly different from the control with 0 mM of NaCl (0 dS m^{-1}) when the salt solution was added incrementally (Rodriguez et al., 1997). Significant differences in the extension rate only occurred when corn seedlings were subjected to salt shock, in which the desired concentration of NaCl was added in one step to the nutrient solution (Rodríguez et al., 1997). Furthermore, the salt shock treatment resulted in a significant decline in root diameter of newly grown roots, whereas this effect was not observed when corn seedling roots were gradually introduced to salinity stress (Rodríguez et al., 1997).

Soybean

Above-ground Biomass

Similar to corn, the growth stage of soybeans (*Glycine max*) dictates its ability to tolerate salinity stress (Table 5). Typically, germination is more tolerant to salt stress than later vegetative growth stages (Phang et al., 2008). However, growth stage tolerance is highly dependent on soybean variety (Phang, et al. 2008). Germination of soybeans grown in a coarse textured soil was reduced in all varieties studied after an EC_e of 8.1 dS m^{-1} , but the rate of reduction varied depending on the cultivar (Abel and MacKenzie, 1964). For example, germination of Lee soybeans was reduced by 6.6% per dS m^{-1} increase after 20 days, whereas germination of N53-509 was reduced by 8.7% per unit increase in salinity (Abel and MacKenzie,

Table 5. Soybean growth responses to salinity from selected publications.

Authors	Life Stage [†]	Parameter [‡]	Medium [§]	EC _x [¶]	Salt [#]	EC _T ^{††} dS m ⁻¹	Slope ^{‡‡} % per dS m ⁻¹
Abel and MacKenzie, 1964	Vegetative	Germination	Coarse, Gypsiferous Soil	EC _e	NaCl	8.1	--
Blanco et al., 2007	Vegetative	Germination	Sandy Loam	EC _i	NaCl:CaCl ₂	2.8	19
Abel and MacKenzie, 1964	Maturity	Plant Density	Silty Clay	EC _e	NaCl:CaCl ₂	5.0	30 ^{§§}
Maas and Hoffman, 1977	Maturity	Yield	Topsoil, Peat	EC _e	NaCl:CaCl ₂	5.0	20
Katerji et al., 2000	Maturity	Yield	Loam	EC _{sw}	NaCl:CaCl ₂	2.0	11.4
Blanco et al., 2007	Vegetative	Height	Sandy Loam	EC _i	NaCl:CaCl ₂	0.9	14
Shalhevet et al., 1995	Vegetative	Height	Peat, Silt Loam, Sand	EC _e	NaCl:CaCl ₂	5.70	5.3
Blanco et al., 2007	Vegetative	Leaf Weight	Sandy Loam	EC _i	NaCl:CaCl ₂	1.0	21
Queiroz et al., 2012	Vegetative	Leaf Area	Nutrient Culture	EC _i	NaCl	--	2.2
Abel and MacKenzie, 1964	Reproductive	Leaf Width	Silty Clay	EC _e	NaCl	6.5	5 ^{¶¶}
Shalhevet et al., 1995	Vegetative	Root Length	Peat, Silt Loam, Sand	EC _e	NaCl:CaCl ₂	5.08	6.6
Bernstein and Ogata, 1966	Reproductive	Nodulation	Field Soils	EC _e	NaCl	7.0	--

† Life stage of corn measurements were collected from

‡ Crop parameter measured.

§ Medium used to grow corn.

¶ Type of salinity measured. EC_e is the electrical conductivity (EC) of a saturated paste extract, EC_i is the EC of the irrigation water applied to corn, and EC_{sw} is the EC of the soil solution within the pores.

Species of salt used to induce salinity.

†† Threshold salinity tolerance reported by the study.

‡‡ Slope of decline observed after threshold salinity tolerance.

§§ Slope of decline applies to Jackson variety soybean.

¶¶ Slope of decline applies to Lee variety soybean.

1964). Blanco et al. (2007) corroborated a reduction in emergence, but observed lower thresholds of EC_i before decline. Reduction in percent emergence and speed of emergence of Conquista soybeans occurred at a threshold EC_i of 2.7 dS m⁻¹ and declined by 20% per unit increase in EC_i after this threshold (Blanco et al., 2007).

Soybean yield response to soil salinity follows similar patterns to corn, but thresholds before yield declines were notably higher, indicating that higher levels of salinity must be reached before declines in soybean productivity occur (Stephun et al., 2005). Typically, discrepancies among results often cited the impact of soybean variety on tolerance to explain the inconsistencies with threshold tolerances and the slopes of yield declines (Abel and MacKenzie, 1964; Katerji et al., 2000; Papiernik et al., 2005). For example, the Lee cultivar (a salt-tolerant variety) declined linearly by 15.6% per unit increase in atm NaCl (Bernstein and Ogata, 1966) or 20% per unit increase in EC_e (Maas and Hoffman, 1977) in a gravel culture. Percent stem dry

matter of the same variety declined by 8.2% per unit increase in EC_e after an EC_T of 5.0 dS m^{-1} in a silty clay soil (Abel and MacKenzie, 1964). Yield of Talon soybeans (a slightly salt-sensitive variety) declined by 11.4% per unit increase in EC_{sw} after an EC_T of 2.0 dS m^{-1} in loam (Katerji et al., 2000), and percent stem dry matter of Jackson soybeans (a salt sensitive variety) declined by 56.7% per unit increase in EC_e in a silty clay soil up to 7.3 dS m^{-1} (Abel and MacKenzie, 1964). After 7.3 dS m^{-1} , complete stand loss of Jackson variety soybeans occurred (Abel and MacKenzie, 1964). While inconsistencies in soybean tolerance exist among cultivars, the generally accepted threshold tolerance and slope of decline for soybeans are 5.0 dS m^{-1} and 20% per unit increase in EC_e , respectively (Maas and Hoffman, 1977).

Similar to corn, soybean height also decreases with increasing salinity because of the decline in turgor pressure attributed to the osmotic adjustment of the plant with increasing soluble salts (Curtis and Läuchli, 1987). Height of vegetative Elf soybeans grown in an equal ratio of perlite, peat, silt loam, and sand declined by 5.3% per dS m^{-1} increase after a threshold EC_e of 5.70 dS m^{-1} (Shalhevet et al., 1995). Alternatively vegetative Conquista variety soybeans in a sandy loam soil declined by approximately 14% per unit increase in EC_i , with a substantially lower threshold of 0.9 dS m^{-1} before decline (Blanco et al., 2007). Height of mature Essex and Manokin varieties grown in a coarse textured soil decreased by 20% at an EC_{sw} of 7 dS m^{-1} compared to the non-saline control (Papiernik et al., 2005), whereas shoot height of NA 4613 soybean plants near maturity in a sandy loam soil irrigated with a solution at 8.0 dS m^{-1} were 75% shorter than controls plants irrigated with water at an EC_e of 0.01 dS m^{-1} (Bustingorri and Lavado, 2014). Similar to yield, while discrepancies in height reductions appear across all studies examined, the general decline in height of soybeans occurs with increasing salinity.

Furthermore, differences among results could be attributed to the different soybean varieties studied (Phang et al., 2008).

Declines in leaf area have been correlated with increasing levels of salinity in soybean plants. Khan et al. (2014) attributed significant declines in leaf area to the low turgor pressure induced by salinity stress, which ultimately resulted in senescence of leaves. Similar to the reduction in photosynthesis with increasing salinity in corn, declines in photosynthesis in soybeans also result from stomatal closure (Queiroz et al., 2012). However, stomatal closure in plants could also be a potential adaptation to acclimate to saline conditions (Queiroz et al., 2012). By closing stomatal apertures, plant transpiration is reduced (Hsiao, 1973), allowing water to accumulate inside the plant (Davenport et al., 1977). Consequently, while the decline in photosynthesis reduces leaf area of the plant (Greenway and Munns, 1980), it is possible that the reduction in leaf growth is a response to decreasing water availability from soluble salts (Davenport et al., 1977). Leaf dry weight of Conquista soybeans grown in sandy loam soils declined by 21% per unit increase in EC_i after a threshold of 1.0 dS m^{-1} (Blanco et al., 2007). Similarly, leaf area of soybean cultivar IAC 17 decreased 2.2% per unit increase in EC_i when irrigated with dissolved NaCl with no observed threshold (Queiroz et al., 2012). Leaf width of Lee soybeans (a salt tolerant variety) declined by approximately 6.5% per unit increase in EC_e after an EC_T of 5.0 dS m^{-1} (Abel and MacKenzie, 1964).

Reductions in nutrient uptake in soybeans have also been previously reported and are likely attributed to declines in N_2 fixation (Delgado et al., 1994). Salinity reduces root nodulation (Bernstein and Ogata, 1966), which reduces plant available N for uptake (Delgado et al., 1994). In effect, declines in root nodulation reduce the efficiency of N-fixation in legumes (Phang et al., 2008). For example, root nodulation of Lee soybeans supplied with only a starter application of

N ($-\text{NO}_3$) was reduced by 6.3% per unit increase in atm NaCl (Bernstein and Ogata, 1966). Yields of $-\text{NO}_3$ soybeans declined by 15.6% per unit increase in atm NaCl because of declines in symbiotic N-fixation (Bernstein and Ogata, 1966). Reductions in yield were reduced to 11.4% per unit increase in atm NaCl when Lee soybeans were supplied adequate amounts of NO_3 (Bernstein and Ogata, 1966). Similarly, nitrogen uptake in Talon variety soybeans declined by 18.1% in loam and 23.6% in clay per unit increase in EC_i (van Hoorn et al., 2001). The declines in N-uptake were verified by relatively constant values of N-content in plant tissues after 79 days (van Hoorn et al., 2001).

Below-ground Biomass

Similar to corn, both root length and biomass of soybeans have declined in response to increasing levels of NaCl, but the reduction in growth is less severe than above-ground components of the plant (Bernstein and Ogata, 1966; Shalhevet et al., 1995; Bustingorri and Lavado, 2011; Queiroz et al., 2012). For example, Elf soybean root growth during vegetative stages in equal parts perlite, peat, silt loam, and sand decreased after a threshold EC_e of 5.08 dS m^{-1} by 6.6% per dS m^{-1} increase (Shalhevet et al., 1995). Similarly, at an EC_e of 4.0 dS m^{-1} in sandy loam soils, root biomass of the NA 4613 cultivar at maturity (R8) was at least 50% of the non-saline control (Bustingorri and Lavado, 2011). Alternatively, Bernstein and Ogata (1966) observed no significant differences in root dry weight up to an EC_e of approximately 15.2 dS m^{-1} , but instead found that root nodulation of soybeans declined non-linearly. Root fresh mass of IAC 17 soybeans was also relatively constant and maintained masses of 2.65 to 3.70 g up to NaCl concentrations of 200 mM ($\text{EC} \approx 25.0 \text{ dS m}^{-1}$; Queiroz et al., 2012). While contrasting results have been reported on root growth in soybeans subjected to salinity stress, it is possible that these discrepancies are attributed to inherent tolerances of different varieties (Phang et al.,

2008). For example, salt-sensitive varieties of soybean demonstrated more pronounced declines in nodulation when compared to salt-tolerant varieties (Abd-Alla et al., 1998). Consequently, it is possible that root growth of more tolerant species is less impacted by salinity (Phang et al., 2008).

Microbial Activity

Similar to the osmotic and specific ion effects impacting crop growth, microbial populations in the soil can also become impacted by increasing levels of salinization (Rath and Rousk, 2015). There are two known mechanisms through which soil microbes acclimate to salinity stress: 1) uptake of K^+ and expulsion of Na^+ from the soil and cell environment, respectively, and 2) production of organic osmolytes for adjustment of intracellular osmolarity (Rath and Rousk, 2015). The former mechanism is commonly used by salt-tolerant or halophytic microorganisms that export Na^+ from the cell using exchange reactions involving the accumulation of hydrogen (H^+) ions (Krulwich, 1983; Rath and Rousk, 2015). The latter mechanism involves the use of biochemical pathways to synthesize compounds, such as sucrose, glucosylglycerol, and ectoine (Oren, 1999), to adjust the osmolarity of the cell to changing osmotic potentials induced by salinity (Empadinhas and da Costa, 2008). Adjusting the osmolarity of the cell maintains cell turgor and prevents water loss (Empadinhas and da Costa, 2008). However, regardless of the mechanism for adaptation to salt stress, energy or adenosine triphosphate (ATP) is required for acclimation (Oren, 1999). For example, the accumulation of osmolytes requires 23 to 79 molecules of ATP, depending on the osmolyte produced (Oren, 1999). Even more, because osmolyte production is energetically expensive, resources that would normally be used for growth must be diverted to survival (Schimel et al., 2007). As a result,

microbial activity, such as respiration of carbon dioxide (CO₂) from the soil, declines (Rath and Rousk, 2015).

Cumulative Respiration

To date, many studies have addressed the response of microbially-mediated carbon (C) fluxes from the soil in response to increasing levels of NaCl. Typically, declines in cumulative microbial respiration (CO₂-C) are attributed to the loss of available substrate because of declines in above-ground biomass and reduced input of organic matter (Rath and Rousk et al., 2015). Additionally, declines in respiration can occur because of reallocation of C for growth instead of respiration (Schimel et al., 2007). However, results of studies assessing microbial respiration in saline soils often have contradictory results (Saviozzi et al., 2011), which are potentially attributed to differences in soil physical and chemical properties (Mavi et al., 2012). For example, microbial respiration in soils with irrigation-induced salinity from NaCl significantly decreased with increasing EC_{1:5} across all textures examined (Setia et al., 2011). However, slopes of decline were steeper in sandy loam soils than clay soils, with slopes of 0.101 and 0.026 mg CO₂-C soil g⁻¹ per unit increase in EC_{1:5}, respectively (Setia et al., 2011). Chowdhury et al. (2011) observed similar differences in cumulative respiration as clay content increased for soils at similar matric potentials: cumulative CO₂-C decreased 2% per unit increase in EC_e in sandy loam soils and 4% per unit increase in EC_e in sand. Differences in cumulative respiration across textures were attributed to differences in osmotic potential of the soil solution (Chowdhury et al., 2011; Setia et al., 2011). In effect, the higher water retention in soils with increasing clay contents resulted in a higher osmotic potential when compared to a coarser textured soil (Setia et al., 2011). The higher osmotic potential reduced the osmotic stress experienced by the microbial population, thus lowering the negative impact of salinity on microbial activity (Setia et al.,

2011). For example, cumulative respiration in sand decreased by 8, 24, and 42% at NaCl and CaCl₂-induced EC_{1.5} of 1.3, 2.5, and 4.0 dS m⁻¹, respectively, compared to a non-saline control at an EC_{1.5} of 0.5 dS m⁻¹ (Mavi et al., 2012). However, the same levels of EC_{1.5} had no significant impact on CO₂-C in sandy clay loam soils, which maintained cumulative respiration above 1.0 mg CO₂-C soil g⁻¹ throughout the entire 42-d incubation study (Mavi et al., 2012).

Alternatively, other studies have reported increases in microbial respiration with increasing salinization. Cumulative CO₂ evolution in soil salinized with NaCl solutions increased up to an EC_e of 4.0 dS m⁻¹, while at an EC_e of 8.0 dS m⁻¹ CO₂-C evolution decreased, but still exceeded cumulative respiration in the non-saline control (Saviozzi et al., 2011). Similarly, Zeng et al. (2013) observed increases in CO₂-C evolution with increasing NaCl-salinization up to EC_{1.5} of 2.83 dS m⁻¹. Increases in cumulative respiration are potentially attributed to increased availability of dissolved organic carbon (DOC), which may become more available because of the dispersive effects of excess Na⁺ ions in the soil (Wong et al., 2013).

Labile Carbon Pools

Dissolved organic carbon is directly related to the labile C pool available for use by microbes for respiration (Saviozzi et al., 2011; Mavi et al., 2012). Typically, labile C is lower in saline soils when compared to non-saline soils because of the reduced soil organic matter (SOM) input from above-ground biomass (Wong et al., 2010; Rath and Rousk, 2015). Consequently, microbial respiration is lower in saline soils because of the lower availability of C resources for mineralization (Wong et al., 2010). However, reductions in labile C in soils impacted by salinity are dependent on soil physical and chemical properties (Bronick and Lal, 2005). For example, labile C pools in loamy sand soils with an NaCl-induced EC_{1.5} of 0.08 dS m⁻¹ totaled 3.96 mg C g⁻¹ soil and was reduced by 12.5% or 0.50 mg C g⁻¹ soil per unit increase in EC_{1.5} up to 5.33 dS

m^{-1} (Setia et al., 2011). In clay soils, labile C reached levels of 4.39 mg C g^{-1} soil and declined by 3.8% or 0.17 mg C g^{-1} soil per unit increase in $\text{EC}_{1:5}$ up to 5.31 dS m^{-1} (Setia et al., 2011).

Differences in the amount of labile C and rates of decline as salinity increases are indicative of the effect of texture on available C in the soil (Setia et al., 2011). Finer textured soils inhibit organic C use by microbial populations because of the incorporation of organic C into stable aggregates (Bronick and Lal, 2005). Availability of C can also be impacted by the chemical composition of salts and ions in solution (Wong et al., 2010). For example, as plant health declines from increasing salinity, root respiration declines, which lowers the partial pressure of CO_2 (Wong et al., 2010). A reduction in the partial pressure of CO_2 can result in increased precipitation of CaCO_3 (Wong et al., 2010). The presence of CaCO_3 (calcium carbonate equivalent or CCE of 9.8%) protects up to 7% or 11.45 mg more of soil organic carbon (SOC) than non-calcareous soils because of the ability of Ca^{2+} to bind SOM into aggregates (Clough and Skjemstad, 2000).

Carbon Mineralization Rate

Declines in the C mineralization rate, or decay rate, have also been negatively correlated to increasing levels of soil salinity. However, similar to respiration and labile C, discrepancies in the rates of decay of C with increasing soluble salts in the soil exist and are likely a result of differences in soil physical and chemical properties (Bronick and Lal, 2005; Wong et al., 2010; Setia et al., 2011). For example, at comparable NaCl-induced $\text{EC}_{1:5}$, the decay rate of labile C decreased linearly by approximately 4.0% per unit increase in clay content of the soil (Setia et al., 2011). At a slightly lower water content, however, the decay rate of labile C in a sandy loam soil was not significantly different in soils artificially salinized with NaCl up to an EC_e of 8 dS m^{-1} and maintained a rate of $0.032 \text{ mg CO}_2\text{-C soil g}^{-1} \text{ d}^{-1}$ (Saviozzi et al., 2011). Turnover of

labile C by microbes was assumed to be higher in textures with more clay because of the reduced effect of salinity (Setia et al., 2011). Additionally, reductions in mineralization rates of C can be attributed to declines in enzymatic activity in saline soils (Batra and Manna, 1997). However, microbial populations in soils predisposed to natural soil salinity may be capable of acclimating to the stressful environmental conditions (Wichern et al., 2006; Chowdhury et al., 2011; Setia et al., 2011). Acclimation to salinity could potentially explain the lack of significant differences in decay rate of labile C observed in one study with salinized soils up to an EC_e of 8.0 dS m^{-1} (Saviozzi et al., 2011).

Community Composition and Structure

The discrepancies in microbial responses to salinity could be attributed to differences in community structure and composition that occur as environmental conditions fluctuate (Allison and Martiny, 2008). Soluble salts impact physical and chemical properties of soil (Bronick and Lal, 2005). As a result, microbial communities may respond differently to environmental stressors (Logue et al., 2015), making inconsistencies in microbial response to salinization possible (Wong et al., 2010). Previous studies have also demonstrated that populations of microorganisms in higher salinity environments can survive stressful conditions (Schimel et al., 2007; Empadinhas and da Costa, 2008; Chowdhury et al., 2011) through acclimation (Allison and Martiny, 2008). For example, ergosterol is a biochemical responsible for membrane rigidity (Abe and Hiraki, 2009). The ratio of ergosterol levels to microbial biomass carbon (MBC) in prokaryotes has been correlated to increasing levels of salinity, potentially indicating a shift in community composition to microbial populations more adapted to saline conditions in the soil (Wichern et al., 2006). However, as a result of acclimation, salinity may no longer be the controlling factor for microbial activity in these soils (Rath and Rousk, 2015) because the

restructure of community composition in response to environmental stressors is often correlated to differences in microbial function (Oren, 1999).

Concluding Remarks

The impact of soil salinity on biology is an immensely complicated interaction. The species of interest, growth stage, nutrient status of the soil, salts contributing to salinity, water availability and climate, disease and pest pressures, and soil texture influence plant response to salinization (Maas and Hoffman, 1977; Volkmar et al., 1997). Furthermore, because soil water and salinity are intricately related (Qureshi et al., 2007), it is difficult to tease apart the effects of salt stress and drought stress (Maas and Hoffman, 1977), especially under field conditions. However, in order to provide meaningful data for this region, these factors must be considered to fully address soil salinity's impact on agricultural production in Richland County, North Dakota.

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PAPER 1. CORN (*ZEA MAYS*) AND SOYBEAN (*GLYCINE MAX*) YIELD RESPONSE TO NATURAL SOIL SALINITY GRADIENTS IN THE NORTHERN GREAT PLAINS

Abstract

Soil salinization is a global issue affecting 831 million ha of arable land and resulting in approximately US \$27.3 billion in crop losses annually. The purpose of this research was to determine the effects of natural gradients in soil salinity on field-grown corn (*Zea mays*) and soybean (*Glycine max*) in silty clay loam and sandy loam soils in southeastern North Dakota. During vegetative and reproductive growth stages of corn and soybean height, nitrogen (N)-content, and leaf area index (LAI) were measured. Yield was hand-harvested at the end of the growing season and assessed against the depth weighted average electrical conductivity (EC_{DWA}) of the root zone. Corn yield did not significantly decline in silty clay loam soils, but declined by 11.8% per unit increase in EC_{DWA} after a threshold salinity (EC_T) of 4.57 dS m^{-1} in sandy loam soil. Soybeans declined by 21% per unit increase in EC_{DWA} after an EC_T of 2.98 dS m^{-1} in sandy loam. Similar to corn, no significant declines in soybean yield were observed when soybeans were planted in silty clay loam soils. The lack of yield declines for both crops in silty clay loam soils may indicate a reduced effect of salinity stress in finer textured soils. The threshold observed for corn and soybean yields in sandy loam soils indicates that crop yields respond differently to texture in saline soils.

Introduction

Soil salinization is a global issue affecting 831 million ha of arable land (Martinez-Beltran and Manzur, 2005) and resulting in approximately US \$27.3 billion in crop losses annually (Qadir et al., 2014). Current salinity thresholds (EC_T) indicate that corn yield begins to decline at an electrical conductivity of a saturated paste extract (EC_e) between 1.3 and 1.7 dS m^{-1}

with yield reductions between 10.5 and 12.0% per unit increase in salinity after this threshold (Maas and Hoffman, 1977; Katerji et al., 2003). Soybean yields begin to decline after a threshold salinity of 5.0 dS m⁻¹ with yield reductions of 20% per unit increase in salinity (Maas and Hoffman, 1977). Studies establishing crop tolerance thresholds have primarily assessed corn and soybean response under greenhouse or controlled plot conditions (Maas, 1984), with salinity induced by chloride salts, like sodium chloride (NaCl), in irrigation water (Maas, 1993). This methodology is problematic because some regions, like the Northern Great Plains, have primary, sulfate (SO₄²⁻)-dominated salinity (Keller et al., 1986). As a result, both osmotic stress and specific ion toxicities experienced by field-grown corn and soybean may differ in these soils because of differences in solubility and anionic constituents, respectively (Curtin et al., 1993). Additionally, because salts originate with depth in this region, differences in the life stage of corn or soybean that experiences salinity may alter the impact of soluble salts on tolerance thresholds (Kaddah and Ghowail, 1964; Maas et al., 1983; Maas, 1993).

Estimates in the Red River Valley of North Dakota indicate that of the 2.6 million ha of arable land, approximately 1.2 million ha are classified as slightly saline, resulting in US \$48 million in crop losses for corn and \$57 million in crop losses for soybean (Hadrach, 2012). Given the extent of sulfate-based salinity and corn and soybean production in this region, obtaining a better understanding of crop productivity in response to soil salinity under regional conditions is vital for preventative and reclamation management of saline soils and economic decisions. The objective of this research was to understand corn and soybean response to natural gradients in soil salinity under field conditions in both silty clay loam and sandy loam soils native to the Red River Valley. Additionally, this research can provide an understanding of the spatial and temporal dynamics of naturally saline soils over two growing seasons. Ultimately, results from

this study are intended to aid producers in farm management of soils with naturally occurring SO_4^{2-} -dominated salinity.

Materials and Methods

Geographical Setting of Research Sites

The research sites consisted of three silty clay loam fields for both the 2014 and 2015 growing seasons and three sandy loam fields in 2014 and two sandy loam fields in 2015. In 2014, corn was planted on silty clay loam fields and soybeans were planted on sandy loam fields. In 2015, the crops were rotated. Each field was 64.7 ha in size and located in Richland County, North Dakota. Silty clay loam fields were mapped as an Antler-Mustinka complex (Fine-loamy, mixed, superactive frigid Aeric to Fine, smectitic, frigid Typic Argiaquolls; USDA-NRCS, 1997; USDA-NRCS, 1999a; USDA-NRCS Web Soil Survey, 2014). Sandy loam fields were mapped as a Wyndmere loam (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll; USDA-NRCS, 1999b; USDA-NRCS Web Soil Survey, 2015).

Silty clay loam fields planted to corn received a total of 43.2 cm of rainfall with an estimated 121.7 cm of potential evapotranspiration (PET) during the 2014 growing season (Figure 1; North Dakota Agricultural Weather Network, NDAWN, 2014). The two sandy loam fields planted with corn received a total of 35.4 cm of rainfall with an estimated 130.2 cm of PET during the 2015 growing season (Figure 1; NDAWN, 2015).

Sandy loam fields planted with soybean received a total of 38.2 cm of rainfall with an estimated 94.6 cm of potential evapotranspiration (PET) during the 2014 growing season (Figure 2; North Dakota Agricultural Weather Network, NDAWN, 2014). During the 2015 growing season, silty clay loam fields planted with soybean received a total of 35.4 cm of rainfall with an estimated 130.2 cm of PET during the 2014 growing season (Figure 2; NDAWN, 2015).

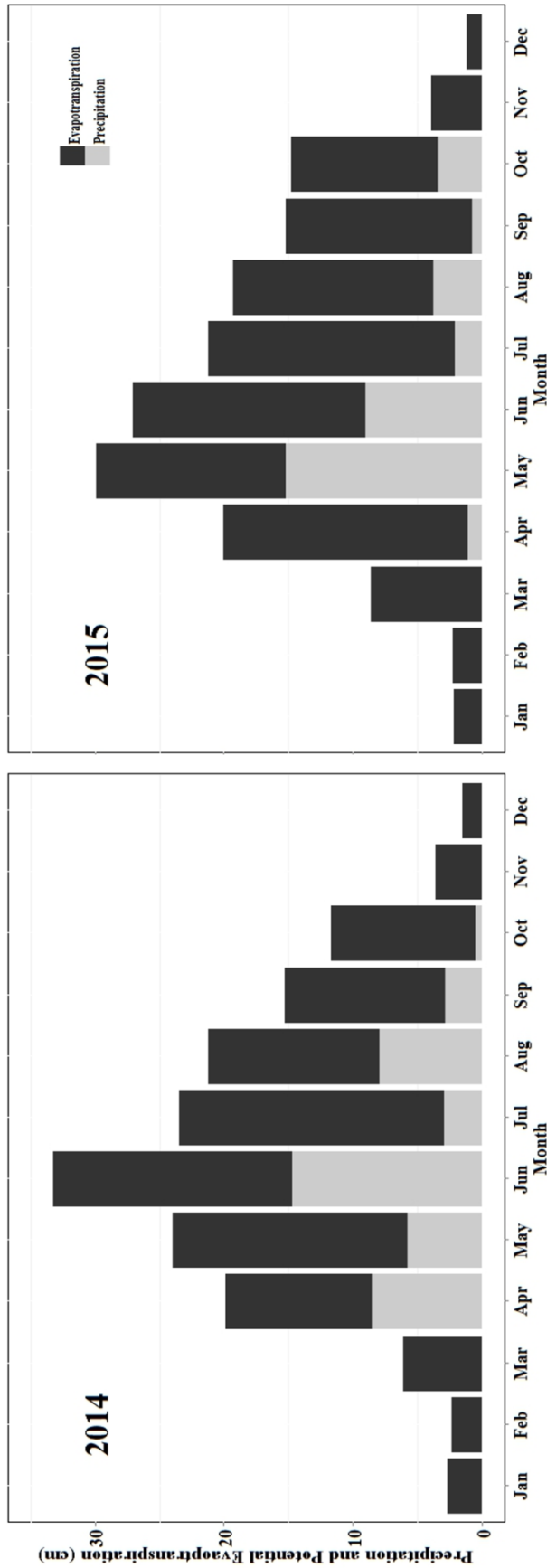


Figure 1. Monthly precipitation and potential evapotranspiration (PET) on silty clay loam fields in 2014 and sandy loam fields in 2015 planted to corn in Richland County, ND. Weather data provided by NDAWN (2014-2015).

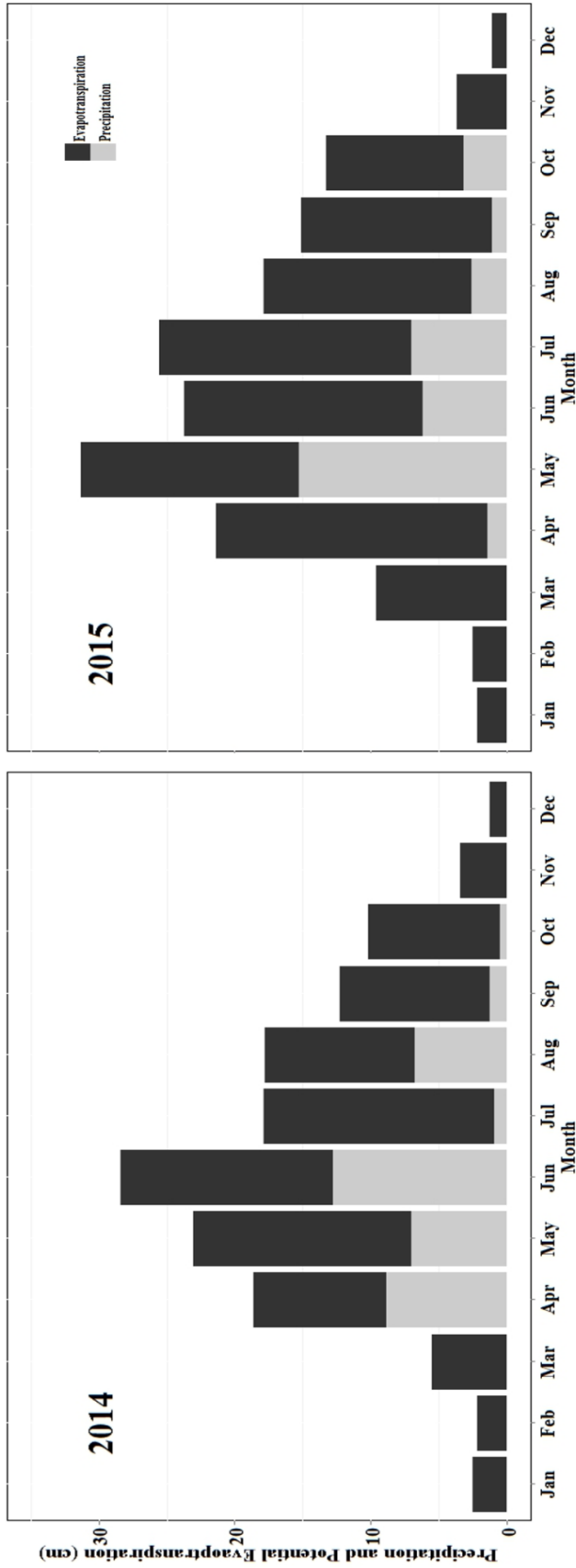


Figure 2. Monthly precipitation and potential evapotranspiration in sandy loam fields in 2014 and silty clay loam fields in 2015 planted to soybean in Richland County, ND. Weather data provided by NDAWN (2014-2015).

A Geonics EM 38 meter (MK2, Geonics Ltd., Canada) was used to continuously map salinity gradients to a 1.2-m depth on each field. Data from the EM 38 readings was converted to apparent electrical conductivity (EC_a) based on previous data collected and interpreted by the Natural Resources Conservation Service (NRCS; K. Anderson, personal communication, 2014). Point data of EC_a was interpolated in ArcMap (10.2, Esri, Redlands, CA) using inverse distance weighted (IDW) interpolation to produce a continuous gradient map of soil salinity on each field (Figure 3). Following interpolation, a total of 15, 100-m transects were constructed each year (for each soil texture) along the most pronounced salinity gradients generated by the IDW-interpolation maps. Transects were constructed within the soil series of interest in the direction of planted rows and composed of four sampling points spaced 25 m apart. Sampling points

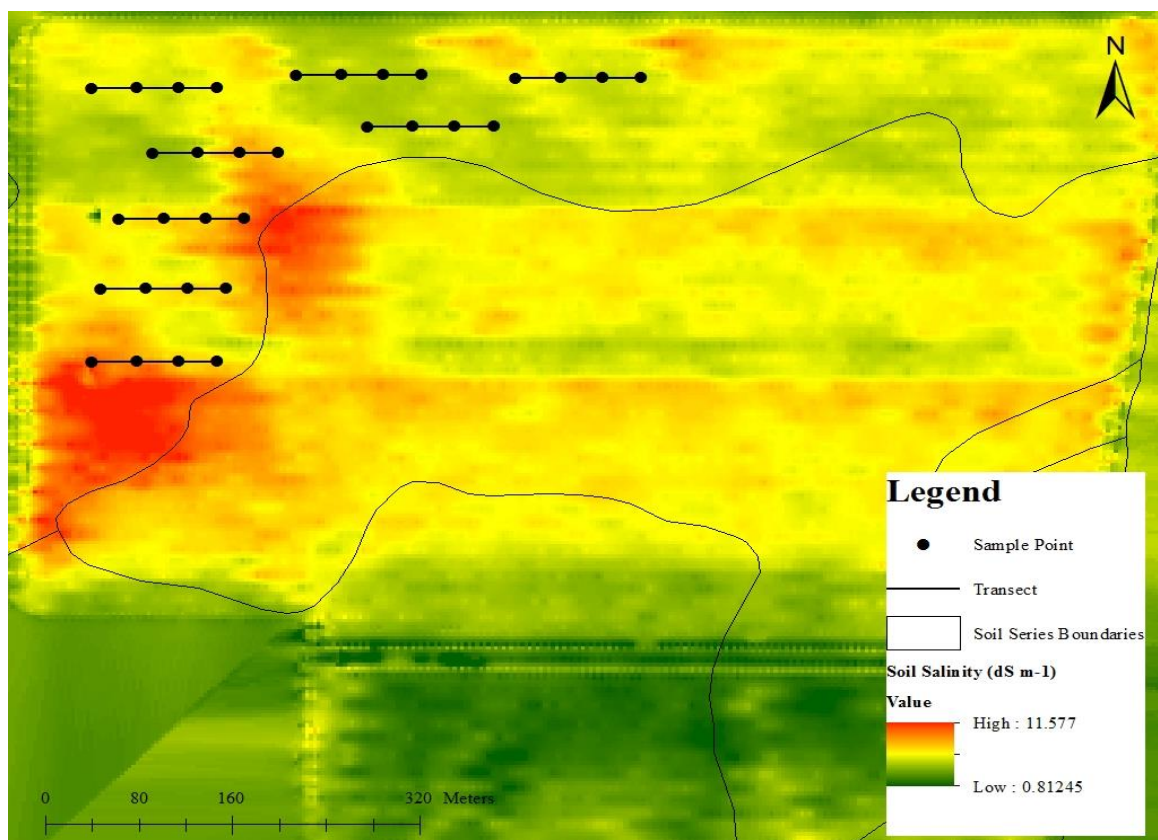


Figure 3. Field-scale salinity gradients on sandy loam field in 2015 generated by the EM 38 meter and interpolated in ArcGIS 10. Transects on each field consisted of four, equally-spaced sampling points within the soil series of interest.

served as central locations for measurements of corn and soybean growth parameters, yield, and salinity.

All three silty clay loam fields were disc-tilled prior to sowing. Three different seed varieties were planted on each silty clay loam field- Pioneer 9917, Pioneer 9675, and Pioneer 9248- on the 23rd, 26th, and 27th of May, respectively. Rows were spaced 0.76 m apart with a planting density of 82,800 seeds ha⁻¹. Fertilizer (140:55:20 NPK) was applied once to silty clay loam fields in 2014 prior to seeding. Harness pesticide was applied prior to sowing, and Roundup Status Pest was applied once during the growing season. Corn was hand harvested on the 20th of October, 2014 on all three fields.

Sandy loam fields planted to corn in 2015 were chisel plowed in the fall the previous year and tilled with a field cultivator and spiral packer in the spring prior to sowing. Corn variety on one field was Pioneer 9284. The remaining field had Pioneer 9917 planted on the western half of the field and Pioneer 9917 on the eastern half. Seeds were planted on the 29th and 30th of May, 2015. Rows were spaced 0.51 m apart with a planting density of 84,000 seeds ha⁻¹. Fields were fertilized the previous fall with 27 kg of P and 27 kg of K deep banded with a chisel plow. Fertilization with 165:40:50 NPK was applied in the spring prior to sowing, and 19 L of 10:34:0 NPK with 0.95 L of Zn was added with a planter. In mid-June, 38 L of 28% side dress was applied. On the 5th of June, 2015, a mixture of RealmQ, AMS, atrazine, and glyphosate was applied as herbicide to both fields. Yield was hand harvested on each sampling point on the 24th September, 2015.

All sandy loam fields planted to soybeans in 2014 had one pass the fall of the previous year with a ripper and packer. Fields had one pass in the spring prior to sowing with a field cultivator and spiral packer. All three fields were planted with Pioneer P10T91R on the 2nd and

3rd of June, 2014. Rows were spaced 0.51 m apart with a planting density of 420,000 seeds ha⁻¹. No fertilizer was applied to soybean fields in 2014. The first application of herbicide was applied on the 7th of July, 2014 and was composed of a mixture of AMS, Assure II, Roundup, Cadet, and Surfactant mixture. The second application was applied on the 23rd of July, 2014 and was composed of AMS, Approach, Roundup, Assure II, and Surfactant mixture. All three fields were sprayed for aphids on the 15th of August, 2014 using a mixture of 0.24 L Lorsban 4E and 0.024 kg Asana XL. Soybeans were hand harvested on the 1st of October, 2014 on all three fields.

Only one silty clay loam field planted to soybean in 2015 was chisel plowed prior to sowing. The remaining two fields were not tilled. One silty clay loam field was planted with BS 1408 on the 2nd of May, and the two remaining fields were planted with Pioneer MN1011CN on the 4th and 5th of May. Rows were spaced 0.38 m apart with a planting density of 215,000 seeds ha⁻¹. Silty clay loam fields were not fertilized in 2015. Authority MTZ pesticide was applied prior to sowing, and Flexsar was applied during the growing season. Yield was hand harvested on the 16th of September, 2015 on all three fields.

Soil Sampling

Four composite soil cores of 6.3-cm diameter were taken from each sampling area of each crop across both the 2014 and 2015 growing seasons using a hydraulic probe following harvest at five depths: 0-15 cm, 15-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm. Laboratory analysis included saturated paste extracts to determine EC_e and pH according to methods by Richards (1954). Particle size distribution was performed on a control section from 0-40 cm based on the vertical section of the profile used for classification (USDA-NRCS, 1997; USDA-NRCS, 1999a, USDA-NRCS, 1999b) according to methods by Gee and Or (2002). One additional core of the same diameter was collected at each sampling point on silty clay loam

fields planted to corn in 2014 at the 0-15, 15-30, and 30-60 cm depths to determine total root length using WinRHIZO software (2012, Regent, Canada). Another core of the same diameter was collected at each sampling point on sandy loam fields planted to soybean in 2014 at the 0-15 and 15-30 cm to determine total root length. In 2015, a 9.0-cm diameter core was taken from silty clay loam fields planted to soybean to determine total root length.

Three low salinity, three medium salinity, and three high salinity samples at the 0-15, 15-30, and 30-60 cm depths on corn fields and 0-15 and 15-30 cm depths on soybean fields were subsampled based on the interpolated EC_a maps and sent to AGVISE (Benson, MN) for cation analysis. Potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were extracted using 1 M ammonium acetate and determined using inductively coupled plasma mass spectrometry.

Volumetric water content (θ_v) was measured in sandy loam soils planted to corn and silty clay loam soils planted to soybean during the 2015 growing season only using a handheld soil moisture probe (GS3, Decagon Devices, Inc., Pullman, WA) at each sampling point five times throughout the growing season. Volumetric water content of sandy loam soils planted to corn were sampled at the V2, V6, V12, R1, and R4 growth stages of corn as determined by growing degree days (GDD; NDAWN, 2015). The depth of θ_v measurements was determined based on the rooting depth of three randomly selected plants on each field at each growth stage sampled. At V2 and V6, θ_v was measured at the soil surface. At V12 and R1, θ_v was measured at the soil surface and 15 cm below the surface. At R4, θ_v was measured at the soil surface, 15 cm below the surface, and 30 cm below the surface. Subsurface measurements of θ_v were taken within a hand-augered hole within the sampling area of each point. Volumetric water content was measured in silty clay loam soils planted to soybean five times throughout the growing season at

the VE, V4, V6, R2, and R6 growth stages of soybean. All θ_v measurements were taken at the soil surface.

Vegetative and Reproductive Stage Crop Parameters

Corn and soybean growth response to soil salinity was quantified throughout the growing season at the V12 and R1 growth stages of corn and the V6 and R6 growth stages of soybean on four randomly selected plants within the sampling area. All crop parameter measurements were taken at the vegetative and reproductive growth stages each year unless otherwise noted. Corn and soybean heights were measured from the base of the plant to the top of the extended leaves. Leaf area index (LAI) was measured on the reproductive growth stages of corn and soybean in silty clay loam soils using a ceptometer (AccuPAR LP-80, Decagon Devices, Inc., Pullman, WA) in the across row position. Due to sampling difficulties, LAI of corn planted in sandy loam soils in 2015 was not measured. Leaf nitrogen (N)-content was estimated using a leaf chlorophyll meter (SPAD-502, Konica Minolta Inc., Europe; Argenta et al., 2004). Yield (kg ha^{-1}) was determined by hand harvesting across five rows, 1.2-m in length for fields planted to corn (D. Franzen, personal communication, 2014) and calculated based on the mass of corn per bushel at 15.5% moisture content (Dorsey-Redding et al., 1991). Soybean yield (kg ha^{-1}) was determined by hand harvesting an area of 2.32 m^2 (D. Franzen, personal communication, 2014) and calculated based on the mass of seeds at 13.0% moisture content (Cox and Jolliff, 1986). Relative yield (Y_R) for each crop was calculated based on the following equation (Maas and Hoffman 1977; Maas, 1993; Eq. 3):

$$Y_R = \frac{Y}{Y_M} * 100\% \quad (3)$$

where Y is the yield (kg ha^{-1}) and Y_M is the maximum yield of corn or soybean at the lowest measured salinity of the root zone (EC_{DWA}).

Statistical Analyses

Corn and soybean yield were analyzed using a modified threshold-slope function (Maas and Hoffman, 1977) and a nonlinear, modified discount response function (MDRF; van Genuchten and Gupta, 1993). The threshold-slope function is a two-piece generalized linear model (Maas, 1993). The first line segment denotes no significant response to salinity up to some threshold, and the second segment denotes the yield reduction per unit increase in EC_e after the threshold is reached (Maas, 1993). The intersection of the two lines is the threshold tolerance of the crop (EC_T), which indicates the maximum salinity that does not cause significant reductions in yield. After this threshold, the relationship between yield and salinity is expressed as (Maas, 1993; Eq. 4):

$$Y_R = 100\% - b(EC_{DWA} - EC_T) \quad (4)$$

where b is the slope of decline in % per $dS\ m^{-1}$ after some threshold salinity (EC_T) with units of $dS\ m^{-1}$, and EC_{DWA} is the depth weighted average EC_e of the root zone in $dS\ m^{-1}$.

The MDRF is the non-linear derivative of the threshold-slope model (van Genuchten and Gupta, 1993; Stepphun et al., 2005; Eq. 5):

$$Y_R = \frac{100\%}{1 + \frac{EC_{DWA}^p}{EC_{50}^p}} \quad (5)$$

where EC_{50} ($dS\ m^{-1}$) is the EC_{DWA} when Y_R is 50%, s is a unitless response curve steepness parameter, and p is a unitless shape parameter equal to the exponent of the product of s and EC_{50} . Both models were used to determine the relationship between Y_R and EC_{DWA} of the root zone of each crop (Maas, 1984; Eq. 6) with

$$EC_{DWA} = \frac{(EC_{0-15} * 15\ cm) + (EC_{15-30} * 15\ cm) + (EC_{30-60} * 30\ cm)}{60\ cm} \quad (6)$$

where EC_{DWA} is the depth weighted average EC_e of the root zone (0-60 cm depth) in $dS\ m^{-1}$,

EC_{0-15} is the EC_e at the 0-15 cm depth, EC_{15-30} is the EC_e at the 15-30 cm depth, and EC_{30-60} is the EC_e at the 30-60 cm depth. The soybean root zone incorporated the 0-15 and 15-30 cm depths only. Depths incorporated into the depth weighted average were based on the distribution of excavated corn and soybean roots from each field, as well as published data reported by Taylor and Klepper (1973), Mengel and Barber (1974), and Gao et al. (2010) on corn and soybean root distribution at each growth stage of sampling.

Crop parameter measurements taken at vegetative and reproductive growth stages were analyzed using linear and threshold-slope models. The V12 stage of corn was analyzed against the EC_{DWA} from 0-30 cm based on the distribution of corn roots observed under field conditions at the V12 life stage. The R1 stage crop parameters were compared against the EC_{DWA} used for the analysis of yield. The V6 stage of soybeans was analyzed against the EC_e at the 0-15 cm depth. The R6 stage of soybeans was compared against the EC_{DWA} used for the analysis of yield. The EC_e of the depth that roots were collected from was used to assess root length response to salinity. The best fit curves for explaining crop responses to salinity were based on statistical significance (p -value < 0.05) and degree of variation explained by the regression model (R^2). All statistical analyses were performed using R Studio (3.2.1, R Foundation for Statistical Computing, Vienna).

Results

Corn

Measured Soil Properties

A detailed summary of all measured soil and crop parameters of corn by soil type and year is listed in Table 6. In general, regardless of texture, EC_e increased with depth (Figure 4 and 5). However, values of EC_e in the sandy loam soils were usually greater than the values of EC_e at

the same depths in the silty clay loam soils. For example, minimum and maximum values of EC_e in the sandy loam soil were 0.22 and 10.41 $dS\ m^{-1}$ which occurred in the 60-90 and 90-120 cm depths, respectively. Minimum and maximum values of EC_e in the silty clay loam soil were 0.23 and 8.15 $dS\ m^{-1}$ which occurred in the 0-30 and 90-120 cm depths, respectively.

On average, Ca^{2+} , Mg^{2+} , and Na^+ increased with depth, whereas K^+ decreased (Table 7).

Calcium was the most abundant cation measured in both the silty clay loam and sandy loam

Table 6. Descriptive statistics of measured soil properties within the 0-30 and 0-60 cm depth ranges and crop parameters measured in silty clay loam (2014) and sandy loam soils (2015) in Richland County, ND.

Property	Unit	Silty Clay Loam (2014)				Sandy Loam (2015)			
		Min	Max	Mean	Std.Dev.	Min	Max	Mean	Std.Dev.
EC_{DWA} (0-30 cm) [†]	$dS\ m^{-1}$	0.26	6.3	2.48	1.46	0.33	6.94	3.06	1.80
EC_{DWA} (0-60 cm) [†]	$dS\ m^{-1}$	0.248	6.13	2.91	1.59	0.34	7.42	3.38	1.91
pH_{DWA} (0-30cm) [†]		7.43	8.32	7.83	0.18	7.31	8.45	7.77	0.28
pH_{DWA} (0-60cm) [†]		6.01	8.23	7.71	1.05	7.37	8.53	7.77	0.26
Clay [‡]	$g\ kg^{-1}$	62	408	306	80.0	64.5	328.3	167.9	296.2
Silt [‡]	$g\ kg^{-1}$	271	622	401	93.4	102.9	511.6	216.7	72.3
Sand [‡]	$g\ kg^{-1}$	175	416	292	48.3	158.5	824.4	615.4	269.1
Height V12 [§]	cm	51.4	147.5	107.7	20.7	102.6	195.3	158.9	18.2
SPAD V12 [¶]		30.4	51.7	42.2	4.8	39.7	58.1	49.1	4.0
Height R1 [§]	cm	158.6	258.1	229.3	22.6	230.3	322.6	271.2	17.3
SPAD R1 [¶]		42.6	62.2	52.1	4.7	54.7	63.5	58.2	1.9
LAI R1 [#]		2.02	5.93	3.87	0.97	--	--	--	--
Root Length (0-15cm) ^{††}	cm	7.5	384.0	104.0	95.2	--	--	--	--
Root Length (15 to 30 cm) ^{††}	cm	9.8	225.3	49.9	39.8	--	--	--	--
Root Length (30 to 60 cm) ^{††}	cm	10.4	183.5	54.1	43.3	--	--	--	--
Yield ^{‡‡}	$kg\ ha^{-1}$	7470	14,300	10,200	1820	6220	13,400	9920	1560

[†] Determined by a saturated paste extract according to methods by Richards (1954).

[‡] Determined using the hydrometer method for particle size distribution (Gee and Or, 2002) on control section of soil profile at each sampling point (USDA-NRCS, 1997; USDA-NRCS, 1999a, USDA-NRCS, 1999b).

[§] Height of corn measured from the base of the plant to the tip of extended leaves.

[¶] Estimated N-content using a leaf chlorophyll meter (Argenta et al., 2004).

[#] Leaf area index measured using a ceptometer in the across row position at the R1 life stage of corn grown in silty clay loam soils.

^{††} Total root length determined using WinRhizo software. In silty clay loam soils, roots were collected from a soil core 6.3 cm in diameter. The length of each core varied based on the depth range.

^{‡‡} Yield calculated based on the mass of corn per bushel at 15.5% moisture content hand-harvested from five rows, 1.2 m in length.

soils, followed by Mg^{2+} and Na^+ . However, the average ratio of exchangeable and solution phase Ca^{2+} and Mg^{2+} concentrations varied between the two textures. For example, the $Ca^{2+}:Mg^{2+}$ at the 0-15 cm depth was 4.28 in silty clay loam soils and 5.80 in sandy loam soils. While not measured in this study, previous data collected by the NRCS-USDA indicated that SO_4^{2-} was the dominant anion. In silty clay loam soils in this study, the concentration of SO_4^{2-} was 10 times higher than Cl^- concentrations in the root zone (USDA-NRCS, 2001), and sandy loam soils had SO_4^{2-} concentrations five times higher than Cl^- (USDA-NRCS, 1996).

Volumetric water content measurements taken in sandy loam soils indicated that, on average, θ_v was at field capacity or close to saturation for all growth stages sampled, except at the R1 stage (Table 8). At this growth stage, the lowest average θ_v throughout the growing season occurred at the 0-15 cm depth, with θ_v increasing above field capacity in the 15-30 cm depth. In general, θ_v increased with depth. The lowest θ_v was $0.095 \text{ cm}^3 \text{ water total cm}^{-3}$ and occurred during the R4 growth stage in the 0-15 cm depth. The maximum θ_v observed was $0.443 \text{ cm}^3 \text{ water total cm}^{-3}$ and occurred during the V12 growth stage in the 15-30 cm depth range.

Crop Parameters

Silty Clay Loam Soil (2014)

In silty clay loam soils, V12 height declined significantly ($p\text{-value} < 0.05$; $R^2 = 12.7\%$) with increasing EC_{DWA} down to 30 cm by 4.2% (5.11 cm) per unit increase in salinity of the root zone (Figure 6). Nitrogen content, as estimated by leaf chlorophyll content, also significantly ($p\text{-value} < 0.05$) declined by 3.0% (1.35 units in N-content) per unit increase in EC_{DWA} down to 30 cm (Figure 7). The depth weighted average EC_e explained 16.1% of the variation in N-content. There were no significant declines in height, LAI, or N-content at the R1 life stage as EC_{DWA} increased up to 6.13 dS m^{-1} down to the 60 cm depth. Total corn root length was highest in the 0-

15 cm depth of the soil where 50% of root length was found. Below 15 cm, the remaining 50% of root length was distributed equally between the 15-30 and 30-60 cm depths. No significant models explaining root length and EC_e were found at any of the depths sampled. However, while not significant, root length depicted a slightly positive trend with increasing EC_e at all depths of

Table 7. Potassium, calcium, magnesium, and sodium concentrations in the root zone of corn in silty clay loam and sandy loam soils in Richland County, ND.

Cation	Depth Range	Unit	Silty Clay Loam (2014)				Sandy Loam (2015)			
			Min	Max	Mean	Std.Dev.	Min	Max	Mean	Std.Dev.
K [†]	0-15 cm	mg kg ⁻¹	145	255	193	33	52	202	100	43
	15-30 cm	mg kg ⁻¹	76	214	131	39	34	156	74	34
	30-60 cm	mg kg ⁻¹	64	200	124	38	28	191	73	43
Ca ^{2+†}	0-15 cm	mg kg ⁻¹	3632	6300	4627	853	1640	5649	4190	1302
	15-30 cm	mg kg ⁻¹	2486	7509	4690	1273	2771	7876	4661	1375
	30-60 cm	mg kg ⁻¹	2401	12830	5108	1875	703	8402	4257	1765
Mg ^{2+†}	0-15 cm	mg kg ⁻¹	434	2399	1081	399	353	1359	722	325
	15-30 cm	mg kg ⁻¹	549	2039	1222	418	442	1656	912	407
	30-60 cm	mg kg ⁻¹	697	2488	1412	485	496	1729	1043	388
Na [†]	0-15 cm	mg kg ⁻¹	18	635	98	122	20	602	164	166
	15-30 cm	mg kg ⁻¹	20	652	139	145	26	611	232	197
	30-60 cm	mg kg ⁻¹	31	787	188	173	27	669	258	190

† Cations extracted with 1 M C₂H₃O₂NH₄ and measured using inductively coupled plasma mass spectrometry.

Table 8. Average volumetric water content measured in the root zone of corn grown in sandy loam soils in 2015 at various growth stages.

Depth Range (cm)	Volumetric Water Content (θ_v) [†]				
	cm ³ water cm ⁻³ total				
	Sampled Growth Stage				
	V2 [‡]	V6	V12	R1	R4
0-15	0.273 ± 0.037 [§]	0.244 ± 0.039	0.309 ± 0.053	0.163 ± 0.033	0.211 ± 0.046
15-30	--	--	0.351 ± 0.054	0.296 ± 0.052	0.268 ± 0.062
30-60	--	--	--	-- [¶]	0.315 ± 0.043
DWA [#]	0.273 ± 0.037 ^{††}	0.244 ± 0.039	0.330 ± 0.109	0.230 ± 0.047	0.278 ± 0.054

† Volumetric water content estimated using handheld soil moisture probe (GS3, Decagon Devices, Inc., Pullman, WA) at each depth of interest.

‡ Vegetative (V) and reproductive (R) growth stages sampled throughout the growing season in 2015. Life stage determined based on growing degree days (GDD).

§ Average $\theta_v \pm$ the standard deviation for both sandy loam fields in 2015.

¶ Volumetric water content in the 30-60 cm depth at the R1 life stage could not be measured.

Depth weighted average θ_v of the root zone, calculated by a modified version of Eq. 1.

†† Depth weighted average $\theta_v \pm$ the standard deviation for both fields in 2015.

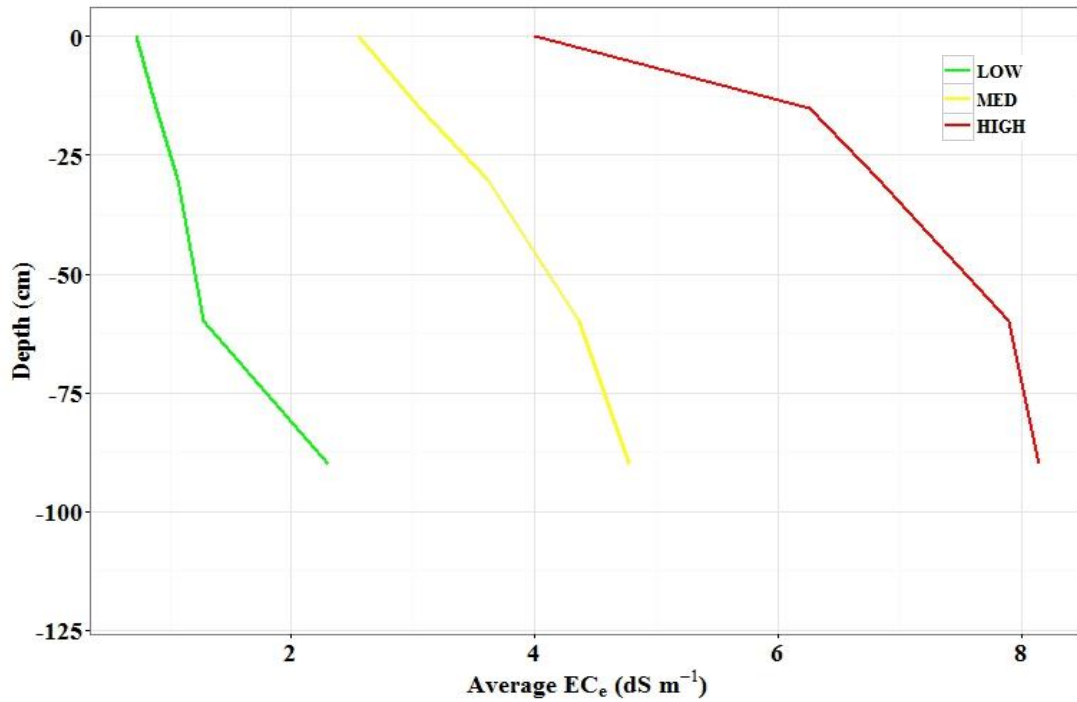


Figure 4. Depth functions of EC_e in silty clay loam soils in 2014. Each depth function represents a single point on a transect. Low, medium, and high levels of salinity had depth weighted average EC_{eS} (EC_{DWA}) down to 120 cm of 1.36, 3.89, and 7.00 $dS m^{-1}$, respectively.

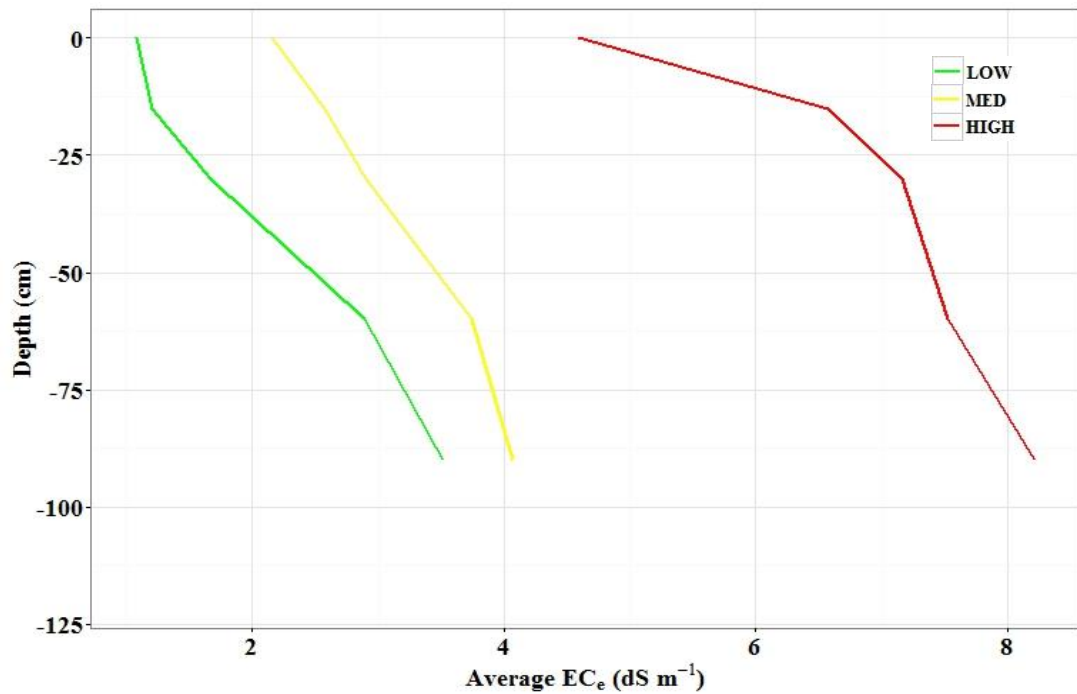


Figure 5. Depth functions of EC_e in sandy loam soils in 2015. Each depth function represents a single point on a transect. Low, medium, and high levels of salinity had depth weighted average EC_{eS} (EC_{DWA}) down to 120 cm of 2.30, 3.27, and 7.12 $dS m^{-1}$, respectively.

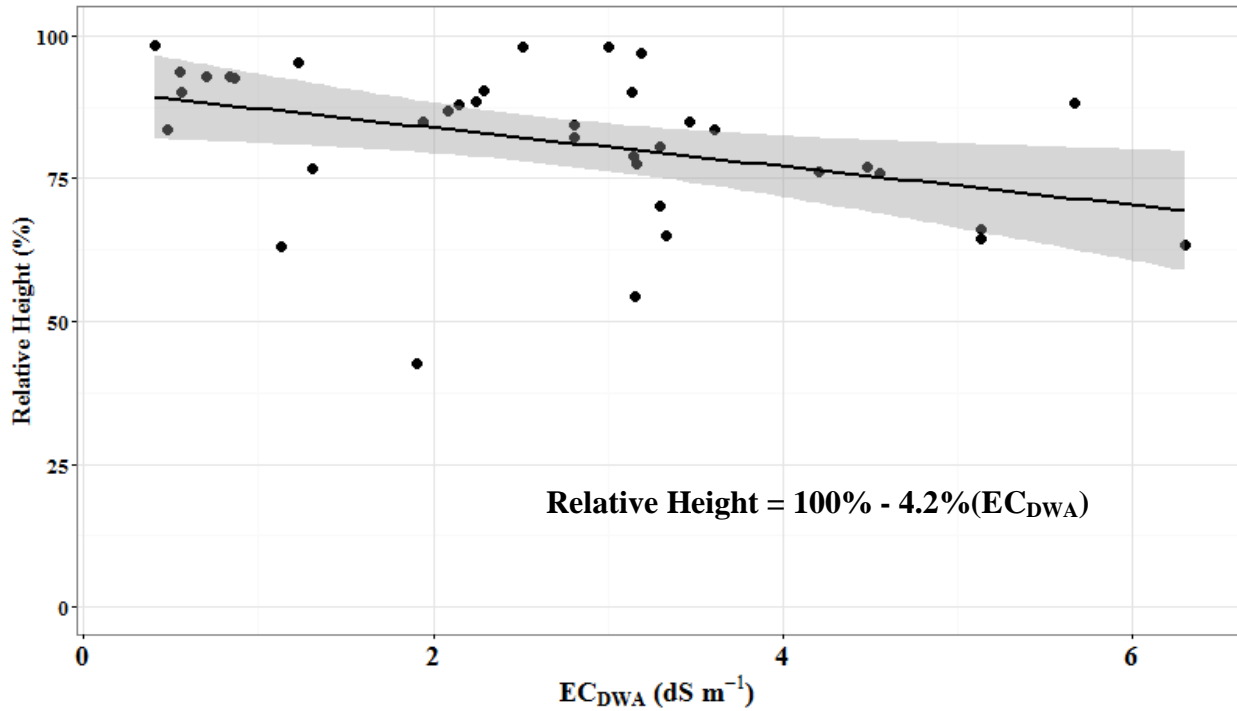


Figure 6. Relative decline in corn height at the V12 stage in silty clay loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Linear model fitted with 95% upper and lower confidence bounds.

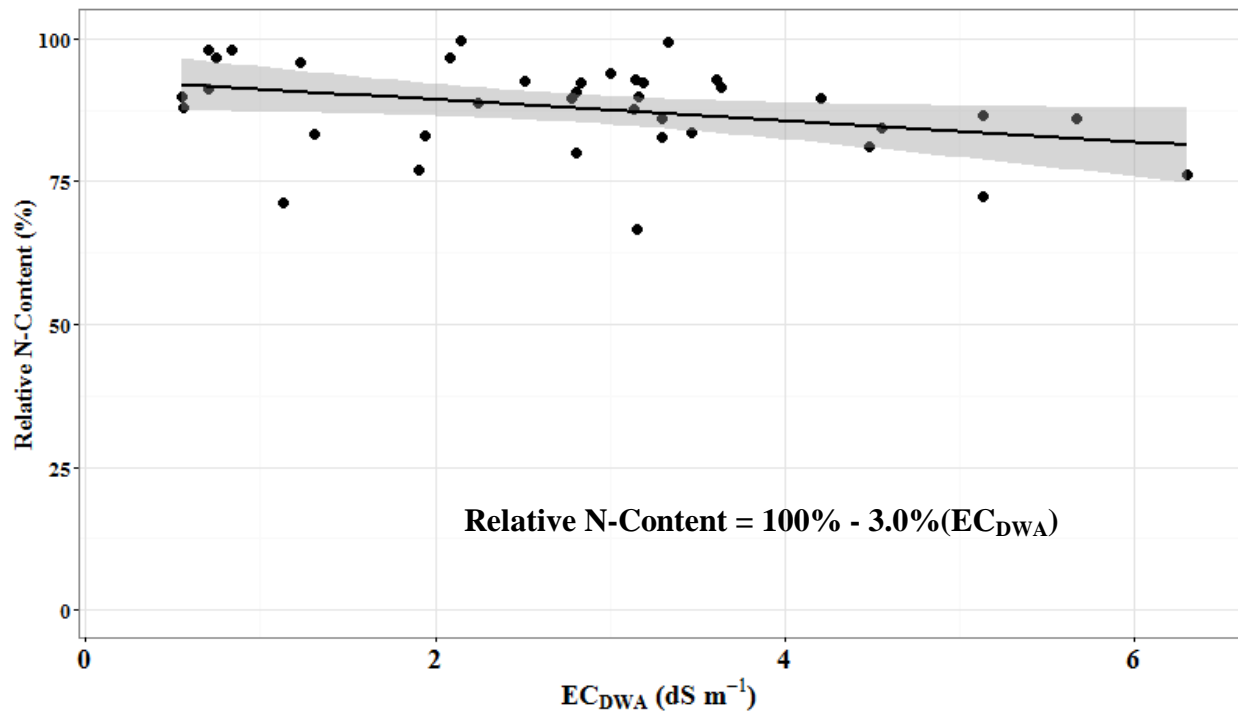


Figure 7. Relative decline in corn leaf SPAD reading at the V12 stage in silty clay loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Linear model fitted with 95% upper and lower confidence bounds.

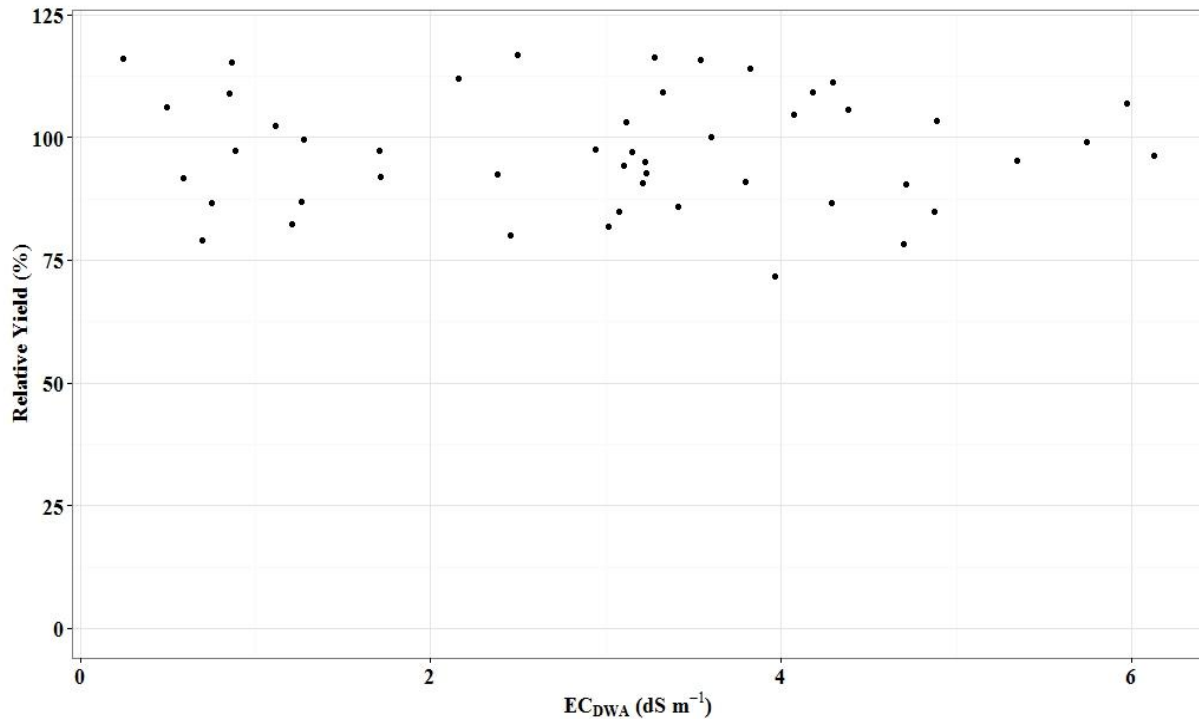


Figure 8. Hand-harvested corn yield in silty clay loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone.

the root zone ($r = +0.09$, $+0.23$, and $+0.10$ for the 0-15, 15-30, and 30-60 cm, respectively). No observed decline in corn yield with increasing salinity occurred in 2014 when corn was grown in silty clay loam soils. Corn maintained relative yields of at least 75% up to an EC_{DWA} of approximately 6.13 dS m⁻¹ (Figure 8).

Sandy Loam Soil (2015)

No crop parameters at either the V12 or R1 stage sampled were negatively impacted by increasing EC_{DWA} in sandy loam soil. No observable declines occurred with increasing EC_{DWA} up to 6.94 and 7.42 dS m⁻¹ for the V12 and R1 life stages, respectively. The threshold-slope model was significant (p -value < 0.001) for predicting corn yield in response to increasing EC_{DWA} down to 60 cm, and EC_{DWA} explained 28.9% of the variation (Figure 9). The model intercept corresponded to 10.7 Mg corn ha⁻¹ and predicted significantly different slopes (p -value < 0.05) in yield after an EC_{DWA} threshold salinity (EC_T) of 4.57 dS m⁻¹. At values below EC_T,

corn yield declined by 0.7% (69.0 kg ha^{-1}) per unit increase in EC_{DWA} . At values greater than or equal to EC_{T} , corn yield declined by 11.8% ($1,240 \text{ kg ha}^{-1}$) per unit increase in EC_{DWA} . The threshold-slope model indicated that EC_{T} was within an interval of 3.21 and 5.94 dS m^{-1} with 95% confidence. At an EC_{DWA} less than EC_{T} , the 95% confidence of the slope of decline was within an interval of -0.5 and +1.4% per dS m^{-1} . The 95% confidence of the slope of decline at an EC_{DWA} greater than or equal to EC_{T} was within an interval of -21.0 and -2.6% per dS m^{-1} . In sandy loam soils, corn yield was slightly positively correlated with clay content ($r = 0.20$) and slightly negatively correlated with sand content ($r = -0.14$). On average, yield was higher on fields with higher clay content by 741 kg ha^{-1} .

Corn yield in sandy loam soil was also significantly predicted by the MDRF model (Figure 10). The estimated value for the steepness parameter (s) was 0.23 as determined by non-linear fitting of the MDRF model. The EC_{50} was estimated from the threshold-slope model at 7.87 dS m^{-1} ($p\text{-value} < 0.001$). The shape of the curve (p) was equal to 6.11. The MDRF model parameters were highly significant ($p\text{-value} < 0.001$), and the model explained more variation in yield with increasing salinity of the root zone ($R^2 = 32.1\%$). The model intercept corresponded to 10.3 Mg ha^{-1} , and yield declined exponentially by a factor of 1.81% per unit increase in EC_{DWA} . The steepest declines were observed after an EC_{DWA} of 4.00 dS m^{-1} .

Residual analysis detected significant heteroskedasticity in both the threshold-slope and MDRF models explaining yield response to EC_{DWA} . Further analysis indicated that residuals of both these models were significantly correlated ($p\text{-value} < 0.05$) to clay content ($r = 0.33$) and sand content ($r = -0.30$). Residuals of the two models were also correlated to the depth weighted average θ_v at the R1 stage ($r = 0.19$), and this correlation was approaching significance ($p\text{-value}$

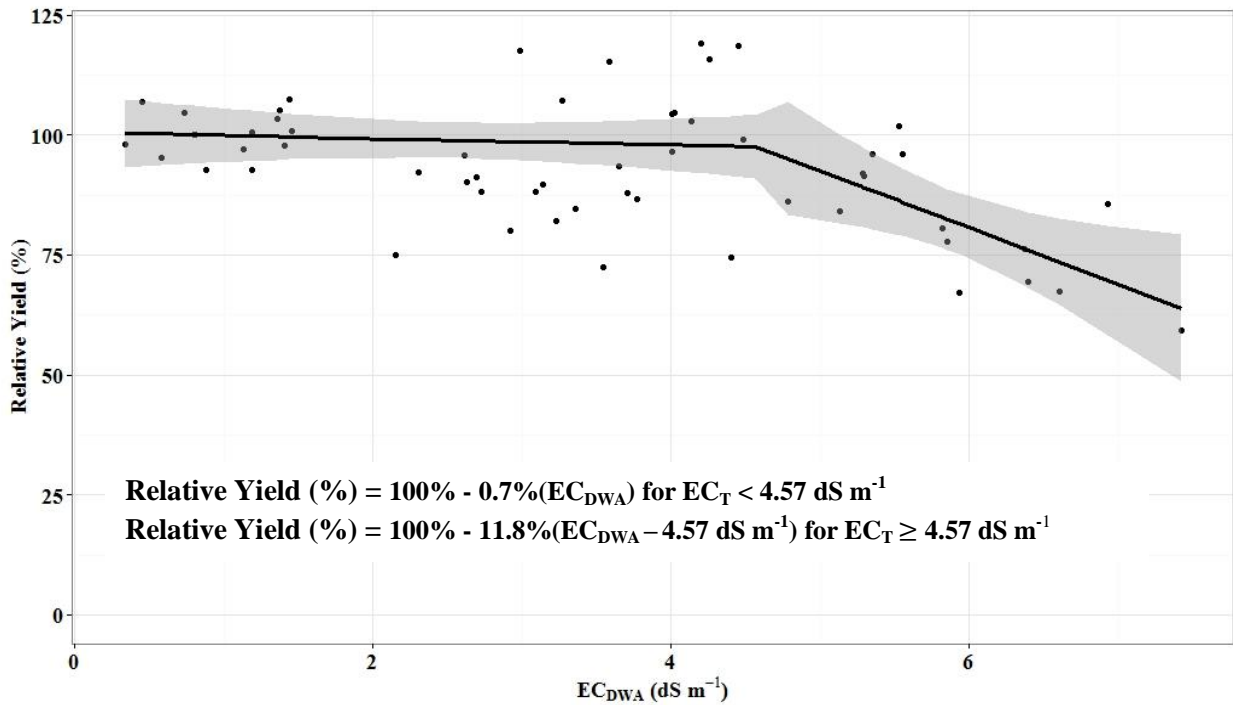


Figure 9. Threshold-slope model of hand-harvested corn yield in sandy loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone. Threshold-slope model fitted with 95% upper and lower confidence bounds. Change in slope occurred after a threshold EC_e (EC_{T}) of 4.57 dS m^{-1} .

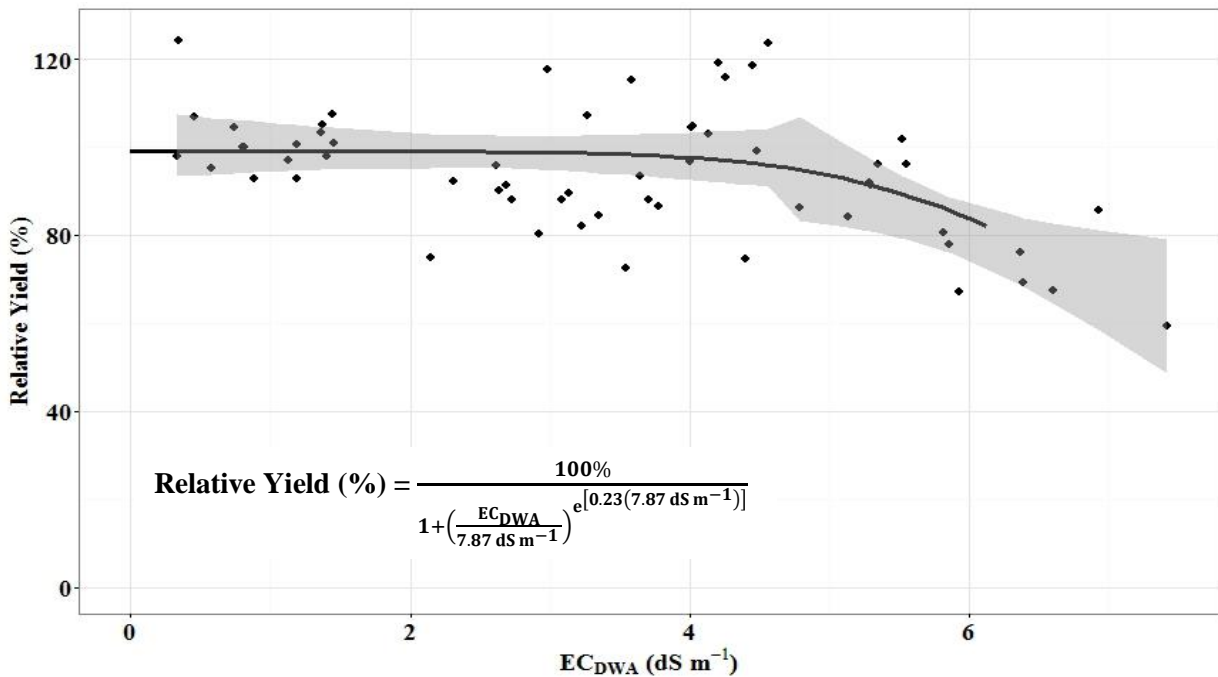


Figure 10. Modified discount response function of hand-harvested corn yield in sandy loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone. Steepest declines occurred after an EC_{DWA} of approximately 4.00 dS m^{-1} . Upper and lower 95% confidence bounds derived from threshold-slope model.

< 0.10). Incorporation of θ_v and EC_{DWA} in a multiple linear regression generated a significant model (p-value < 0.001) explaining 21.5% of the variation in yield.

Table 9. Descriptive statistics of measured soil properties within the 0-15 and 0-30 cm depth ranges and crop parameters measured in sandy loam (2014) and silty clay loam soils (2015) in Richland County, ND.

Property	Unit	Sandy Loam (2014)				Silty Clay Loam (2015)			
		Min	Max	Mean	Std.Dev.	Min	Max	Mean	Std.Dev.
EC_e (0-15 cm) [†]	dS m ⁻¹	0.30	4.68	1.65	1.21	0.38	4.93	2.08	1.13
EC_{DWA} (0-30 cm) [†]	dS m ⁻¹	0.36	5.23	1.94	1.47	0.37	5.76	2.40	1.23
pH _e (0-15 cm) [†]		6.95	8.26	7.69	0.27	7.24	8.53	7.92	0.302
pH _{DWA} (0-30 cm) [†]		7.04	8.10	7.66	0.25	7.21	8.45	7.88	0.29
Clay [‡]	g kg ⁻¹	81.1	313	155	579	62.1	408	306	800
Silt [‡]	g kg ⁻¹	111	593	266	110	271	623	401	934
Sand [‡]	g kg ⁻¹	225	767	579	150	175	416	293	483
Height V _n [§]	cm	15.1	34.9	25.3	4.7	21.9	56.0	33.2	6.8
SPAD V _n [¶]		19.3	37.2	30.5	3.4	24.0	37.3	32.1	2.9
Height R6 [§]	cm	26.2	60.2	42.0	8.5	35.2	110.2	70.5	17.2
SPAD R6 [¶]		42.6	62.2	52.1	4.7	28.3	47.3	42.4	3.11
LAI R6 [#]		0.59	3.58	1.71	0.71	0.10	2.35	1.25	0.61
Root Length (0-15 cm) ^{††}	cm	22.5	515.6	258.2	158.0	92.6	1018.8	44.1	269.0
Root Length (15- 30 cm) ^{††}	cm	46.5	513.6	219.9	149.6	61.3	695.9	214.7	134.6
Yield ^{‡‡}	kg ha ⁻¹	1,810	6,630	4,130	944	2,220	8,060	6,170	1,670

[†] Determined by a saturated paste extract according to methods by Richards et al. (1954).

[‡] Determined using the hydrometer method for particle size distribution (Gee and Or, 2002) on control section of soil profile at each sampling point.

[§] Height of soybean measured from the base of the plant to the tip of extended leaves.

[¶] Estimated N-content using a leaf chlorophyll meter.

[#] Leaf area index using a ceptometer in the across row position.

^{††} Total root length determined using WinRhizo software. In sandy loam soils, roots were collected from a soil core 15 cm in length and 6.3 cm in diameter. In silty clay loam soils, roots were collected from a soil core 15 cm in length and 9.0 cm in diameter.

^{‡‡} Yield calculated based on the mass of soybean per bushel at 13.0% moisture content hand-harvested from an area of 2.32 m².

Soybean

Measured Soil Properties

A detailed summary of all measured soil and plant parameters by soil type and year is listed in Table 9. In general, regardless of texture or salt level, EC_e increased with depth (Figure 11 and 12). However, values of EC_e in sandy loam soils were usually greater than values of EC_e at the same depths in the silty clay loam soils. For example, minimum and maximum values of EC_e in the sandy loam soil were 0.30 and 11.5 $dS\ m^{-1}$ which occurred in the 0-15 and 90-120 cm depths, respectively. Minimum and maximum values of EC_e in the silty clay loam soil were 0.23 and 8.18 $dS\ m^{-1}$ which occurred in the 0-30 and 90-120 cm depths, respectively.

Calcium, magnesium, and sodium also generally increased with depth, whereas potassium decreased (Table 10). Calcium was the most abundant cation measured in both the sandy loam and silty clay loam soils, followed by Mg^{2+} and Na^+ .

Volumetric water content measurements taken in the silty clay loam soil indicated that, on average, θ_v at the soil surface was at field capacity or close to saturation for all growth stages sampled (Table 11). The maximum θ_v observed was 0.531 cm^3 water total cm^{-3} and occurred during the V4 growth stage. The minimum θ_v observed was 0.175 cm^3 water total cm^{-3} , which is

Table 10. Calcium, magnesium, and sodium concentrations in the root zone of soybean grown in sandy loam (2014) and silty clay loam (2015) soils in Richland County, ND.

Property	Depth Range	Unit	Sandy Loam (2014)				Silty Clay Loam (2015)			
			Min	Max	Mean	Std.Dev.	Min	Max	Mean	Std.Dev.
$K^{+\dagger}$	0-15 cm	$mg\ kg^{-1}$	85	209	123	34	107	291	170	35
	15-30 cm	$mg\ kg^{-1}$	48	125	71	22	58	192	114	31
$Ca^{2+\dagger}$	0-15 cm	$mg\ kg^{-1}$	1950	6250	4092	1176	3348	6693	4725	1019
	15-30 cm	$mg\ kg^{-1}$	3397	11563	5402	1849	2080	17337	4978	2882
$Mg^{2+\dagger}$	0-15 cm	$mg\ kg^{-1}$	252	1405	591	294	504	1724	1097	351
	15-30 cm	$mg\ kg^{-1}$	334	1781	759	345	388	2031	1216	442
$Na^{+\dagger}$	0-15 cm	$mg\ kg^{-1}$	18	230	76	62	17	274	88	70
	15-30 cm	$mg\ kg^{-1}$	24	491	144	117	21	337	120	88

\dagger Cations extracted with 1 M $C_2H_3O_2NH_4$ and determined using inductively coupled plasma mass spectrometry (ICP).

Table 11. Average volumetric water content measured in the root zone of soybean grown in silty clay loam soils in 2015 at various growth stages.

Depth Range (cm)	Volumetric Water Content (θ_v) [†]				
	cm ³ water cm ⁻³ total				
	Sampled Growth Stage				
	VE [‡]	V4	V _n	R2	R6
0-15	0.350 ± 0.048 [§]	0.363 ± 0.049	0.472 ± 0.040	0.311 ± 0.072	0.369 ± 0.044
15-30	--	--	--	-- [¶]	-- [¶]

[†] Volumetric water content estimated using handheld soil moisture probe (GS3, Decagon Devices, Inc., Pullman, WA) at each depth of interest.

[‡] Vegetative (V) and reproductive (R) growth stages sampled throughout the growing season in 2015.

[§] Average $\theta_v \pm$ the standard deviation for both fields in 2015.

[¶] Volumetric water content at the 15-30 cm depth at the R2 and R6 stage could not be measured.

approaching the wilting point of silty clay loam soils. The minimum θ_v occurred during the V4 growth stage of soybeans.

Crop Parameters

Sandy Loam Soil (2014)

In sandy loam soils, N-content was the only crop parameter at the V6 growth stage to significantly decline in response to increasing EC_e down to 15 cm. Nitrogen content, as estimated by leaf chlorophyll content, declined linearly by 2.1% (0.77 SPAD units) per unit increase in EC_e of the 0-15 cm depth (p -value < 0.05; $R^2 = 5.6\%$; Figure 13). Declines occurred after the lowest observed salinity of 0.30 dS m⁻¹. At the R6 growth stage, soybean height and N-content significantly declined in response to increasing EC_{DWA} down to 30 cm. Height declined linearly by 12.8% (7.0 cm) per unit increase in EC_{DWA} after a threshold EC_{DWA} (EC_T) of 2.96 dS m⁻¹ (Figure 14). The threshold-slope model was significant (p -value < 0.05) and explained 8.8% of the variation in soybean height. The threshold-slope model indicated that EC_T was within an interval of 1.60 and 4.31 dS m⁻¹ with 95% confidence. The 95% confidence of the slope of decline at an EC_{DWA} greater than or equal to EC_T was within an interval of -26.8 and -1.1% per

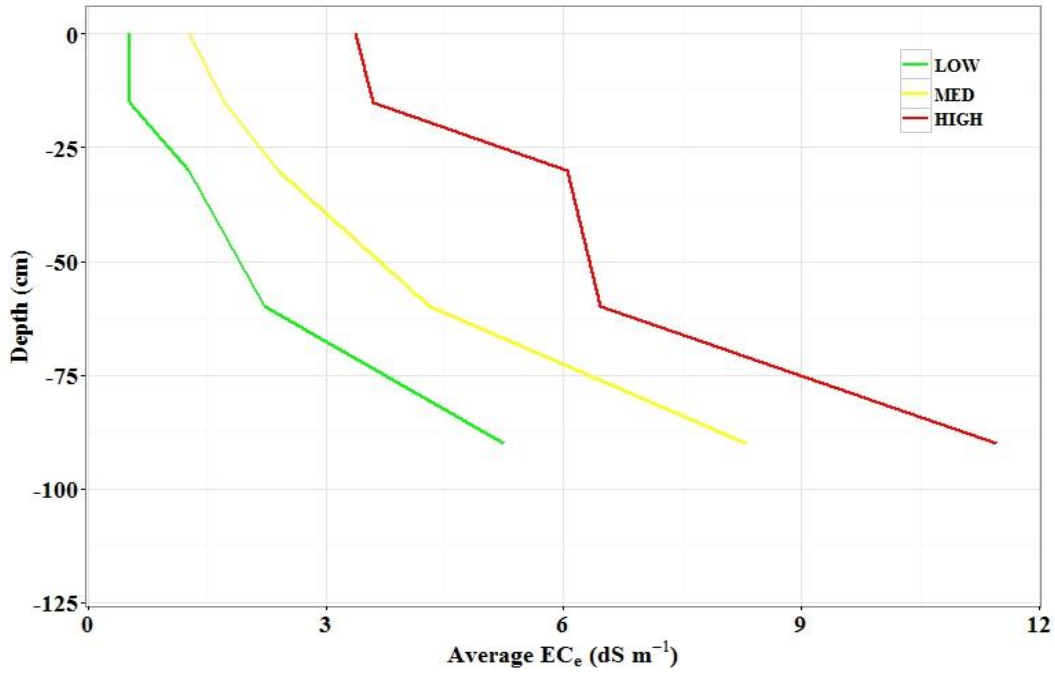


Figure 11. Depth functions of EC_e in sandy loam soils in 2014. Each depth function represents a single point on a transect. Low, medium, and high levels of salinity had depth weighted average EC_e s (EC_{DWA}) down to 120 cm of 2.31, 4.13, and 6.90 $dS\ m^{-1}$, respectively.

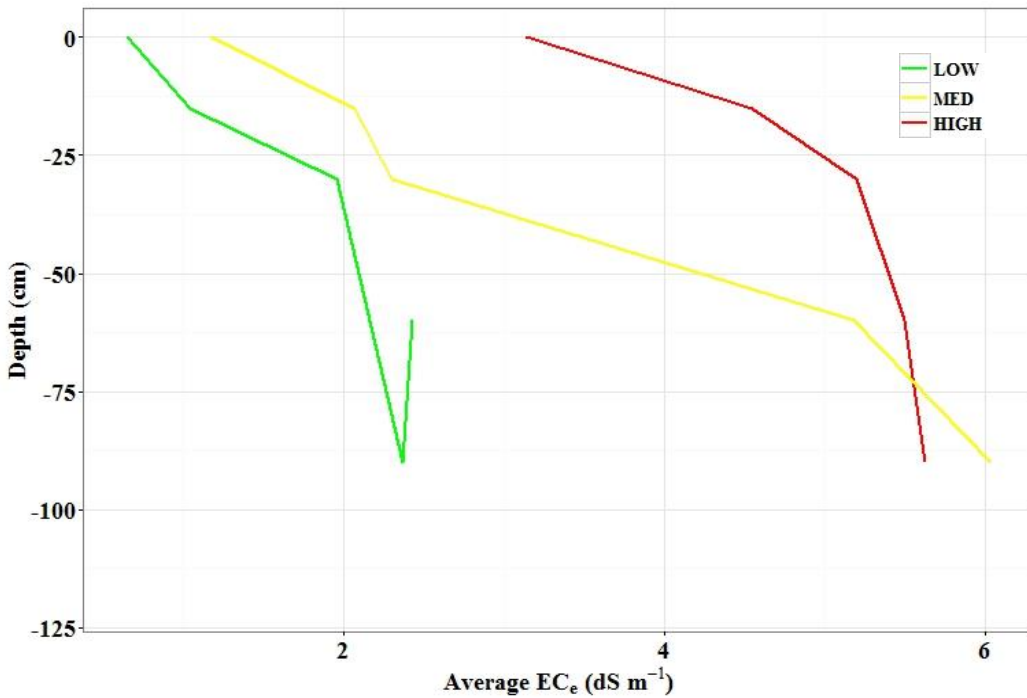


Figure 12. Depth functions of EC_e in silty clay loam soils in 2015. Each depth function represents a single point on a transect. Low, medium, and high levels of salinity had depth weighted average EC_e s (EC_{DWA}) down to 120 cm of 1.90, 3.79, and 5.04 $dS\ m^{-1}$, respectively.

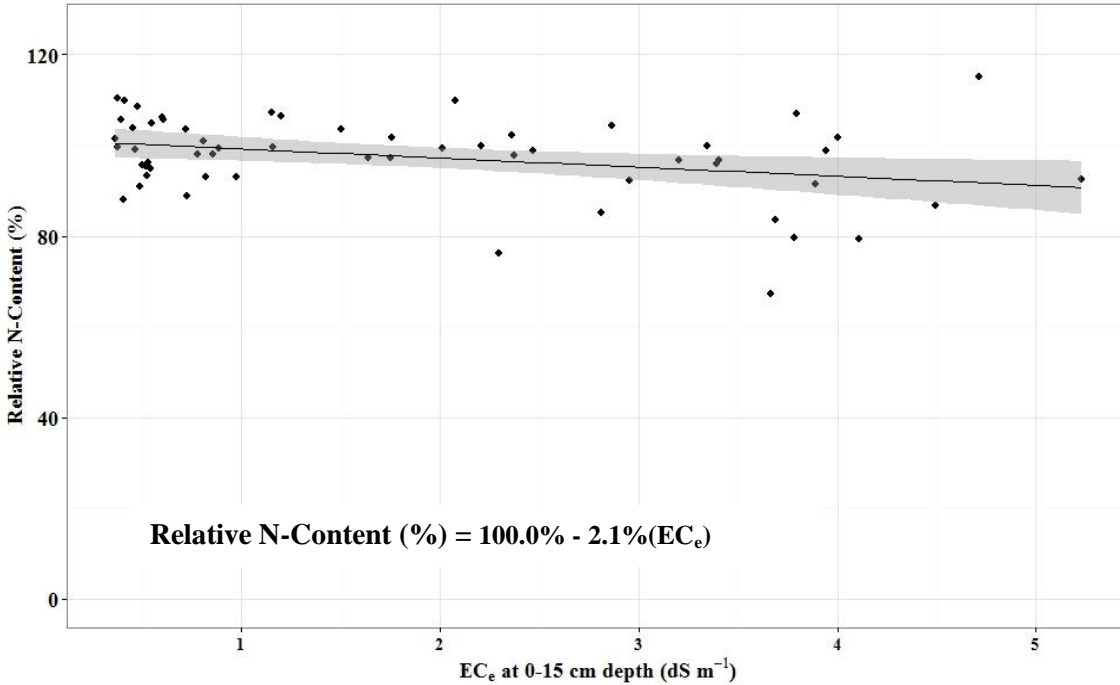


Figure 13. Relative decline in soybean leaf SPAD reading at the V6 stage in sandy loam soils in Richland County, ND with increasing EC_e of the 0-15 cm root zone. Best fit line fitted with 95% upper and lower confidence bounds.

dS m⁻¹. Nitrogen content at the R6 life stage declined linearly by 1.3% (0.59 SPAD units) per unit increase in EC_{DWA} down to 30 cm (Figure 15). The linear model was significant (p-value < 0.01), and EC_{DWA} explained 10.7% of the variation in N-content. Root length was distributed equally between 0-15 and 15-30 cm depths in sandy loam soils. No significant models explaining root length and EC_e were found at any of the depths sampled. However, while not significant, distribution of roots in the 0-15 cm depth increased as EC_e of the 15-30 cm depth increased.

The threshold-slope model was highly significant (p-value < 0.0001) for predicting soybean yield in response to increasing EC_{DWA} down to 30 cm in sandy loam soils (Figure 16). The depth weighted average EC_e of the root zone explained 27.2% of the variation in soybean yield. The model intercept corresponded to 1.8 Mg ha⁻¹, and a significantly different (p-value < 0.0001) slope of yield decline was predicted after an EC_T of 2.98 dS m⁻¹. At values below EC_T, soybean yield declined by 1.8% (32.0 kg ha⁻¹) per unit increase in EC_{DWA}. At values greater than

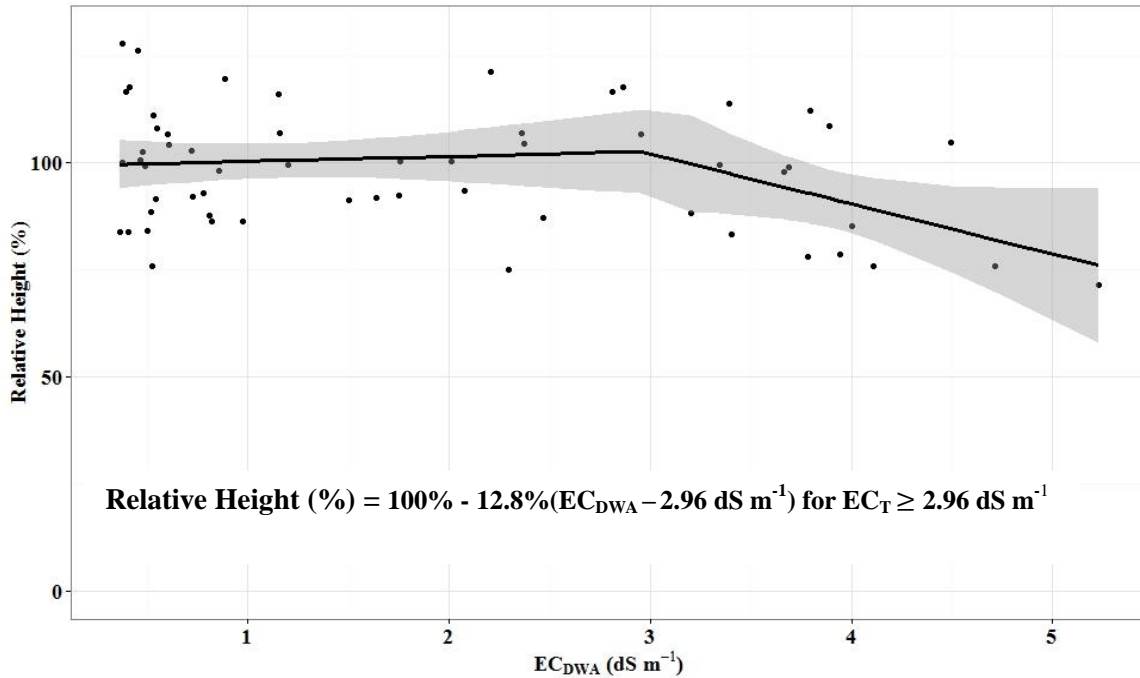


Figure 14. Threshold-slope model of height at the R6 stage of soybeans grown in sandy loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Threshold-slope model fitted with 95% upper and lower confidence bounds. Change in slope occurred after a threshold EC_e (EC_T) of 2.96 dS m⁻¹.

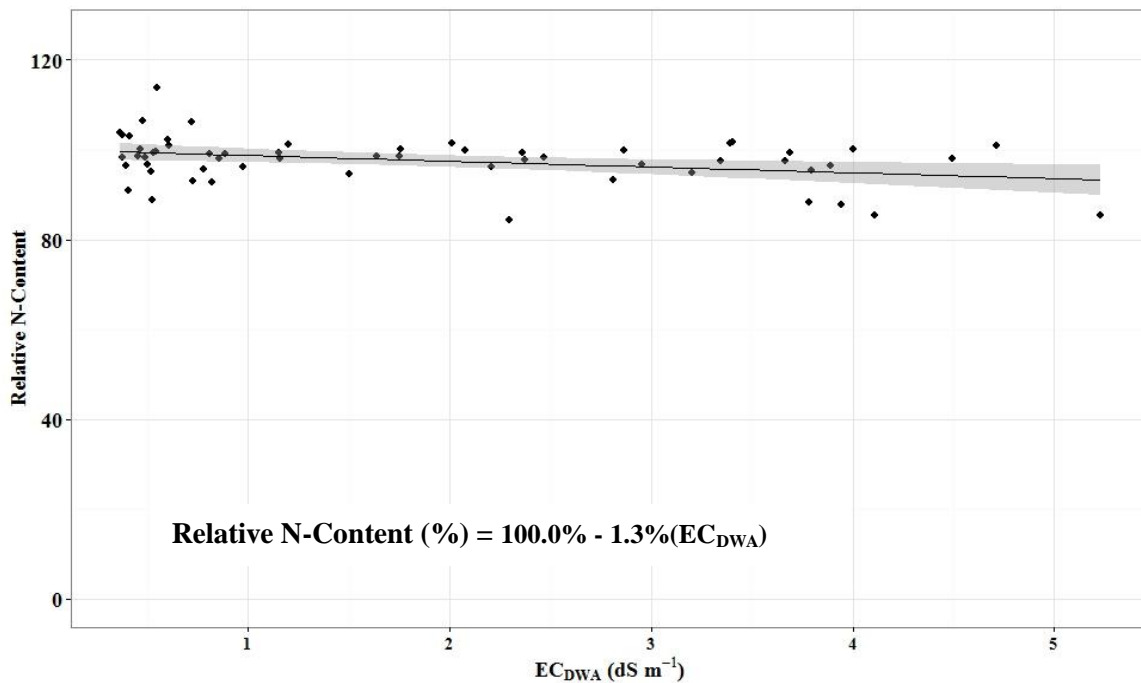


Figure 15. Relative decline in N-content at the R6 stage of soybeans grown in sandy loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Best fit line fitted with 95% upper and lower confidence bounds.

or equal to EC_T , soybean yield declined by 21.0% (384 kg ha^{-1}) per unit increase in EC_{DWA} . The threshold-slope model indicated that EC_T was within an interval of 1.80 and 4.17 dS m^{-1} with 95% confidence. The 95% upper and lower confidence bounds for the slope of decline after EC_T were -38.3 and -3.7% per dS m^{-1} increase, respectively.

Soybean yield in sandy loam soil was also significantly predicted by the MDRF model (Figure 17). The model intercept corresponded to $1.8 \text{ Mg soybeans ha}^{-1}$. The estimated value for the steepness parameter (s) was 0.33 as determined by non-linear fitting of the MDRF model. The EC_{50} was estimated from the threshold-slope model at 5.00 dS m^{-1} . The value of p was calculated at 5.21. The MDRF model parameters were highly significant ($p\text{-value} < 0.001$), and the model explained more variation in yield with increasing salinity of the root zone ($R^2 = +30.8\%$). The model intercept corresponded to 1.8 Mg ha^{-1} , and yield declined exponentially by a factor of 1.66% per unit increase in EC_{DWA} . The steepest declines were observed after an EC_{DWA} of 3.00 dS m^{-1} .

Residual analysis detected significant heteroskedasticity in both threshold-slope and MDRF models explaining yield response to EC_{DWA} . Further analysis indicated that residuals of both yield models were significantly correlated ($p\text{-value} < 0.05$) to clay content ($r = +0.30$) and approached a significant correlation ($p\text{-value} < 0.10$) to sand content ($r = -0.22$). Incorporation of clay content and EC_{DWA} into a multiple linear regression generated a highly significant model ($p\text{-value} < 0.0001$) explaining 35.5% of the variation in yield. In the multiple linear regression, soybean yield declined by 10.0% per unit increase in EC_{DWA} when clay content was constant. When EC_{DWA} was constant, soybean yield increased by 1.5% per $\text{g clay soil kg}^{-1}$ increase.

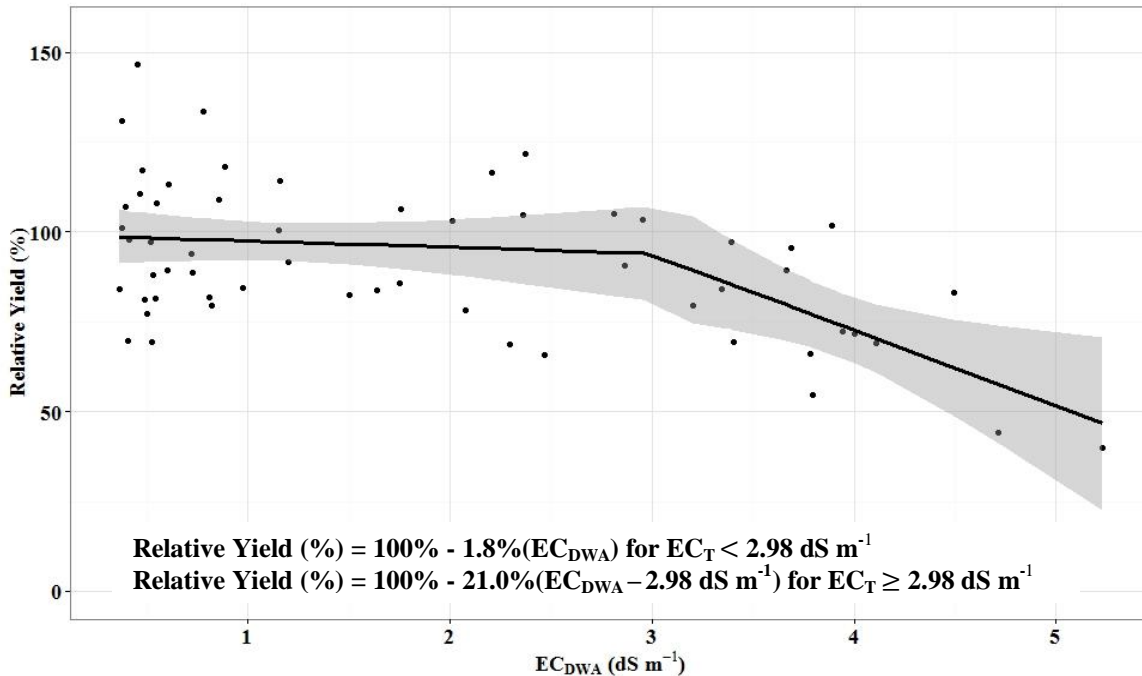


Figure 16. Threshold-slope model of hand-harvested soybean yield in sandy loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone. Best fit line fitted with 95% upper and lower confidence bounds. Change in slope occurred after a threshold EC_e (EC_{T}) of 2.98 dS m^{-1} .

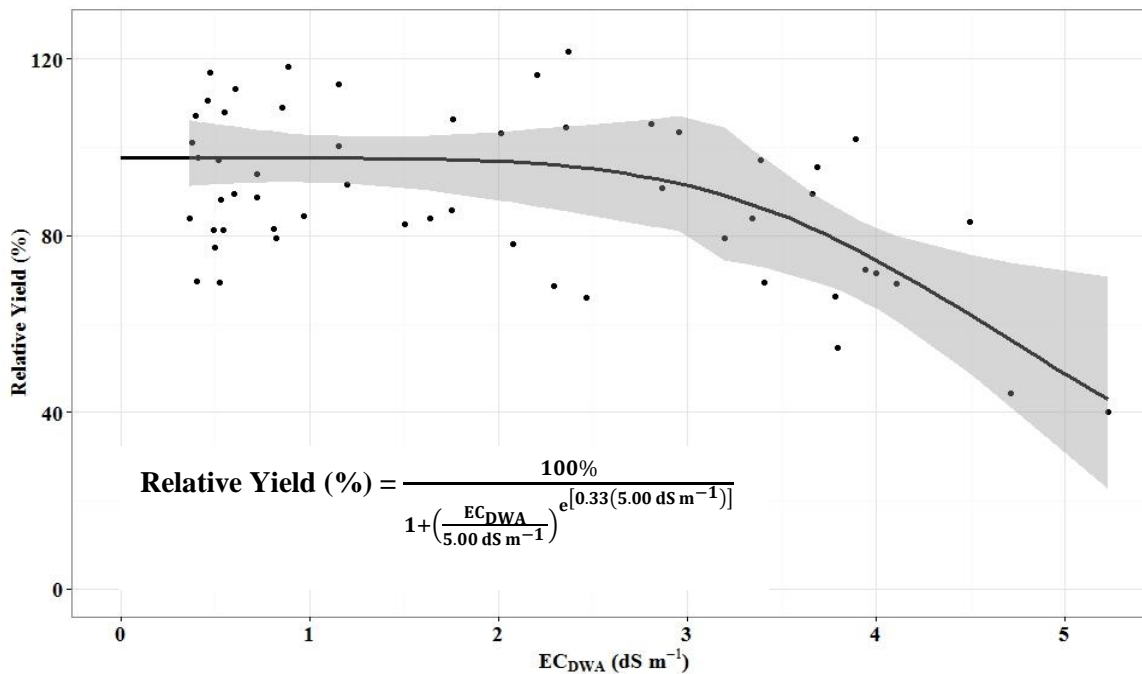


Figure 17. Modified discount response function of hand-harvested soybean yield in sandy loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone. Steepest declines occurred after an EC_{DWA} of approximately 3.00 dS m^{-1} . Confidence intervals derived from threshold-slope model.

Silty Clay Loam Soil (2015)

In silty clay loam soils, no significant declines in soybean height or N-content at the V6 stage were observed. Soybean height at the R6 stage was best predicted by a threshold-slope model. The model was significant (p -value < 0.01) and explained 10.4% of the variation in soybean height (Figure 18). Significantly different slopes in height were observed after an EC_T of 2.76 dS m^{-1} . The 95% upper and lower confidence bounds for EC_T were $+1.15$ and -4.38 dS m^{-1} , respectively. Soybean height declined by 11.6% (10.6 cm) per unit increase in EC_{DWA} at values of EC_{DWA} greater than or equal to EC_T . Nitrogen content at the R6 stage of soybeans declined linearly by 1.8% (0.81 SPAD units) per unit increase in EC_{DWA} (Figure 19). The linear model was significant (p -value < 0.05) and explained 8.7% of the variation in N-content. Highly significant declines (p -value < 0.0001) in LAI were also observed at the R6 stage of soybeans grown in silty clay loam soils (Figure 20). Leaf area index declined by 13.4% (0.24 LAI units) per unit increase in EC_{DWA} . The model explained 23.6% of the variation in LAI.

Similar to root distribution in sandy loam soils, the root length in silty clay loam soils was equally distributed between the 0-15 and 15-30 cm depths. Root length in both the 0-15 and 15-30 cm depths could not be explained by increasing EC_e of the respective depths. However, root length distribution in the 0-15 cm depth was significantly correlated to EC_e of the 15-30 cm depth below ($r = +0.34$). No significant declines in soybean yield were observed in silty clay loam soils up to an EC_{DWA} of 5.76 dS m^{-1} (Figure 21).

Discussion

Corn

Silty Clay Loam Soil (2014)

Both height and N-content at the V12 stage of corn grown in silty clay loam soils declined linearly in response to increasing EC_{DWA} down to 30 cm. The decline in these crop

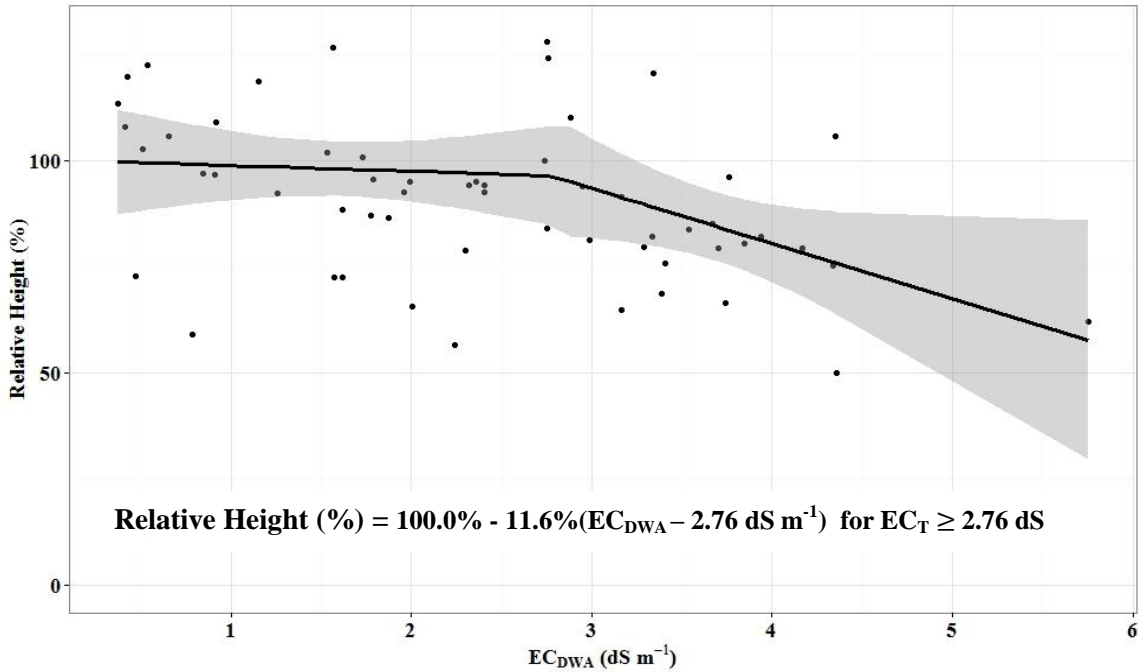


Figure 18. Threshold-slope model of height at the R6 stage of soybeans grown in silty clay loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Relative height declined linearly after a threshold depth weighted average EC_{DWA} (EC_T) of 2.76 dS m⁻¹. Best fit line fitted with 95% upper and lower confidence bounds.

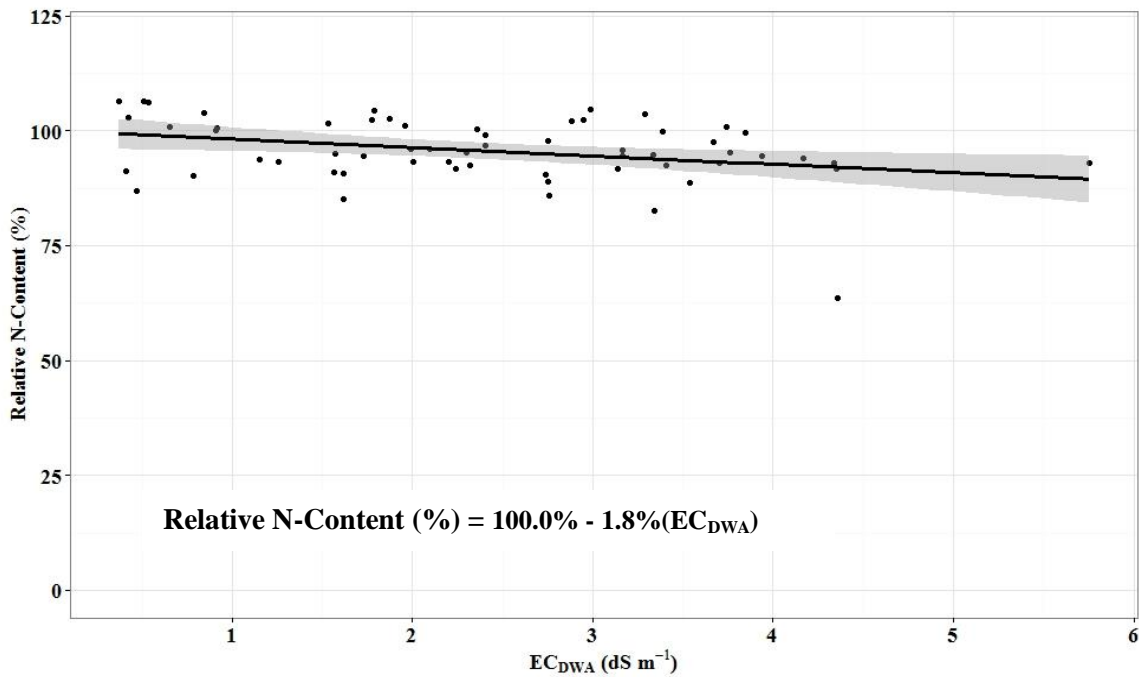


Figure 19. Relative decline in N-content at the R6 stage of soybeans grown in silty clay loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Best fit line fitted with 95% upper and lower confidence bounds.

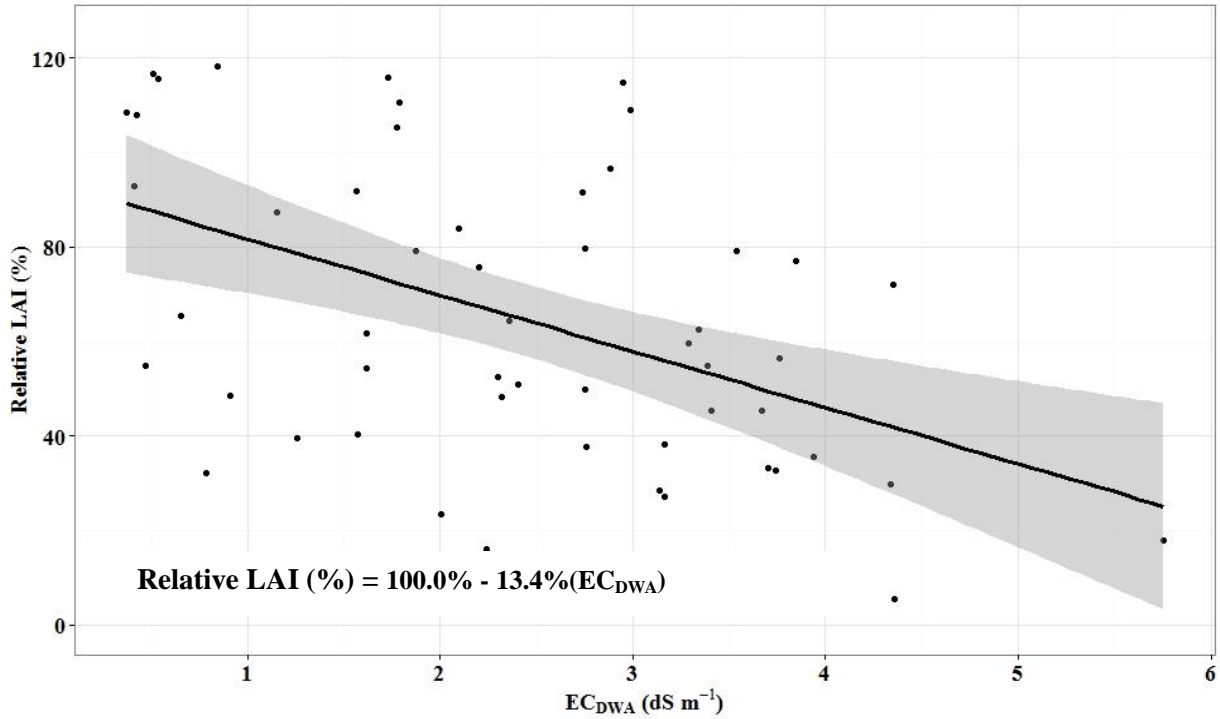


Figure 20. Relative declines in LAI at the R6 stage of soybeans grown in silty clay loam soils in Richland County, ND with increasing depth weighted average EC_e (EC_{DWA}) down to 30 cm. Best fit line fitted with 95% upper and lower confidence bounds.

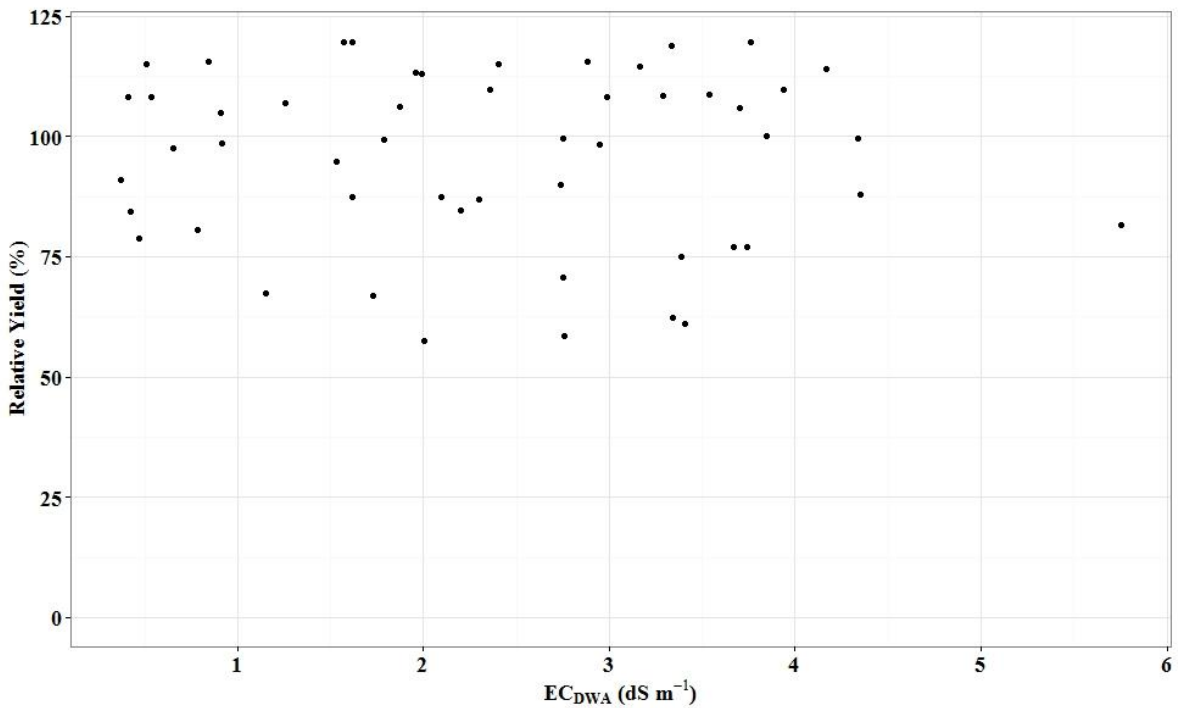


Figure 21. Hand-harvested soybean yield in silty clay loam soils in Richland County, ND as a function of the depth weighted average EC_e (EC_{DWA}) of the root zone.

parameters at the vegetative growth stage sampled is likely attributed to the decreased tolerance of corn at vegetative life stages to increasing salinization (Maas et al., 1983). Declines in height have been previously observed, but the rates of relative decline and thresholds before decline varied depending on the study. Shalhevet et al. (1995) reported decreases in height of 6.9% per unit increase in EC of NaCl and CaCl₂ irrigation water (EC_i) when vegetative corn was grown in a mixture of peat, silt, and sand. However, the declines did not occur until after an EC_T of 4.02 dS m⁻¹ (Shalhevet, 1995). Similarly, vegetative corn in a sandy loam soil declined by 13% per unit increase in EC_i after a threshold of 1.9 dS m⁻¹ (Blanco et al., 2007).

Discrepancies among thresholds and slopes of decline could be attributed to differences in salt constituents. Previous studies focused on the impacts of chloride salts on crop yield, which may have a greater impact on crop parameters once the salinity threshold is reached. Alternatively, the differences could be a result of the soil type used in the study. For example, Shalhevet et al. (1995) studied vegetative corn grown in a mixture of peat, silt loam, and sand, and Blanco et al. (2007) studied vegetative corn height in sandy loam. Because of the increased water holding capacity of finer textured soils (Setia et al., 2011), the soil solution is effectively diluted, and the EC of the soil solution is lower (Bernstein, 1975). Consequently, a higher EC would be necessary to impair biological growth and development in finer textured soils (Richards, 1954).

While silty clay loam soils used in this study justify the smaller slope observed, there is still no explanation for why vegetative corn height did not demonstrate some tolerance up to a threshold EC as previous studies noted. One potential explanation is the period of time that vegetative corn was subjected to salinity. Previous studies induced salinity with saline irrigation water after corn was established or fully germinated (Shalhevet et al., 1995) providing a non-

saline seed bed for corn to establish growth (Maas, 1984). It is possible that because the field-grown corn in this study was impacted by salinity at planting, the threshold tolerance was lower than previous studies reported. Salinity has been demonstrated to reduce seedling growth by 44 to 59% relative to a non-saline control after 0.7 dS m^{-1} (Maas et al., 1983). Furthermore, after this threshold, seedling growth of corn declined by 4.9% per unit increase in salinity (Maas et al., 1983).

While nutrient uptake is generally diminished by increasing levels of soluble salts in the soil (Fageria et al., 2011), contradictory results have been reported on N-content of corn with increasing levels of salinity. For example, no significant differences were observed in leaf and root N-content of corn regardless of the salts contributing to salinity (Tas and Basar, 2009). Alternatively, Bassuony et al. (2008) found total N-content reductions of 1.4% per unit increase in EC. Nitrogen content of corn in this study did demonstrate significant linear declines, but were steeper than reductions reported by Bassuony et al. (2008). The differences in the percent reduction could be attributed to differences in anion constituents. However, chloride (Cl^-) generally inhibits nitrate (NO_3^-) uptake (Tas and Basar, 2009). Consequently, it is unknown why declines in N-content of corn plants would have higher rates of reduction in soils of this study given that SO_4^{2-} is the dominate anion.

The lack of decline in height, LAI, and N-content at the reproductive stage of corn grown in silty clay loam soils was corroborated by a previous study that observed no declines in mature corn growth when subjected to an EC_e of 9.3 dS m^{-1} during the reproductive growth stages (Maas et al., 1983). The same study also reported that corn at the tasseling and grain-filling stages of its life cycle maintained 90 to 100% relative grain yield if salinity during the vegetative stages did not exceed 3.0 dS m^{-1} or salinity during the grain filling stage did not exceed 9.3 dS m^{-1} (Maas et

al., 1983). Average measured EC_{DWA} of the root zone in silty clay loam soils during vegetative growth of corn was 2.48 dS m^{-1} . During reproductive stages, the average EC_{DWA} of the root zone was 2.91 dS m^{-1} . Consequently, the lack of decline in crop parameters at the reproductive growth stage and corn yield could be a result of salinity in the rooting depths; on average, the root zone salinities did not exceed thresholds at vegetative and reproductive growth stages throughout the growing season to induce growth and yield reductions.

Another factor potentially influencing the lack of decline in reproductive crop parameters and yield of corn in silty clay loam soils is precipitation patterns. Silty clay loam fields received slightly higher total precipitation (43.2 cm) and less PET (121.7 cm) in 2014 compared to sandy loam fields in 2015. Coupled with higher water retention of finer textured soils (Li et al., 2013), the excess water in these soils during the 2014 growing season could be effectively diluting the soil solution (Setia et al., 2011). By consequence, salinity experienced by the plant could become reduced, leading to less detrimental impacts on plant growth and development. Again, this is attributed to the water holding capacity of finer textured soils which can dilute soil solution salinity (Richards, 1954; Setia et al., 2011). With this dilution effect, plants in coarser textured soils experience higher values of EC in the soil solution (EC_{sw}) in comparison to finer textured soils at the same salt content (Bernstein, 1975). As a result, plants in finer textured soils require higher threshold values of EC before negative impacts on plant growth and development occur (Richards, 1954).

The lack of significant patterns in corn root length and EC_e and the positive correlation of root length with EC_e at different depths within the root zone contradicted most publications assessing corn root length in response to increasing salinization. For example, corn root length in a solution culture with 100 mM of NaCl ($EC \approx 12.5 \text{ dS m}^{-1}$) declined by 22.5% relative to the

control (Hoque et al., 2015). Similarly, Shalhevet et al. (1995) observed declines in root length of 9% per unit increase in EC_e when corn was grown in a mixture of peat, silt, and sand. However, the lack of observed declines in root length with increasing EC_e in this study may be a result of the ionic composition of salinity in the study region. For example, 10 mM of Ca^{2+} ($EC \approx 1.0 \text{ dS m}^{-1}$) reduced both Na^+ replacement of Ca^{2+} on the plasmalemma of roots and K^+ efflux from the cytosol (Cramer et al., 1985). Zidan et al. (1990) found that the rate of elongation of corn roots in a solution with 100 mM of NaCl decreased by 75% relative to the control. However, the rate of decline in elongation was substantially reduced to 44% with the addition of 10 mM of calcium chloride ($CaCl_2$; Zidan et al., 1990). Magnesium is also an abundant salt constituent in the study region (Table 7; Keller et al., 1986). Similar to Ca^{2+} , Mg^{2+} is also capable of displacing Na^+ from the root membrane, but its effects are reduced in comparison to Ca^{2+} (Kinraide, 1998; Tas and Basar, 2009).

The lack of declines in root length could also be attributed to the ability of roots to better withstand salinity stress (Munns, 2002). For example, Rodriguez et al. (1997) demonstrated that if roots were slowly subjected to salinity stress, no significant differences in the rate of root extension relative to a non-saline control occurred. Consequently, significant declines in total root length may not have been observed in this study because of the abundance of Ca^{2+} and Mg^{2+} in these soils (Table 7), coupled with an extended period of time for roots to become acclimated to increasing levels of salinity in the root zone (Rodríguez et al., 1997).

Sandy Loam Soil (2015)

In sandy loam soils, no significant declines in crop parameters occurred at either the V12 or R1 life stage sampled. The lack of observed declines in this soil type is counterintuitive, given that salts are more concentrated in the soil solution in coarser textured soils because of

lower water retention (Richards, 1954; Bernstein, 1975; Setia et al., 2011). However, the lack of observed declines could be attributed to the precipitation and PET patterns in 2015.

Precipitation in 2015 was highest during May and June, when corn was in its vegetative stages and approaching reproductive growth (Figure 1). The increased precipitation during these months may have alleviated water deficits induced by salinity (Charles and Dukes, 2009). As a result, there would be no observed declines in any crop parameters at the vegetative and reproductive life stages sampled with increasing salinity. The decrease in precipitation and relatively constant rates of PET after June could also explain why yield declines were observed, despite a lack of decline in other crop parameters. Corn is relatively tolerant to moisture stress during its early vegetative and ripening stages (Doorenbos et al., 1979). However, during tasseling to ear formation (VT-R3), moisture deficits can cause the greatest declines in yield (Cakir, 2004). During these critical stages in July and August 2015, estimated PET was at its highest with low rainfall compared to the preceding months. Consequently, while the stress did not impede crop parameters like height or N content, precipitation and PET during 2015 may have contributed to the yield decline observed at the end of the growing season because of moisture stress at those critical stages of corn development.

Significant, exponential declines in yield of field-grown corn with decreasing precipitation during drought-sensitive, reproductive growth stages have been previously reported by Bergamaschi et al. (2007). In this study, the potential moisture stress induced by weather conditions was evidenced by volumetric water content (θ_v) measurements taken in July, which, on average, were the lowest θ_v throughout the 2015 growing season (Table 8). Additionally, the significant multiple linear regression model generated when both EC_{DWA} and θ_v at the R1 life

stage were incorporated into a yield response model supports the importance of adequate moisture at these critical stages of corn growth and development.

The intercepts of the threshold-slope and MDRF models (10.6 Mg ha^{-1} and 10.4 Mg ha^{-1} , respectively) were consistent with the average yield (10.1 Mg ha^{-1}) for Richland County in 2015 (National Agricultural Statistics Service; USDA-NASS, 2015). The slope of decline after EC_T in the threshold-slope model was 11.8% per dS m^{-1} increase. Similar slopes have been reported by previous studies, with declines between 10.5 and 12.0% (Maas and Hoffman, 1977; Katerji et al., 2003). Similarities in slopes between studies indicate that corn yield declines by approximately the same rate after some threshold tolerance, regardless of the ions contributing to salinity.

The previous threshold tolerance for corn was established at 1.7 dS m^{-1} (Maas and Hoffman, 1977). Conversely, corn grown in sandy loam soils in this study did not respond to salinity until an EC_{DWA} of 4.57 dS m^{-1} using the threshold-slope model. Parameters of the MDRF model were also higher than previously reported for corn response to increasing salinity. Model parameters in this study were 7.87 dS m^{-1} , 6.11, and 0.23 for EC_{50} , p , and s , respectively. Stepphun et al. (2005) reported values for corn of 5.54 dS m^{-1} , 2.75, and 0.183 for EC_{50} , p , and s , respectively. The lower values reported by Stepphun et al. (2005) indicate a lower tolerance of corn to soil salinity. However, in an assessment of four salinity trials by Maas and Hoffman (1977), van Genuchten and Gupta (1993) reported a range of p between 2.42 and 6.74, indicating that higher values of p have been previously observed for corn yield in response to increasing salinity.

Models generated in this study estimated higher values of EC_T than previous studies reported. Increased tolerance of corn to sulfate salinity is possible given the benefits of Ca^{2+} , Mg^{2+} , and SO_4^{2-} to plant growth and development (Curtin et al., 1993) since each of these

constituents is considered a macronutrient to most plants (Cramer et al., 1995; Kowalska, 2005; Jezek et al., 2015). For example, SO_4^{2-} is commonly used in many plant biochemical reactions that help regulate physiological function in plants (Kowalska, 2005) and is also associated with defense compounds that can alleviate abiotic stressors in plants (Leustek and Saito, 1999).

However, SO_4^{2-} -salinity has demonstrated negative effects on corn growth during germination and seedling stages. The weight of germinating corn seedling tops was reduced to 52% relative to the control when plants were irrigated with a 2 MPa ($\text{EC} \approx 0.8 \text{ dS m}^{-1}$) solution of Na_2SO_4 (Kaddah and Ghowail, 1964). The negative influence of SO_4^{2-} -salinity at early vegetative stages could explain the significant declines in V12 crop parameters in silty clay loam soils. Height of mature corn in response to sulfate salinity has also been previously reported, but declines were observed at 50 mM of Na_2SO_4 ($\text{EC} \approx 12.3 \text{ dS m}^{-1}$; Umair et al., 2014). In both silty clay loam and sandy loam soils, EC_{DWA} did not exceed this salinity, even at the maximum values of salinity measured (Table 6). Consequently, it is possible that the lack of observed declines during the reproductive stage in both soil types sampled resulted from levels of sulfate salinity that were not high enough to induce reductions in plant growth.

Calcium is an integral component of cell wall structure and turgidity (Cramer et al., 1995). Calcium can ameliorate Na^+ -induced salinity stress on plant physiology (Lahaye and Epstein, 1969) by displacing Na^+ ions on the cell membranes of roots (Kinraide, 1998; Tas and Basar, 2009). Calcium can also improve uptake of K^+ in corn (Kahn and Hanson, 1957). In saline soils composed of Na^+ , competition between Na^+ and K^+ can reduce the influx of K^+ into the plant (Bernstein, 1975). Potassium is essential for many biochemical reactions, as well as cell turgor maintenance (Gupta and Huang, 2014). In corn, 200 mg L^{-1} of Ca^{2+} ($\text{EC} \approx 0.5 \text{ dS m}^{-1}$) can

enhance the rate of K^+ uptake into roots (Kahn and Hanson, 1957), which could potentially reduce declines in plant function associated with the replacement K^+ with Na^+ in saline soils.

While Ca^{2+} does appear to have an ameliorative effect in saline soils, it is important to note that in soils dominated by sulfate, Ca^{2+} deficiencies can occur as a result of ion pairing and the relatively low solubility of gypsum ($CaSO_4 \cdot 2H_2O$; Curtin et al., 1993). As a result, as salinity increases and, by consequence, concentrations of SO_4^{2-} increase, it is difficult to tease apart effects of salinity with those of Ca deficiency (Curtin et al., 1993). Ultimately, whether Ca^{2+} is available to alleviate salinity stress depends on its presence in solution. Ion pairing of SO_4^{2-} and Ca^{2+} and precipitation of gypsum removes Ca^{2+} from solution (Curtin et al., 1993; Springer et al., 1999), which makes it unavailable to alleviate salinity stress and be adsorbed by the plant. Consequently, the presence of excessive SO_4^{2-} in soils in this study region (Keller et al., 1986) could potentially be limiting the beneficial effects of Ca^{2+} and sources of Ca^{2+} for plant growth (Curtin et al., 1993). Alternatively, ion pairing of Ca^{2+} and SO_4^{2-} and precipitation of gypsum could also be reducing the osmotic stress experienced by the plant as Ca^{2+} is removed from solution (Janzen and Chang, 1987).

The presence of gypsum could explain the higher EC_T values observed in this study. Typically, gypsum can increase EC_T by approximately 2 dS m^{-1} (Maas, 1993). Incorporation of this assumption into the EC_T values generated by models in this study decreases the values of EC_T to 2.57 and 2.00 dS m^{-1} for the threshold slope and MDRF models, respectively, which are more comparable to threshold values previously established by Maas and Hoffman (1977) and Katerji et al. (2003).

Magnesium is also a vital macronutrient for plants and is a major component in enzyme activation and chlorophyll structure (Jezek et al., 2015). Magnesium deficiency in plants can be

induced by salinity dominated by NaCl (Bernstein, 1975) as high chloride concentrations can degrade chlorophyll and diminish photosynthetic capacity (Tavakkoli et al., 2011). Additions of Mg^{2+} with $MgSO_4$ nutrient solutions to both leaves and roots of Mg^{2+} -deficient maize plants increased both SPAD chlorophyll readings and photosynthetic activity (Jezek et al., 2015). Consequently, the relatively high concentrations of Mg^{2+} could reduce declines in photosynthesis commonly associated with increasing salinization from NaCl.

However, specific ion effects of Mg^{2+} have been reported (Bernstein and Hayward, 1958). The top weight of germinating corn was more inhibited by $MgSO_4$ at -2 and -3 MPa (EC ≈ 0.8 and 1.2 dS m^{-1} , respectively) compared to isosmotic concentrations of NaCl, $CaCl_2$, $MgCl_2$, or Na_2SO_4 (Kaddah and Ghowail, 1964). The declines in biomass were attributed to Mg-induced Ca-deficiency (Kaddah and Ghowail, 1964).

Alternatively, indirect benefits of Ca^{2+} and Mg^{2+} may also have contributed to the higher EC_T in sandy loam soils. Both Ca^{2+} and Mg^{2+} have demonstrated an ability to improve soil structure (Bronick and Lal, 2005). Divalent cations can act as cementing agents for aggregates, and the formation of aggregates is beneficial for infiltration and water retention in soils (Bronick and Lal, 2005). Based on the results from corn grown in silty clay loam soils, an increase in the water holding capacity of soils may dilute the concentration of salt constituents in the soil solution, potentially lowering the osmotic stress experienced by the plant (Setia et al, 2011).

Despite the heteroskedasticity, correlations of residuals to soil physical properties, and low R^2 values of the models, both the threshold-slope and MDRF could adequately explain the response of corn yield to salinity. The dependence of salinity under field conditions on physical and chemical conditions in the soil could contribute to the low variation explained by models in this study. Properties measured in this study, like clay and sand content, as well as unmeasured

properties, like temperature, the interaction of salinity and water content, and aggregate stability are all properties that influence solvation of salts in the soil and, by consequence, the ions in solution contributing to soil salinity (Curtin et al., 1993 Rengasamy, 2010; Setia et al., 2011).

These properties may be confounding variables that should likely be included in the regression analyses to potentially reduce the heteroskedasticity within the models and improve the variation in yield explained. However, the addition of more factors in the model could be problematic to the original objectives of the study because producers may not necessarily have extensive data from the root zone of their fields. Even more, as factors such as temperature and water content fluctuate with time, salinity becomes an immensely complicated and interactive soil chemical condition throughout the growing season. Consequently, a more in-depth model containing multiple variables reduces and complicates the applicability of the results of this study to producers managing these problem soils.

Between the threshold-slope and MDRF models, the MDRF may be more ecologically relevant to corn yield response to salinity under field conditions (van Genuchten and Gupta, 1993). The MDRF model is potentially more relevant because of the cumulative effects of soil salinity on plant growth. For example, increasing salinization can reduce biomass (Wong et al., 2010), resulting in increased exposure of the soil surface. Exposure of the soil surface enhances evaporation (Shah et al., 2007). In the study region, groundwater is naturally saline (Benz et al., 1961). As a result, increases in evaporation from groundwater can enable more salts to accumulate in the root zone (Li et al., 2013) and exacerbate drought stress already experienced by the plant. Other potential added stressors attributed to salinity include pest pressures and nutrient deficiencies (Maas, 1993). In effect, salinity could cumulatively intensify plant decline

after some threshold because plant function declines from several secondary effects attributed to increasing salinization.

While the chemical composition of salinity in this region does seem conducive for increased tolerance, this general conclusion cannot be made. Both the threshold-slope and MDRF models depicted significant heteroskedasticity, and residuals were correlated to both texture and water content at the R1 growth stage. Consequently, it is difficult to differentiate effects of salinity on corn yield with other measured parameters under field conditions.

Soybean

Sandy Loam Soil (2014)

Nitrogen content was the only crop parameter at the V6 stage of soybean grown in sandy loam soils to significantly decline with increasing EC_{DWA} down to 15 cm. The decline in N-content during the vegetative growth stage is potentially explained by the adverse effects of salinity on root nodules. Root nodulation of soybeans is strongly inhibited by soil salinity (Delgado et al., 1994), and the impact of salinity on nodulation may be more pronounced at salt-sensitive vegetative stages of growth (Phang et al., 2008). While root nodulation was not measured in this study, Bernstein and Ogata (1966) observed nonlinear declines in the number of nodules with increasing concentrations of NaCl. At the highest salinity observed ($EC_e \approx 9.0 \text{ dS m}^{-1}$), the number of nodules declined to 46% relative to the non-saline control, despite no observed impacts on soybean root development (Bernstein and Ogata, 1966). Declines in root nodulation decrease plant uptake of N (Delgado et al., 1994) because of a decreased capacity for N_2 fixation (Bernstein and Ogata, 1966). Consequently, soybean plants that rely on N_2 fixation for N supplies become adversely affected by increasing levels of salinization.

The presence of both gypsum and calcite (CaCO_3) has also demonstrated detrimental impacts on root nodulation in soybean (Franzen and Richardson, 2000). The sandy loam soils in this study are classified as calcic (USDA-NRCS, 2014) and cation analysis indicated high levels of Ca^{2+} (Table 10). Consequently, N-content declines observed could also be the result of a combination of salinity, calcite, and gypsum interactions inhibiting root nodulation.

Both soybean height and N-content at the R6 growth stage declined with increasing EC_{DWA} down to 30 cm. Similar to N-content declines at the V6 stage, N-content during reproductive growth also declined linearly. Again, the decline could be attributed to reductions in nodulation with increasing salinity (Bernstein and Ogata, 1966) that lower the ability of soybeans to fix N_2 for growth (Delgado et al., 1994).

Soybean height at the R6 stage in sandy loam soils was predicted by a threshold-slope model of decline. Previous studies have also demonstrated declines in height during reproductive phase growth, but slopes vary among studies and no thresholds were reported. For example, mature soybean height at 7 dS m^{-1} was 20% lower than the non-saline control plant (Papiernik et al., 2005). Similarly, height of mature soybeans in sandy loam soils was 75% shorter at 8.0 dS m^{-1} than the control plant at 0.01 dS m^{-1} (Bustingorri and Lavado, 2014). Declines in soybean height with increasing salinity were expected, given that excess salts reduce the osmotic potential of the soil solution (Maas and Nieman, 1978). The reduction in osmotic potential forces plants to produce osmolytes to alter the osmotic potential of cells (Gupta and Huang, 2014) and restore water movement back into the plant (Horie et al., 2012). However, osmotic adjustments are energetically expensive and may result in diversion of resources for osmotic adjustment that are normally used for growth (Läuchi and Epstein, 1984).

It is uncertain why height declines were observed during reproductive growth stages and not vegetative growth stages, as vegetative stages tend to be more salt-sensitive (Phang et al., 2008). A potential explanation for this discrepancy is the precipitation patterns in 2014. For example, during vegetative stages (May through early July), precipitation was at its highest (Figure 2). It is possible that high rainfall during vegetative growth stages reduced the impact of salinity by dilution of excess salts in the soil. As a result of dilution, salinity experienced by the plant would be reduced (Richards, 1954; Bernstein, 1975; Setia et al., 2011). Even more, the increased precipitation could have provided more water to the plant, which was originally unavailable due to the reduction in osmotic potential of the soil solution from salinity (Shereen and Ansari, 2001).

Increased water availability could also explain the lack of significant decline observed in LAI with increasing salinity at the R6 stage. Typically, salinity induces stomatal closure, which reduces photosynthetic activity (Queiroz et al., 2012) and ultimately results in senescence of leaves (Khan et al., 2014). The purpose of stomatal closure is to decrease the water lost to transpiration during drought conditions (Davenport et al., 1977). However, high rainfall during vegetative stages may have alleviated drought stress and consequently reduced senescence of leaves that would have resulted in lower LAI readings. Furthermore, despite low rainfall conditions during reproductive stage growth in this study, a previous study reported that declines in leaf width of soybeans did not occur until after a threshold salinity of 5.0 dS m^{-1} , which is close to the maximum EC_{DWA} down to 30 cm measured in this study.

The lack of significant decline in root length with increasing salinity in sandy loam soils contradicts results of most previously published studies. For example, Shalhevet et al. (1995) observed declines in root growth of soybeans subjected to NaCl:CaCl₂ salinity of 6.6% per unit

increase in EC_e after an EC_T of 5.08 dS m^{-1} . Similarly, root biomass of soybeans in sandy loam soils was 50% of the non-saline control at 8.0 dS m^{-1} (NaCl; Bustingorri and Lavado, 2011). However, salinity levels in the 0-15 and 15-30 cm depths did not exceed the EC_T reported by Shalhevet et al. (1995). Consequently, it is possible that salinity levels in this study were not high enough to induce reductions in root length. Furthermore, a study by Bernstein and Ogata (1966) observed no declines in root growth of soybeans grown in saline soils compared to those of non-saline soils. As a result, it is possible that roots subjected to salinity are more capable at acclimating to increasingly saline conditions than above-ground plant components (Hsiao and Xu, 2000; Munns, 2002).

The intercept of the threshold-slope and MDRF models ($1.8 \text{ Mg soybeans ha}^{-1}$) was slightly lower than the average soybean yield (2.4 Mg ha^{-1}) for Richland County in 2014 (National Agricultural Statistics Service; USDA-NASS, 2014). The slope of decline after EC_T in the threshold-slope model was 21.0% per dS m^{-1} increase. Similar slopes have been reported by previous studies with declines between 11.4 and 20% (Maas and Hoffman, 1977; Katerji et al., 2000). Similarities in slopes between studies indicate that soybean yield declines by approximately the same rate after some threshold tolerance, regardless of the ions contributing to salinity. The previous threshold tolerance for soybean was established at 5.0 dS m^{-1} (Maas and Hoffman, 1977), but lower thresholds between 2.0 and 3.5 dS m^{-1} have been reported (Bernstein, 1975; Katerji et al., 2000). Soybeans grown in sandy loam soils in Richland County responded negatively to salinity after an EC_{DWA} of 2.98 dS m^{-1} in the threshold-slope model and approximately 3.00 dS m^{-1} in the MDRF model. Parameters of the MDRF model were also lower than previously reported estimates for soybean response to increasing salinity. Model parameters in this study were 5.00 dS m^{-1} , 5.21, and 0.33 for EC_{50} , p , and s , respectively. Stepphun et al.

(2005) reported values of 7.16 dS m⁻¹, 8.85, and 0.305 for EC₅₀, *p*, and *s*, respectively. The higher values reported by Stepphun et al. (2005) indicate a higher tolerance of soybean to soil salinity.

Models generated in this study estimated lower values of EC_T than previous studies reported. Decreased tolerance of soybean to sulfate salinity is one possible explanation for the discrepancies in EC_T between studies. For example, CaSO₄·2H₂O induces mineral deficiencies in soybean plants after two weeks in nutrient solutions (Leggett and Gilbert, 1969). Evidence suggests that excess Ca²⁺ in the external solution (20 mg L⁻¹ or EC ≈ 0.52 dS m⁻¹) of soybean roots can decrease Mg²⁺ uptake (Leggett and Gilbert, 1969) and cause Mg-deficiencies (Key et al., 1962). Furthermore, Mg-deficiencies are enhanced in sandy soils (Hellal and Abdelhamid, 2013). Magnesium deficiencies in this study were potentially evidenced by SPAD readings. While used as an estimation of N-content, SPAD meters actually measure chlorophyll content, and a major component of chlorophyll is Mg²⁺ (Jezek et al., 2015). Consequently, SPAD readings in this study may be indicative of declines in Mg²⁺ content in soybeans with increasing salinity.

Ion pairing of Ca²⁺ or Mg²⁺ with SO₄²⁻ in soils, as well as precipitation of CaSO₄·2H₂O could also exacerbate salinity stress experienced by soybeans. For example, ion pairing and precipitation reduce the available Ca²⁺ and Mg²⁺ in solution (Springer et al., 1999). Reduced concentrations of these cations can exacerbate Na⁺-induced dispersion because Na⁺ is now available for adsorption onto soil particle surfaces (Springer et al., 1999). Because dispersion ultimately impacts soil water movement (Wong et al., 2010), osmotic stress could become more pronounced in SO₄²⁻-dominated soils.

Alternatively, the abundance of Mg^{2+} in sandy loam soils of Richland County (Table 10) could also contribute to the lower EC_T observed in this study. Fresh weight of seeds and pods of soybeans salinized with $MgSO_4$ decreased to 17% at 1.08 dS m^{-1} relative to the non-saline control at 0.28 dS m^{-1} (Nukaya et al., 1982). Contrary to Leggett and Gilbert (1969), Nukaya et al. (1982) found that Ca^{2+} content in leaves of soybeans was lower than the control content, indicating that excess Mg^{2+} inhibited plant uptake of Ca^{2+} . Furthermore, the lower Ca^{2+} content of leaves induced by high levels of Mg^{2+} occurred in soybeans salinized by both $MgSO_4$ and $MgCl_2$, which indicated an ion toxicity specific to Mg^{2+} (Nukaya et al., 1982).

Another potential explanation for the lower EC_T observed could be the result of the variety of soybeans tested. Several previously published studies stress the importance of soybean variety in salt tolerance studies (Abel and MacKenzie, 1964; Papiernik et al., 2005; Phang et al., 2008). However, no salinity studies using Pioneer P10T91R variety soybeans are known. Because no comparisons can be made, it is difficult to validate this conclusion.

The lower observed EC_T may also be a consequence of the methods used to estimate soil salinity. Typically, the EC_e of a saturated paste is approximately half the EC of soil water at field capacity (Richards, 1954; Ayers and Westcott, 1985; Maas, 1993). Under the assumption that water content in sandy loam soils in 2014 were maintained at field capacity, EC_T would likely be closer to 5.96 dS m^{-1} , which is much closer to the previously accepted threshold tolerance reported by Maas and Hoffman (1977). However, volumetric water content was not measured in sandy loam soils during the 2014 growing season. Consequently, there is no evidence to warrant the adjustment of EC_T .

Despite the heteroskedasticity, correlations of residuals to soil physical properties, and low R^2 values of the models, both the threshold-slope and MDRF could adequately explain

the response of soybean yield to salinity. Similar to factors potentially affecting corn yield, soybean yield may also be influenced by other variables under field conditions. Again, these properties may be confounding variables that should likely be included in the regression analyses to potentially reduce the heteroskedasticity within the models and improve the variation in yield explained. However, more in-depth models reduce the applicability of results from this study to producers managing saline soils.

Similar to results of corn yield, the MDRF model is potentially more relevant because of the cumulative effects of soil salinity on soybean yield. For example, salinity increases cation concentrations in the soil solution contributing to both osmotic stress and specific ion toxicities (Maas and Niemen, 1978). Cation salinity in Richland County is dominated by Ca^{2+} (Table 7). If Ca^{2+} is available in solution, it can complex with phosphorus (Jackman and Black, 1951). Precipitation of calcium phosphate removes plant available phosphorus from the soil, which could lead to phosphorus deficiencies (Curtin et al., 1993). The presence of CaCO_3 could also be contributing to declines in N_2 -fixation as a result of a reduction in soybean root nodulation (Franzen and Richardson, 2000). Coupled with pest pressures (Maas, 1993) and an increased risk for iron deficiency chlorosis (Franzen and Richardson, 2000), salinity may cumulatively intensify soybean decline after some threshold from secondary factors attributed to increasing salinization.

Silty Clay Loam Soil (2015)

Declines in crop parameters of soybeans grown in silty clay loam soils only occurred at the R6 life stage. The lack of declines during vegetative growth is counterintuitive given that vegetative stages of soybean are more susceptible to deleterious effects of salinity (Phang et al., 2008). However, the lack of declines during vegetative stages may be attributed to precipitation

patterns. Similar to rainfall in sandy loam soils the previous year, soybeans in silty clay loam soils received the highest amount of precipitation during vegetative stages. After May, precipitation was substantially reduced (Figure 2). Consequently, increased precipitation may have contributed to higher water availability for plant growth during vegetative growth stages (Shereen and Ansari, 2001). Increased water availability, as evidenced by θ_v in the root zone during the VE, V4, and V6 stage (Table 11), would reduce the concentration of salts in the soil solution (Richards, 1954; Setia et al., 2011). As a result, salinity experienced at the vegetative stages would not impact growth and development to the same extent as soybeans grown in soils at lower water content.

During reproductive growth, θ_v of the root zone began to decline, indicating a reduction in the amount of available water (Table 11). The decline in available water at the onset of reproductive stages could explain why significant declines were observed at the R6 stage sampled. Drought and salinity stress trigger osmoregulation in plants in an effort to acclimate to lower osmotic potentials of soil solution and reduce water loss (Munns, 2002). As a result, resources normally directed towards growth and development must now be used for turgor maintenance (Läuchi and Epstein, 1984). The reallocation of resources could ultimately be the cause of declines in crop parameters at the R6 stage sampled. However, given the intricate relationship between salinity and soil water (Qureshi et al., 2007), it is difficult to differentiate whether the declines were attributed to salinity, drought, or a combination of both factors.

Root length was not significantly explained by increasing EC_e . Similar to root length in sandy loam soils, the lack of significant decline could be attributed to the ability of roots to better withstand salinity stress (Hsiao and Xu, 2000; Munns, 2002). However, significant increases were observed in root length distributions in the 0-15 cm depth when EC_e in the 15-30 cm depth

below increased. Increases in lateral growth of roots have been observed in *Zea mays* (Al-Khateef et al., 1989). Changes in EC_e with depth caused changes in root distribution by concentrating roots in layers above (Al-Khateef et al., 1989). The concentration of roots in the less saline layer (0-15 cm depth) is potentially evidenced by the higher average root length observed in these layers in silty clay loam soils (Table 9). The average root length decreased in the 15-30 cm depth, which was generally more saline than the 0-15 cm depth (Table 7).

It is uncertain why declines were not observed in soybean yield in silty clay loam soils, despite the significant declines in vegetative components of the plant during reproductive growth. One potential explanation is that declines observed during reproductive growth were an adaptive response to reduce the negative impacts of salinity on soybean yield. For example, declines in LAI of plants under salinity stress are a possible mechanism to reduce water loss through transpiration (Cutler et al., 1977). In effect, declines in crop parameters observed at the R6 stage may have been a response of soybeans to ensure successful yield. Reallocation of resources under drought stress has been observed in *Arabidopsis* (Ma et al., 2014). Despite declines in height at maturity, production of *Arabidopsis* flowers was maintained under moderate drought stress (gravimetric water content equal to 40% of field capacity) relative to the well-watered control (gravimetric water content equal to 85-90% of field capacity; Ma et al., 2014). However, this does not explain why yield was maintained up to 5.76 dS m^{-1} in silty clay loam soils, but not in sandy loam soils up to similar values of EC_{DWA} .

Variety plays a pivotal role in soybean tolerance to salinity and drought (Phang et al., 2008). Consequently, the lower EC_T observed in this study could be the results of the variety tested. However, it is difficult to verify this conclusion as previous studies have not used varieties studied in this research.

Another potential explanation for the lower EC_T observed is the texture itself. At the same salt content on a dry soil basis and same volumetric water content, the EC of soil water is five times higher in coarser textured soils compared to finer textured soils (Bernstein, 1975). Consequently, it is possible that salinity in silty clay loam soils induces moderate drought stress, whereas salinity in sandy loam soils induces severe drought stress. Under severe drought stress (gravimetric water content less than 40% of field capacity), flower yield of *Arabidopsis* declined and plant mortality increased when compared to the well-watered control and moderate drought stress treatments (Ma et al., 2014). In effect, soybean plants in sandy loam soils may not have been capable of adapting to drought conditions to the same extent of soybeans grown in silty clay loam soils because the drought stress induced by salinity was too severe for acclimation.

Chemical properties of the two textures studied may have also resulted in the differences in yield response of soybeans. Silty clay loam soils in this study were not classified as calcic (USDA-NRCS, 2015). The presence of calcite can hinder nodulation in soybeans (Franzen and Richardson, 2000) and result in declines in N_2 fixation for plant growth (Delgado et al., 1994). Consequently, despite salinity stress, the lack of calcite in silty clay loam soils in this study may have reduced the stress imposed on soybean plants in 2015.

Conclusions

The previously established threshold salinity tolerance for corn was substantially lower than the threshold observed in this study when corn was grown in sandy loam soils. However, once the threshold was reached, corn yield declined by approximately the same rate as previous studies reported. In silty clay loam soils, corn yield did not appear to be impacted by increasing salinization up to 6.00 dS m^{-1} . The results of corn grown in sandy loam soils indicate that corn

yield does not respond to salinity under field conditions at the same threshold tolerance observed in studies with salinity induced by NaCl.

The previously established threshold salinity tolerance for soybean was substantially higher than the threshold observed in this study when soybeans were grown in sandy loam soils. However, once the threshold was reached, soybean yield declined by approximately the same rate as previous studies reported. In silty clay loam soils, soybean yield did not respond to increasing salinization up to 5.76 dS m^{-1} . The results of soybeans grown in sandy loam soils indicated that soybean yield responds to salinity under field conditions at a lower threshold tolerance than studies with salinity induced by NaCl.

The lack of significant declines in corn and soybean yield in silty clay loam soils may be attributed to the dilution effect on soluble salts in soils that contain more water. Additionally, results suggest that texture plays an important role in crop tolerance to salinity. However, all of these interpretations are still hypotheses that require further testing. Furthermore, given different weather conditions, corn or soybean variety, or soil conditions, thresholds and slopes of decline are subject to change. Despite this variability, the results of this study can allow improved farm-management decisions for saline soils. Future research should address how salinity fluctuates under field conditions, as this knowledge may provide insight into the variation in yield across natural gradients in soil salinity. Additionally, an understanding of the spatial and temporal variation in salinity could increase the applicability of results of field studies assessing crop response to salinity across growing seasons.

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PAPER 2. MICROBIAL RESPIRATION UNRESPONSIVE TO INCREASING EC_e IN SULFATE-DOMINATED, SALINE SOILS

Abstract

An understanding of agro-ecosystem functions affected by increasing levels of soil salinity is critical for land management. This laboratory study determined CO₂ fluxes from soil samples collected along 100-m transects from a naturally saline, sandy loam soil, incubated at 50% water holding capacity over 45 d. In non-saline soils (EC_e < 2.0 dS m⁻¹), cumulative respiration (CO₂-C) and labile C were 0.40 and 0.36 mg CO₂-C g⁻¹, respectively. The labile C mineralization rate was 0.11 mg CO₂-C g⁻¹ d⁻¹. In saline soils (EC_e ≥ 2.0 dS m⁻¹), cumulative respiration and labile C were 0.30 and 0.27 mg CO₂-C g⁻¹, respectively. The decay rate of labile C was 0.11 mg CO₂-C g⁻¹ d⁻¹. Cumulative CO₂-C and labile C were significantly higher in non-saline soils, whereas the labile C mineralization rates did not vary between non-saline and saline conditions. However, regression analysis indicated no significant linear relationship between CO₂-C and EC_e, indicating that microbial activity does not significantly respond to sulfate salinity up to 4.38 dS m⁻¹. Instead, CO₂-C was best predicted by sand content and dissolved organic carbon concentrations. Consequently, microbial populations in these soils may have shifted towards more resilient communities to withstand increasing soil EC_e. As a result, populations would no longer be controlled by increasing salinization, but instead, factors that control availability of labile C for respiration.

Introduction

Microbial activity is a primary indicator of soil health (Kennedy and Papendick, 1995). Consequently, abiotic stressors reducing microbial function ultimately degrade soil ecosystems. Soil salinization is one such abiotic stressor previously demonstrated to impair microbial activity

(Rath and Rousk, 2015). Soil salinization is the accumulation of excess soluble salts in the soil profile (Keller et al., 1986). In North Dakota's Red River Valley, 1.2 million ha of arable land are classified as slightly saline, which reduces agricultural income by US \$150 million annually (Hadrich, 2012). Given the extent of agricultural production in this region and the percentage of sulfate-based salinity in the Northern Great Plains (NGP), it is important to better understand the impact of excess soluble salts on microbial processes as this could provide insight into ecosystem function and crop productivity.

Commonly, microbial activity is quantified by microbial respiration of carbon dioxide (CO₂; Rath and Rousk, 2015). The abundance of previous literature assessing microbial respiration in response to increasing concentrations of sodium chloride (NaCl) reported a negative relationship where microbial respiration declined as concentrations of dissolved NaCl increased (Chowdhury et al., 2011; Setia et al., 2011a; Setia et al., 2011b; Asghar et al., 2012; Elmajdoub et al., 2014). However, there are two issues with applying the results of chloride (Cl⁻)-based salt studies to soils in the NGP. Firstly, the use of dissolved NaCl in irrigation water to induce salinity is not indicative of the geochemistry of salinization in this region. Salinity in the Northern Great Plains is dominated by sulfate (SO₄²⁻) salts (Keller et al., 1986). Accordingly, there are potential differences in both osmotic stress and specific ion toxicities on microbial populations as a result of differences in solubility and anion constituents, respectively (Rath and Rousk, 2015). Secondly, a "shock effect" is produced when non-saline soil is irrigated with saline solutions (Setia et al., 2011b), which simulates secondary salinization (irrigation with poor-quality water; Rengasamy, 2010). However, the NGP has primary salinization, in which soluble salts accumulate in the soil from capillary rise of water originating from a naturally saline water table (Abrol et al., 1998). Because salinity is endemic to this region, microorganisms

potentially have adaptations which help regulate the cells to decreasing osmotic potentials in the soil solution (Schimel et al., 2007). Soil carbon (C) pools also respond differently to different salt constituents contributing to salinity. For example, excess sodium (Na^+) in the soil causes dispersion (Wong et al., 2009), which can increase the amount of labile C available for microorganisms to metabolize (Oades, 1984). Alternatively, calcium (Ca^{2+}) and magnesium (Mg^{2+}) have demonstrated flocculating abilities in soils (Bronick and Lal, 2005). As soil particles become flocculated, labile C is incorporated into aggregates which makes it unavailable for use by microbes (Clough and Skjemstad, 2000).

The purpose of this study was to assess impacts of sulfate-dominated salinity on microbial respiration in a naturally saline, sandy loam soil native to the Red River Valley of North Dakota. The results of this study are to determine if microbial activity can be used as a measure of salinity's impact on ecosystem function and land productivity in this region.

Materials and Methods

Research Site

Soil was collected from a research field located within 20 km of Mooreton, North Dakota in Richland County. The 64.7-ha field for this research was in a corn (*Zea mays*)-soybean (*Glycine max*) rotation, with soybeans planted the year of sampling. The region has a semi-arid climate, with rainfall typically averaging 50 cm annually (Baker, 1967). Mean annual temperatures are 5.6 °C (Baker, 1967). Prior to sampling in 2014, the field received approximately 28 cm of rainfall at an average temperature of 13.3 °C over the seven-month growing season (NDAWN, 2014). The soil series was mapped as the Wyndmere loam (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll; USDA-NRCS Web Soil Survey, 2014) with

the A horizon of this series described as a moderately alkaline, fine sandy loam with weak, coarse, subangular blocky structure on 0 to 3 percent slopes (USDA-NRCS, 1999).

Soil Sampling and Metrics

A Geonics EM 38 was used to continuously map salinity gradients to a 1.2-m depth on the 64.7 ha field. Data from EM 38 readings was interpolated using inverse distance weighted (IDW) interpolation in ArcMap (10.2, Esri, Redlands, CA) to produce a continuous gradient map of soil salinity on the field. Following interpolation, a total of five, 100-m transects were constructed along the most pronounced salinity gradients generated by the IDW-interpolation map. Transects were constructed within the soil series of interest and composed of four sampling points spaced 25 m apart. Following soybean harvest, four composite samples from the 0 to 15 cm depth were collected at each point along the transects for a total of 20 samples. Samples were air-dried and sieved to pass through a 2-mm mesh prior to all analyses. The electrical conductivity (EC) of each sample was determined using a saturated paste extract (EC_e) and a 1:5 soil to water slurry ($EC_{1:5}$; Richards et al., 1954). Electrical conductivity using 1:5 soil to water slurries were performed for literature comparison purposes. Particle size distribution was performed on a control section from 0-40 cm based on the vertical section of the profile used for classification (USDA-NRCS, 1999) according to methods by Gee and Or (2002). Samples were sent to AGVISE (Benson, MN) for cation and anion analysis. Calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), and sodium (Na^+) were extracted using 1 M $C_2H_3O_2NH_4$ and determined using inductively coupled plasma mass spectrometry. Concentrations reported for each cation included both the solution phase and exchange phase concentrations. Chloride (Cl^-) was extracted using 0.5 M K_2SO_4 and measured by potentiometric titration. Sulfate was extracted using 0.01 M KCl and determined by the turbidometric method. Concentrations of sulfate-sulfur

(SO₄²⁻-S) exceeding 60 mg kg⁻¹ were listed as 60+ mg kg⁻¹. Supernatants from centrifuged 1:1 soil to water slurries were filtered and used to determine dissolved organic carbon (DOC) using the Shimadzu TOC-V CPH/CPN Total Organic Carbon Analyzer based on procedures by Dilling and Kaiser (2002). Soil organic matter (SOM) was estimated by loss on ignition (LOI) as described by Ben-Dor and Banin (1989). Finally, calcium carbonate equivalent (CCE) was measured according to procedures reported by the Martin and Reeve (1955). Soil property information can be found in Table 12.

Incubation Setup and Metrics

Fifty grams of air-dried, 2-mm sieved soil was brought to 50% water holding capacity (WHC) in a 0.5-L mason jar with a rubber septum to allow for gas extraction. Three replicates of each sample were used for a total of 60 incubation chambers. Throughout the incubation, samples were kept at 50% water holding capacity (WHC) on a mass basis in a temperature controlled room at 25 °C. One milliliter of gas was extracted from the headspace of each jar and injected into an infrared gas analyzer (IRGA) using a flow-through system (GasHound, LiCOR Co., Lincoln, NE) on days 1, 5, 10, 25, 35, and 45 of the incubation.

A standard curve was generated to determine the mass of CO₂ in mg for a given volume injected. Mass of CO₂ was determined by integrating the area under the curve for each sample and comparing to standard curves. The mass of CO₂-C per g of soil was calculated as follows (Eq. 7):

$$mg\ CO_2 - C\ soil\ g^{-1} = \frac{M_{CO_2} * 12.01\ mg\ C * V_H}{V_{std} * 50\ soil\ g^{-1}} \quad (7)$$

where M_{CO_2} is the mass of CO₂ in mg calculated from the standard curve, V_H is the volume of headspace in the incubation chamber, and V_{std} is the volume of 1 mol of CO₂ at 1 atm and 25 °C based on the Ideal Gas Law.

Data Analysis

Cumulative microbial respiration was calculated as the summation of the measured mg CO₂-C g⁻¹ across the 45-d incubation period and incorporated into the first order kinetic equation to estimate the decay rate and labile C pools in each incubation chamber (Mora et al., 2007; Eq. 8), such that

$$y = C_o(1 - e^{-k*x}) \quad (8)$$

where y is the cumulative respiration and C_o is the labile or mineralizable C, both with units of mg CO₂-C g⁻¹, k is the mineralization rate or decay rate of labile carbon with units of mg CO₂-C g⁻¹ d⁻¹, and x is the time in d.

Samples were divided into subgroups based on EC_e as either non-saline (EC_e < 2.0 dS m⁻¹) or saline (EC_e ≥ 2.0 dS m⁻¹) according to classifications by Richards (1954). Of the original 20 samples, ten were classified as non-saline, with the remaining ten classified as saline. An analysis of variance (ANOVA) was used to determine if significant differences between cumulative respiration, labile C pools, and decay rate of labile C were observed between non-saline and saline soils. Linear regression was used to determine the relationship between CO₂-C and EC_e. Both ANOVA and regression analyses were considered significant at p-values < 0.05. All statistical analyses were performed using R Studio (3.2.1, R Foundation for Statistical Computing, Vienna).

Results and Discussion

Microbial Respiration

Average cumulative respiration in non-saline soil was 0.40 mg CO₂-C g⁻¹ and was significantly higher (p-value < 0.05) than CO₂-C in saline soils (0.30 mg CO₂-C g⁻¹). On average, cumulative respiration was 25% higher in soils classified as non-saline (Figure 22). The

significantly higher cumulative respiration observed in non-saline soils coincided with most studies reporting a reduction in cumulative respiration as salinity increased (Chowdhury et al., 2011; Setia et al., 2011a; Setia et al., 2011b; Asghar et al., 2012; Elmajdoub et al., 2014).

Table 12. Descriptive statistics of measured physical and chemical properties for the non-saline and saline soil samples.

Property	Unit	Non-Saline Soils				Saline Soils			
		Min.	Max.	Mean	Std.Dev.	Min.	Max.	Mean	Std.Dev.
EC _e [†]	dS m ⁻¹	0.80	2.45	1.34	0.6	2.54	4.38	3.10	0.6
pH [†]		7.76	8.22	7.96	0.2	7.57	8.06	7.74	0.2
EC _{1:5} [‡]	dS m ⁻¹	0.15	0.37	0.25	0.08	0.35	1.13	0.58	0.23
Ca ^{2+§}	mg kg ⁻¹	2870	6250	5170	1100	4510	6050	5670	464
K [§]	mg kg ⁻¹	122	218	170	34.8	115	200	164	29.5
Mg ^{2+§}	mg kg ⁻¹	515.0	1240.0	819	247	604	1410	1020	218
Na ^{+§}	mg kg ⁻¹	34.0	91.0	57.2	16.5	61.0	230	105	52.8
Cl ^{-¶}	mg kg ⁻¹	1.0	27.0	8.0	7.0	4.5	11.0	8.3	2.0
SO ₄ ^{2-#}	mg kg ⁻¹	15.0	60+	--	--	60+	60+	--	--
CCE ^{††}	%	0.5	4.0	1.6	1.1	1.4	4.0	2.9	1.0
SOM ^{‡‡}	g kg ⁻¹	0.96	0.98	0.97	0.00	0.97	0.98	0.97	0.00
DOC ^{§§}	mg L ⁻¹	29.8	43.2	37.4	5.1	19.8	39.8	27.5	6.7
Clay ^{¶¶}	g kg ⁻¹	150	250	185	34	168	250	209	30
Silt ^{¶¶}	g kg ⁻¹	53	2775	223	72	150	313	227	49
Sand ^{¶¶}	g kg ⁻¹	50	748	592	510	438	625	564	57
Respiration ^{##}	mg CO ₂ -C g ⁻¹	0.20	0.57	0.40	0.10	0.19	0.36	0.297	0.06
Labile C ^{†††}	mg CO ₂ -C g ⁻¹	0.19	0.52	0.36	0.10	0.17	0.33	0.271	0.06
Decay Rate ^{‡‡‡}	mg CO ₂ -C g ⁻¹ d ⁻¹	0.07	0.14	0.11	0.03	0.07	0.23	0.11	0.05

† Determined by a saturated paste extract according to methods by Richards et al. (1954).

‡ Determined by a 1:5 soil to water slurry according to methods by Richards et al. (1954).

§ Cations were extracted using 1 M C₂H₃O₂NH₄ and determined by inductively coupled plasma mass spectrometry. Values represent both the solution phase and exchange phase concentrations.

¶ Extracted using 0.5 M K₂SO₄ and measured by potentiometric titration.

Extracted using 0.01 M KCl and measured by the turbidometric method. Concentrations exceeding 60 mg kg⁻¹ were reported as 60+ mg kg⁻¹.

†† Estimated by loss on ignition (LOI) as described by Ben-Dor and Banin (1989).

‡‡ Measured on the filtered supernatant of 1:1 soil to water slurry and measured using a Shimadzu TOC-V CPH/CPN Total Organic Carbon Analyzer (Dilling and Kaiser, 2002).

¶¶ Determined by the hydrometer method for particle size distribution (Gee and Or, 2002) on a control section of the soil profile (USDA-NRCS, 1999).

Summation of CO₂-C measured across 45 d incubation

††† Calculated as the intercept (C₀) of Eq. 2 (Mora et al., 2007).

‡‡‡ Calculated as the slope of decline (k) in CO₂-C from Eq. 2 (Mora et al., 2007).

However, the values of cumulative respiration at different levels of EC_e or $EC_{1.5}$ varied across all studies examined. For example, CO_2 -C in sand declined by 8% at $EC_{1.5}$ of 1.3 dS m^{-1} and 42% at $EC_{1.5}$ of 4.0 dS m^{-1} relative to the non-saline control ($EC_{1.5}$ of 0.5 dS m^{-1} ; Mavi et al., 2012).

Cumulative respiration was not significantly impacted by similar values of $EC_{1.5}$ in sandy clay loam soils (Mavi et al., 2012). Similarly, at $EC_{1.5}$ of approximately 5.0 dS m^{-1} , CO_2 -C decreased by 56% in loamy sand, 29% in sandy loam, 24% in sandy clay loam, and 20% in clay relative to a non-saline control soil with no salt added (Setia et al., 2011a). The results of these studies indicated that finer textured soils may alleviate salinity stress on microbial respiration (Setia et al., 2011a).

Labile Carbon Pools

Average labile C (C_o) under non-saline conditions was significantly higher (p-value < 0.05) than labile C under saline conditions (0.36 and $0.27 \text{ mg CO}_2\text{-C g}^{-1}$, respectively). On average, labile C pools were 25% higher in soils classified as non-saline (Figure 22). Lower fractions of labile C were also observed by Setia et al. (2011a). Labile C in a sandy loam, saline soil ($EC_{1.5}$ of 5.33 dS m^{-1}) had 64% lower labile C than the non-saline control ($EC_{1.5}$ of 0.8 dS m^{-1}). The magnitude of difference between labile C in saline soils and the control depended on texture, with higher clay contents resulting in a smaller decline in labile C with increasing salinity (Setia et al., 2011a). Saline soils tend to have lower C inputs because of decreased above-ground biomass (Wong et al., 2010). Decreased biomass was demonstrated by the lower average soybean yield observed on the saline samples in this study ($1,980$ vs. $1,630 \text{ kg ha}^{-1}$ for non-saline and saline soils, respectively). As a result of lower organic matter input, less DOC is produced (Asmar et al., 1994). Dissolved organic C is the water soluble fraction of the labile C pool and the most readily available C fraction for use by microorganisms (Mavi et al., 2012).

Consequently, lower DOC inputs equate to a lower C fraction available for microbes to metabolize and respire (Wong et al., 2010), which validates the significantly smaller labile C pools and DOC observed in this study. Alternatively, the lower C pools in the saline soils could have been attributed to increases in aggregation as a result of increased salinity (Dalal and Mayer, 1986). The higher clay and Ca^{2+} observed in the saline soils (Table 12) would enhance stable aggregate formation, thus inhibiting organic C use by microbial populations (Bronick and Lal, 2005).

Decay Rate of Labile Carbon

Average decay rate of labile C (k) in both non-saline and saline soils was $0.11 \text{ mg CO}_2\text{-C soil g}^{-1} \text{ d}^{-1}$, with no significant differences in decay rates observed ($p\text{-value} > 0.60$; Figure 22). The lack of significant differences observed between the decay rates of labile C in non-saline and saline soils indicates that salt concentrations had no influence on decomposition of labile C. Consequently, it is possible that excess soluble salts in saline soils do not lower the ability of microorganisms to respire (Wichern et al., 2006; Chowdhury et al., 2011). Instead, the lower cumulative respiration observed in the saline soils may be attributed to indirect impacts of excess soluble salts on available C in the soil (Oades, 1984; Dalal and Mayer, 1986; Bronick and Lal, 2005) or reduced microbial biomass with increasing salinity (Chowdhury et al., 2011). Saviozzi et al. (2011) confirmed the results of this study and found no significant differences in the rates of decomposition in sandy loam soils up to an EC_e of 8.0 dS m^{-1} . However, rates of labile C decay reported by Saviozzi et al. (2011) were approximately half the average rate observed in this study. Chowdhury et al. (2011) observed no significant declines in cumulative respiration when the ratio of respiration to microbial biomass was studied. In effect, while microbial biomass decreased with increasing salinity, activity per unit biomass remained constant

(Chowdhury et al., 2011). The results indicate the potential adaptation of microbial populations in saline environments (Wichern et al., 2006; Chowdhury et al., 2011).

Regression Analysis

The linear regression analysis of cumulative respiration and EC_e was not significant (p-value > 0.10) and explained only 7.0% of the variation in cumulative respiration with increasing EC_e (Figure 23). The inability of EC_e to significantly predict CO_2 -C potentially indicates that within the range of salinity assessed, microbial CO_2 -C does not respond to increasing sulfate-dominated salinity. However, this interpretation contradicts the results of the ANOVA, as well as previous studies that observed significant linear declines in CO_2 -C as salinity increased. For example, Setia et al. (2011a) found declines of $0.033 \text{ mg } CO_2\text{-C g}^{-1}$ per unit increase in $EC_{1.5}$. Another study demonstrated that cumulative respiration in sandy loam soils declined by approximately $0.10 \text{ mg } CO_2\text{-C g}^{-1}$ per unit increase in EC_e , but the slope of decline varied depending on water content (Chowdhury et al., 2011). It is possible that the established salinity classifications used in the ANOVA are not applicable to microorganisms in the soil, likely because these classifications were arbitrarily assigned (Richards et al., 1954). As a result, salinity class may not be biologically relevant. Alternatively, the higher clay content observed in saline soils used in this study may have alleviated the salinity stress experienced by microorganisms (Setia et al., 2011a), consequently impacting the linear decline of CO_2 -C with increasing EC_e observed in this study.

Cumulative respiration of soils in this study was better predicted by sand content and the concentration of DOC. The multiple linear regression model was significant (p-value < 0.10) and explained 21% of the variation in CO_2 -C. Both sand content and DOC concentration were positively correlated with CO_2 -C. Sand content increased cumulative respiration by 0.60 mg

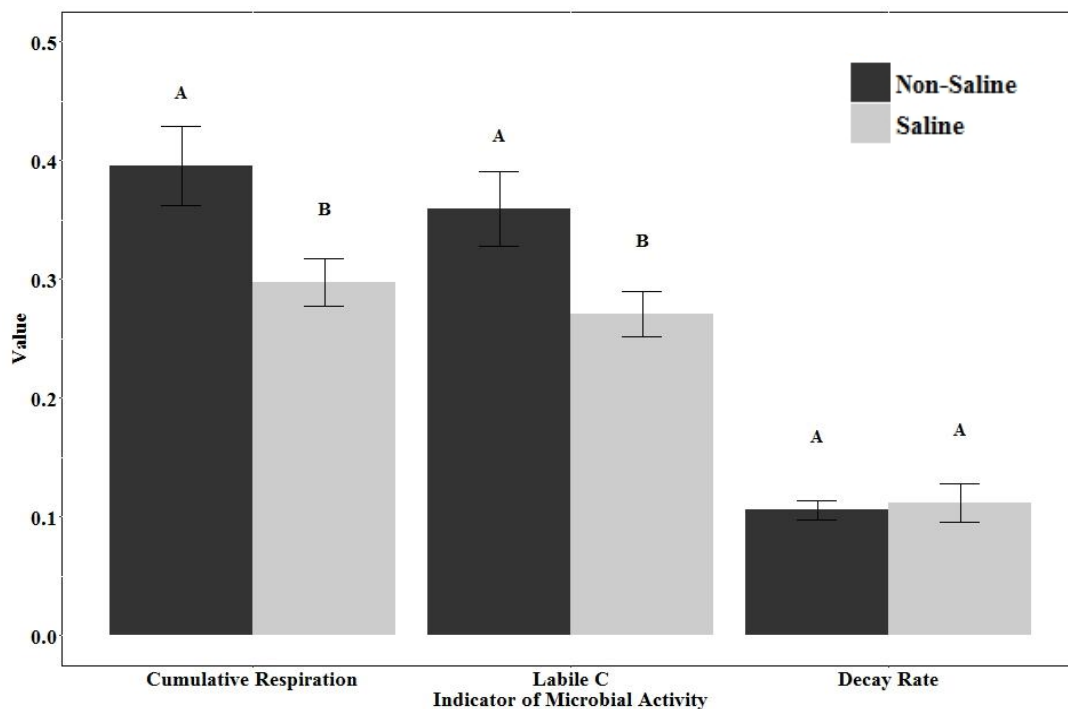


Figure 22. Cumulative microbial respiration (mg CO₂-C g⁻¹), labile C pools (mg CO₂-C g⁻¹), and decay rate of labile C (mg CO₂-C g⁻¹ d⁻¹) and their corresponding standard errors in non-saline (EC_e < 2.00 dS m⁻¹) and saline soils (EC_e ≥ 2.00 dS m⁻¹). Significant differences among variables denoted by different letters.

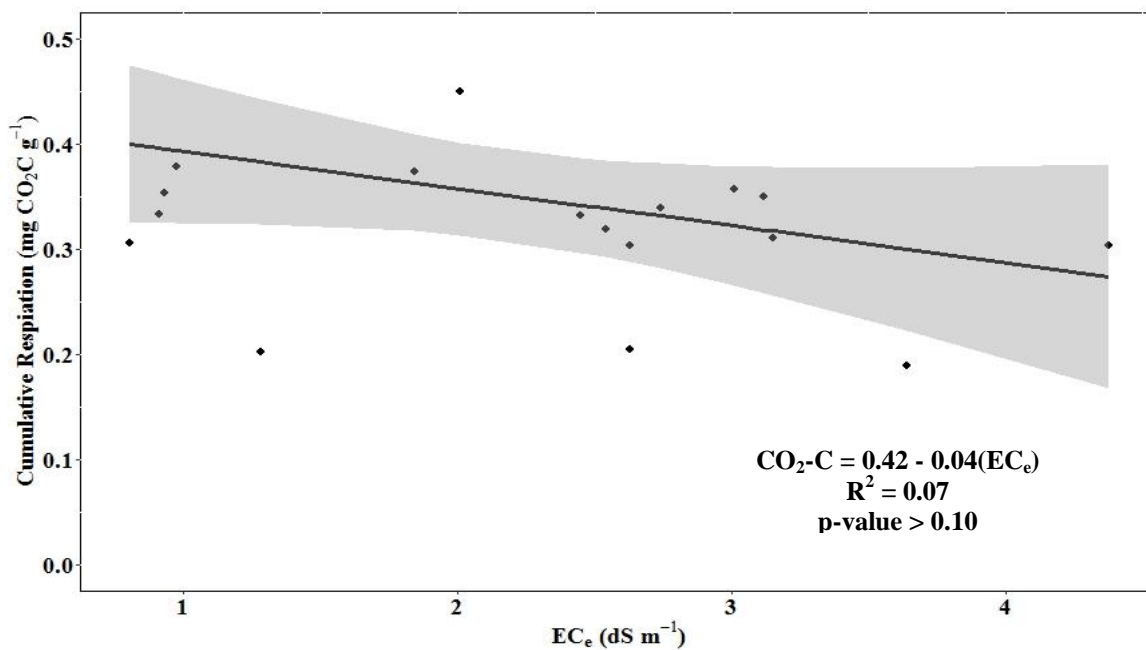


Figure 23. Cumulative CO₂-C g⁻¹ as a function of EC_e with 95% upper and lower confidence bounds. Cumulative CO₂-C flux from the soil depicted a negative relationship with increasing EC_e up to 4.38 dS m⁻¹, but the decline was not significant and explained little variation in microbial respiratory patterns.

CO₂-C per mg sand soil kg⁻¹, and DOC increased cumulative respiration by 0.47 mg CO₂-C per mg DOC L⁻¹. However, the positive correlation between sand and CO₂-C contradicts previous studies demonstrating a larger negative impact of salinity in coarser textured soils (Setia et al., 2011a). Instead, the significance of the multiple linear regression in explaining CO₂-C could indicate that C availability for microbial mineralization may be the controlling factor in saline soils of this region. Dissolved organic carbon is directly related to the labile C fraction microbes can metabolize (Mavi et al., 2012). Sand content has been linked to increased substrate availability because of the lower degree of aggregation in coarser textured soils (Bronick and Lal, 2005; Mavi et al., 2012). In effect, despite increasing salinity, respiration in these soils may be no longer be controlled by salinity. Instead, factors influencing the availability of labile C would control microbial respiration (Rath and Rousk, 2015). One previous study has significantly linked sand, particulate organic carbon, and humus-C to CO₂ emission in amended, saline soils (Setia et al., 2011b).

The influence of available C on cumulative respiration in sulfate-dominated, saline soils could be also a consequence of the community structure and composition of microorganisms in soil environments. Changes in microbial community structure and composition can occur as soluble salts in the soil accumulate (Chowdhury et al., 2011). Previous studies have demonstrated that populations of microorganisms in higher salinity environments can become acclimated to drought conditions (Schimel et al., 2007; Empadinhas and da Costa, 2008), which was also evidenced in this study by the lack of significant differences observed between the decay rate of labile C in non-saline and saline soils. In effect, salinity may no longer be the controlling factor for microbial respiration once microbes have become acclimated to this type of

environment (Rath and Rousk, 2015). Based on the results of this study, factors controlling microbial activity (respiration) in saline soils would become impacted by abiotic parameters of soil that influence the availability of C for microbial use (Mavi et al., 2012; Rath and Rousk, 2015). However, changes in community composition can be associated with changes in microbial function (Oren, 1999). Consequently, it is possible that changes in ecosystem function may occur as a result of changes in community composition from acclimation (Allison and Martiny, 2008).

Conclusions

Sulfate-dominated salinity up to 4.38 dS m^{-1} (EC_e) could not significantly explain microbial respiration from soils in the southeastern Red River Valley of North Dakota. The inability of EC_e to explain cumulative respiration in these soils is potentially attributed to differences in microbial community structure and composition, likely resulting from the acclimation of microbial populations to increasing sulfate salinization of the landscape. However, results of this study contradict previous research that has observed significant negative declines in cumulative respiration with increasing salinity (Chowdhury et al., 2011; Setia et al., 2011a; Setia et al., 2011b; Asghar et al., 2012; Elmajdoub et al., 2014). Extensive research must continue to assess microbial response to salinity because compositional and structural changes in microbial populations in response to abiotic stressors are not well understood (Rath and Rousk, 2015), and differing salt compositions across studies further complicates comparisons. Greater insight into the responses of soil organisms to fluctuating EC_e would ultimately provide a greater understanding of changes in microbial function attributed to soil salinization and, by consequence, agro-ecosystem function and land productivity.

Acknowledgements

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PAPER 3. STEPS FOR THE IMPROVEMENT OF SOIL SALINITY MEASUREMENTS DURING UNSATURATED SOIL WATER CONDITIONS

Abstract

Soil salinization is a global issue, affecting 831 million ha of arable land and resulting in approximately US \$27.3 billion in annual crop losses. Currently, little research, if any, has examined the effect of fluctuations in volumetric soil water content (θ_v) on soil salinity. Given the extent of soil salinization, it is important to better understand salinity under field conditions, as this knowledge is more pertinent to producers managing saline soils. The purpose of this research was to simulate the effect of increasing volumetric water content (θ_v) on the electrical conductivity of the soil solution (EC_{sw}) in a silty clay loam and sandy loam sulfate-dominated systems. Deionized (DI) water was added incrementally to salt mixtures to simulate an increasing degree of saturation in soil pore space. The EC_{sw} was measured after each addition of DI water. Results of this study suggested that the EC_{sw} decreased exponentially as θ_v approached pore saturation. Conversely, values of EC_{sw} up to four times greater than EC_e at values of θ_v slightly above the wilting points (θ_{WP}) of the soils. Nonlinear regression analysis indicated that the total volume of water in soil pore spaces significantly explained the ratio of EC_{sw} and mass of salt present (M_m). A paired t-test demonstrated that the EC_{sw} at field capacity (θ_{FC}) is statistically different than the EC from a saturated paste extract (EC_e) in sandy loam soils. Results of this study demonstrate the importance of incorporating θ_v in salinity measurements and provide evidence that EC_e may not be indicative of EC_{sw} at θ_{FC} in the sandy loam soil.

Introduction

Soil salinity is a substantial contributor to land degradation and, consequently, a major threat to soil health. Globally, estimates indicate that 831 million ha of land are affected by soil

salinity (Martinez-Beltran and Manzur, 2005) and that 50% of arable land will be impacted by salinity by 2050 (Wang et al., 2003). The global extent of salinization has both social and economic implications. Given the propensity of soil salinity to impede agricultural productivity, its impacts threaten both the global food supply and agricultural profits. Qadir, et al. (2014) estimated crop declines attributed to soil salinity result in profit losses of US\$ 27.3 billion annually. Accordingly, it is vital to the sustainability of agriculture to improve our current understanding of soil salinity's effect on crops under field conditions in order to properly manage and mitigate future crop productivity losses attributed to salinization.

Understanding the impact of soil salinity under field conditions is extremely complicated due to inherent variability in soil physical, chemical, and biological properties (Maas, 1993; Volkmar et al., 1997; Rengasamy, 2010). However, it is imperative to obtain a more fundamental understanding of crop responses to natural variation in soil salinity as these results are more pertinent to producers managing saline soils. Previous literature rarely addresses crop response to salinity under field conditions. Consequently, the applicability of these results for producers managing saline soils is limited.

A major limitation to assessing crop response to salinity under field conditions is the lack of knowledge on how soil salinity fluctuates with varying volumetric water content (θ_v) of the soil. Commonly, soil salinity is measured by the electrical conductivity (EC_e) of a saturated paste extract (Richards, 1954). While this method facilitates cross-comparison of multiple samples, it does not take into consideration the salinity at unsaturated water contents in the field (Bernstein, 1975; Rengasamy, 2010). Because θ_v is not incorporated into EC_e , the values of salinity produced from a saturated paste extract are not necessarily indicative of salinity of soil water contents under field conditions (Rengasamy, 2010). In effect, the salinity within soil pores

(EC_{sw}) that crops experience is some fraction or multiple of the EC_e value depending on the water content of the soil (Richards, 1954; Bernstein, 1975). Consequently, the purpose of this study was to examine how EC_{sw} changes as water content fluctuates by varying the volume of water in the pore spaces that would normally be present under field conditions in silty clay loam and sandy loam soils under a laboratory setting.

Materials and Methods

Soil Sampling

The research sites consisted of three silty clay loam fields and two sandy loam fields located in Richland County, ND. Silty clay loam fields were mapped as an Antler-Mustinka complex (Fine-loamy, mixed, superactive frigid Aeric to Fine, smectitic, frigid Typic Argiaquolls; USDA-NRCS, 1997; USDA-NRCS, 1999a; USDA-NRCS Web Soil Survey, 2015). Sandy loam fields were mapped as a Wyndmere loam (Coarse-loamy, mixed, superactive, frigid Aeric Calciaquoll; USDA-NRCS, 1999b; USDA-NRCS Web Soil Survey, 2015). A Geonics EM 38 meter (MK2, Geonics Ltd., Canada) was used to continuously map salinity gradients to a 1.2-m depth on each field. Data from the EM 38 was converted to apparent electrical conductivity (EC_a) based on previous data collected and interpreted by the Natural Resources Conservation Service (NRCS; K. Anderson, personal communication, 2014). Point data of EC_a was interpolated in ArcMap (10.2, Esri, Redlands, CA) using inverse distance weighted (IDW) interpolation to produce a continuous gradient map of soil salinity on each field. Three low, medium, and high salinity composite soil samples were taken from each field at the 15-30 cm depth based on interpolated EC_a maps.

Soil Chemical Analysis

Saturated paste extracts were performed on each sample to determine EC_e according to methods by Richards (1954). Extracts of the saturated pastes were analysed for cations using atomic adsorption spectrometry based on procedures by Wright and Stuczynski (1996). Only calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were measured. After determining EC_e and conducting cation analysis, water from extracts was evaporated from the solutions in pre-weighed 250-mL beakers at room temperature to produce precipitated salt mixtures. Following evaporation, beakers containing the salts were weighed to determine the mass of salt present in each sample (M_m ; total dissolved solids). Deionized (DI) water was then added in increments to salts based on the volumes of water estimated to be in soil pore space at varying θ_v , as calculated in the following section.

Volume of Water in Soil during Unsaturated Conditions

Bulk density (ρ_B) was measured at sample locations on each field at the 15-30 cm depth using a cylinder of known volume (Eq. 9)

$$\rho_B = \frac{\text{Mass Oven Dried Soil}}{\text{Volume of Cylinder}} \quad (9)$$

Particle density (ρ_s) was measured on each composite sample based on methods by Flint and Flint (2002). The ρ_B and ρ_s were measured three times, and the average values for each sample were used for all subsequent calculations. Measurements of ρ_B and ρ_s were used to calculate the volumetric water content at saturation (θ_s), such that (Hillel, 1998; Eq. 10)

$$\theta_s = 1 - \frac{\rho_B}{\rho_s} \quad (10)$$

Estimation of the volume of water corresponding to the calculated θ_v was based on a 200-g mass of soil which was the same mass of soil used for a saturated paste extract. The volume of water (V_s) required to saturate 200 g (M_s) of soil was calculated as follows (Eq. 11):

$$V_S = \frac{M_S}{\rho_B} * \theta_S \quad (11)$$

Texture of each sample was measured using the hydrometer method (Gee and Or, 2002). Final measurements for sand, silt, clay, ρ_B , and EC_e were used in the Soil-Plant-Air-Water (SPAW) Field Soil Water Characteristics program (6.02.74, USDA Agricultural Research, Washington) to verify calculated values of θ_s for each sample (Eq. 2). Additionally, SPAW was used to generate soil water retention curves to estimate the wilting point (θ_{WP}) and field capacity (θ_{FC}) of each sample.

Calculation of Water Content in the Soil Pores during Unsaturated Conditions

The volume of water in the soil pores at saturation (V_{ps}) was calculated as the difference between V_S (Eq. 11) and the volume of water in the soil at the wilting point (V_{WP}). The V_{WP} was calculated in a similar fashion as V_s (Eq. 12):

$$V_{WP} = \frac{M_s}{\rho_B} * \theta_{WP} \quad (12)$$

where θ_{WP} is the volumetric water content at the wilting point of the soil generated by SPAW.

The volume of water in the pore spaces at saturation (V_{ps} ; Eq. 13) was

$$V_{ps} = V_S - V_{WP} \quad (13)$$

Fractions of V_{ps} were used to vary the water content in the soil solution to simulate unsaturated conditions (Eq. 14):

$$V_p = (\%) * (V_{ps}) \quad (14)$$

The fractions used were 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (or saturation of the soil pore space). Fractions of water were added incrementally to salts created after extract solutions were evaporated at room temperature (25 °C). Solutions with added DI water were equilibrated for 24 h before adding the next increment of water. Following each addition of water, EC_{sw} was measured using a conductivity/TDS meter (Con 11 Series, Oakton, Illinois).

After saturation was attained, the extracts were evaporated and additions of water were repeated. The evaporation/wetting process was repeated three times and averages of the measured values of EC_{sw} were used for statistical analysis. The amount of water in the soil pores was related back to the θ_v of the soil based on the following equation (Eq. 15):

$$\theta_v = \frac{V_{WP} + V_p}{V_s} * \theta_s \quad (15)$$

Wilting point, and not residual water content, was used as the lower boundary for plant available water because the purpose of this study was to determine how fluctuations in water content alter solution salinity with respect to plants. Consequently, at values of θ_v below the wilting point, the EC_{sw} of water remaining in the soil solution is irrelevant to the plant because it is not extractable (Hillel, 1998).

Analysis of Results

Linear and non-linear regression analyses were used to determine the significance of observed patterns in EC_{sw} and θ_v . In order to compare across varying salt content, EC_{sw} was normalized across all samples by dividing by the mass of salt in each sample (M_m). For comparisons across texture, θ_v was normalized by converting θ_v to the cumulative amount of water present at the time of EC_{sw} measurement (V_C). Paired t-tests were used to determine if significant differences exist between the EC_{sw} at field capacity (θ_{FC}) and EC_e in silty clay loam and sandy loam soils. Results were considered significant at p-values < 0.05. All statistical analyses were performed using R Studio software (3.2.1, R Foundation for Statistical Computing, Vienna).

Results and Discussion

Soil Chemical and Physical Properties

Electrical conductivity of composite samples ranged between 0.49 and 1.40 dS m⁻¹ for low salinity samples, 1.03 and 3.47 dS m⁻¹ for medium salinity samples, and 3.09 and 5.10 dS m⁻¹ for high salinity samples (Table 13). In general, EC_e was higher for all levels of salinity in sandy loam soils. Calcium was the dominant cation in each sample regardless of texture, followed by Mg²⁺ then Na⁺. Calculated θ_v at saturation (θ_s) was usually higher in silty clay loam soils than sandy loam soils. Accordingly, θ_v at field capacity (θ_{FC}) and wilting point (θ_{WP}) were also higher in silty clay loam soils (Table 14).

Water Content and EC_{sw}

Regardless of salinity level, samples of both soil textures demonstrated significant exponential declines in EC_{sw} as θ_v increased (Figure 24). Values of EC_{sw} were higher across all

Table 13. Descriptive statistics of soil chemical and physical properties of composite soil cores from the 15-30 cm depth at low, medium, and high levels of salinity in silty clay loam and sandy loam soils.

Property	Unit	Silty Clay Loam				Sandy Loam			
		Min	Max	Mean	Std.Dev.	Min	Max	Mean	Std.Dev.
EC _e [†]	dS m ⁻¹	0.49	4.61	2.38	1.49	0.59	5.10	2.47	1.75
Ca ^{2+‡}	mg L ⁻¹	76	454	329	153	80	488	309	189
Mg ^{2+‡}	mg L ⁻¹	37	547	245	187	23	400	208	164
Na ^{+‡}	mg L ⁻¹	4	335	119	125	10	173	98	75
M _m [§]	g	0.01	0.20	0.10	0.06	0.02	0.16	0.08	0.06
Sand [¶]	g kg ⁻¹	264	390	346	39	496	786	683	122
Silt [¶]	g kg ⁻¹	286	420	359	54	82	263	159	73
Clay [¶]	g kg ⁻¹	222	383	296	63	102	243	159	52
ρ _B [#]	g cm ⁻³	1.06	1.33	1.18	0.07	1.14	1.51	1.40	0.15
ρ _s ^{††}	g cm ⁻³	2.05	2.30	2.15	0.09	2.01	2.40	2.27	0.14

[†] Electrical conductivity from a saturated paste extract (Richards, 1954) of three composite cores taken from the 15-30 depth on each field. Statistics performed on all levels of salinity.

[‡] Cation concentrations determined on extract from saturated paste using atomic adsorption (Wright and Stuczynski, 1996).

[§] Mass of salt in evaporated extracts.

[¶] Particle size distribution determined by hydrometer method (Gee and Or, 2002).

[#] Bulk density on field samples using an Al ring to determine volume. Samples were oven dried at 105 °C for 48 hr to determine mass.

^{††} Particle density measured based on methods by Blake and Hartge (1986).

Table 14. Volumetric water content in the silty clay loam and sandy loam soils at saturation, field capacity, and wilting point based on modeled data and corresponding volumes of water associated with water contents as calculated by Eq. 4.

Sample	Texture [†]	Salt Level [‡]	V_e [§] cm ³	θ_s [¶] cm ³ water cm ⁻³ total	V_s [#] cm ³	θ_{FC} [¶] cm ³ water cm ⁻³ total	V_{FC} [#] cm ³	θ_{WP} [¶] cm ³ water cm ⁻³ total	V_{WP} [#] cm ³
1	SCL	Low	104.0	0.473	85.6	0.364	62.6	0.229	41.4
2	SCL	Med	103.5	0.511	88.7	0.365	63.3	0.230	45.1
3	SCL	High	103.0	0.454	78.2	0.363	60.2	0.236	40.6
4	SCL	Low	96.5	0.488	91.1	0.300	53.6	0.208	38.8
5	SCL	Med	93.0	0.481	90.3	0.294	50.8	0.236	44.3
6	SCL	High	101.0	0.492	92.0	0.296	55.4	0.148	27.7
7	SCL	Low	93.0	0.440	77.9	0.309	51.1	0.159	24.7
8	SCL	Med	88.0	0.437	67.6	0.310	46.7	0.169	26.1
9	SCL	High	90.0	0.494	89.6	0.324	52.3	0.169	30.7
10	SL	Low	77.0	0.354	47.0	0.195	25.9	0.123	16.3
11	SL	Med	85.0	0.373	54.5	0.225	32.9	0.138	20.2
12	SL	High	86.0	0.341	51.5	0.258	38.9	0.166	25.0
13	SL	Low	68.0	0.357	47.5	0.137	18.2	0.087	11.6
14	SL	Med	72.0	0.386	53.8	0.137	19.1	0.082	11.4
15	SL	High	68.0	0.370	49.0	0.161	21.3	0.114	15.1

† Texture classification according to NRCS-USDA Official Series Descriptions. SCL is silty clay loam and SL is sandy loam.

‡ Salt level corresponds to relative electrical conductivity of saturated paste extract (EC_e).

§ Volume of water added to saturated paste to saturate approximately 200 g of 2-mm sieved soil.

¶ Volumetric water content at saturation (θ_s), field capacity (θ_{FC}), and wilting point (θ_{WP}) estimated by SPAW (6.02.74, USDA Agricultural Research, Washington).

Volume of water in soil pore space calculated by Eq. 4 for volumetric water contents at saturation, field capacity, and wilting point.

values of θ_v and among all salinity levels in the sandy loam soil extracts. The increase in EC_{sw} with decreasing θ_v is intuitive given that a lower volume of water will concentrate salts in solution (Bernstein, 1975; Setia et al., 2011). Normalization of individual samples for cross comparison generated a similar pattern of exponential decline (Figure 25). Nonlinear regression analysis indicated that the cumulative volume of water significantly (p -value < 0.00001) explained 91% of the variation in the ratio of $EC_{sw}:M_m$. Variation in $EC_{sw}:M_m$ is potentially explained by the differences in conductivity attributed to different ions (Tölgyessy et al., 1993). For example, Mg^{2+} ions contribute $2.60 \mu S \text{ cm}^{-1}$ per mg L^{-1} in solution, whereas Na^+ contributes 2.13 cm^{-1} per mg L^{-1} in solution (Tölgyessy et al., 1993). Complete chemical analysis on

individual extracts was not performed, and consequently, deviations from the best fit line may be indicative of varying concentrations of ions in solution.

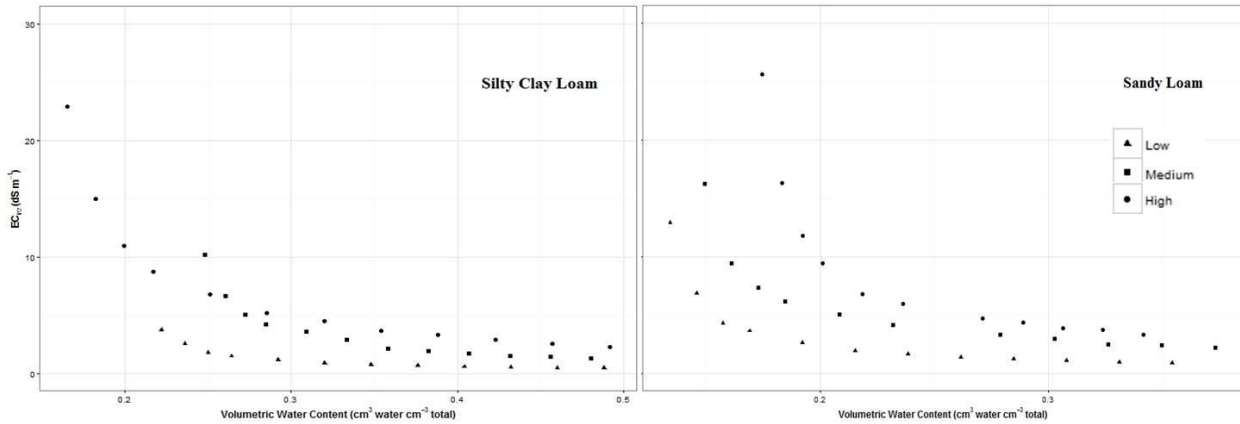


Figure 24. Fluctuations in electrical conductivity of the soil solution (EC_{sw}) as volumetric water content (θ_v) changes in silty clay loam (left) and sandy loam (right) soils. In silty clay loam soils, low, medium, and high levels of salinity are equivalent to electrical conductivities from saturated paste extracts (EC_e) of 0.49, 2.42, and 3.09 $dS\ m^{-1}$, respectively. In sandy loam soils, these levels are equivalent to 1.40, 3.22, and 3.47 $dS\ m^{-1}$, respectively.

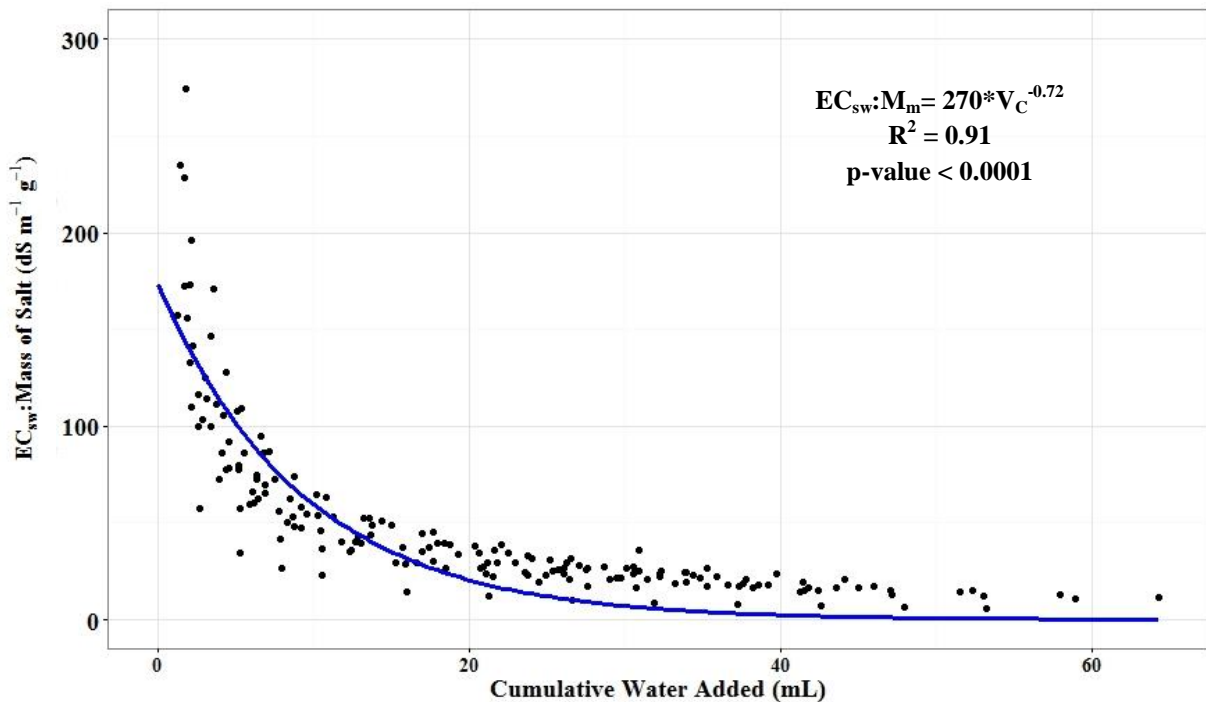


Figure 25. Nonlinear regression analysis of the ratio of EC_{sw} and mass of salt (M_m) as a function of the cumulative amount of water added (V_C). Samples were standardized to account for varying masses of salt in each extract. Samples of different textures were combined by assessing the $EC_{sw}:M_m$ ratio against the total volume of water added

Comparison of EC_{sw} and EC_e

Paired t-tests between EC_{sw} at θ_{FC} and EC_e of silty clay loam and sandy loam soils indicated that only EC_{sw} and EC_e were significantly different (p -value < 0.001) in the sandy loam soil. On average, EC_{sw} at θ_{FC} in the sandy loam was two times higher than values of EC_e (Figure 26). Significant differences between EC_{sw} and EC_e were validated by previous studies reporting that EC_e is approximately half EC_{sw} at θ_{FC} (Richards, 1954; Ayers and Westcott, 1985; Maas, 1993).

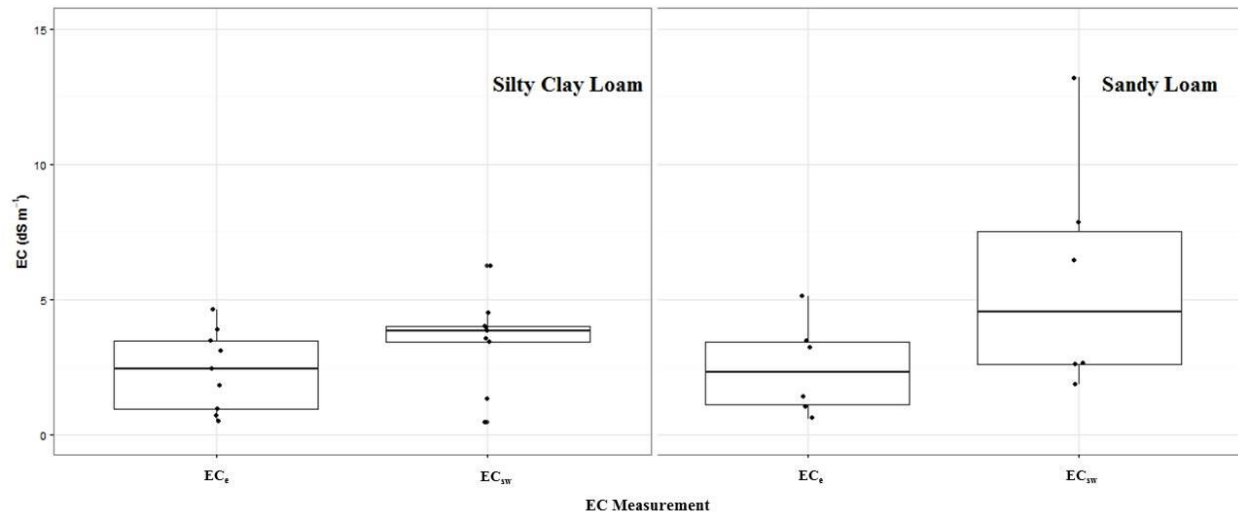


Figure 26. Boxplots of electrical conductivity measured by saturated paste extract (EC_e) and at field capacity in soil pore water (EC_{sw}) in the silty clay loam and sandy loam soils. Points represent individual sample readings.

The presence of gypsum ($CaSO_4 \cdot 2H_2O$) could account for the discrepancies in EC_{sw} and EC_e (USDA-NRCS, 1999a). Typically, $CaSO_4 \cdot H_2O$ dissolution is assumed to occur during the saturated paste process because of the addition of excess water to saturate the ground sample (Bernstein, 1975). As a result, $CaSO_4 \cdot H_2O$ dissolution can contribute an additional 2.2 dS m^{-1} to the total conductivity of the solution (Maas, 1993). Under field soil-water conditions, $CaSO_4 \cdot H_2O$ is considered a sparingly soluble salt (Bernstein, 1975) and thus the dilution effect produced in saturated paste extracts (Richards, 1954; Maas, 1993) may have increased dissolved

CaSO₄·2H₂O in extract solutions, increasing EC_e in the silty clay loam soil to values more comparable to EC_{sw} at θ_{FC}.

A potential explanation for the significant differences in EC_e and EC_{sw} in the sandy loam soil is ρ_B. In the sandy loam soil, residuals of the paired-t-test were significantly (p-value < 0.05) correlated to ρ_B (r = 0.90). The positive correlation with ρ_B indicates that as ρ_B increases, the deviation between EC_e and EC_{sw} increases. Under field conditions, increases in bulk density equate to decreased total pore space and thus less water is needed to saturate that particular volume of soil. When soils are ground for saturated paste procedures, the ρ_B of the field sample becomes irrelevant. However, because particles are substantially more exposed in the ground and sieved sample of the saturated paste, more water would be needed to saturate the sample (Richards, 1954). In effect, the increase in water added to saturate sandy loam samples diluted the extract of the saturated paste because, under field conditions, less water would be needed to saturate the same mass of soil. As a result, the values of EC_e measured from a saturated paste extract would be lower than EC_{sw} measured at θ_{FC} (Figure 26). The dilution effect would be enhanced in coarser textured soils given that these soils generally have higher ρ_B (Table 13) and lower particle specific surface areas (Banin and Amiel, 1970). Both of these physical properties decrease the amount of water required for saturation (as indicated by the lower average θ_s measured in sandy loam soils of this study; Table 13). The dilution effect from saturated paste procedures would be reduced in finer textured soils because of their generally lower ρ_B values (Table 11) and higher particle specific surface area (Banin and Amiel, 1970). In effect, more water would be needed to saturate finer textured soils than the same mass of coarse textured soil. The dilution effect is also supported by the differences between the calculated volume of water in soil pore spaces at saturation for 200 g of soil (V_S) and the amount of water added to the

saturated paste extract to saturate the ground sample (V_e). On average, the difference in V_e and V_s was 12.2 mL in silty clay loam soils, whereas the difference doubled in sandy loam soils (Table 14). Consequently, while EC_e would still be slightly lower from dilution regardless of texture, the increase in water necessary to saturate finer textured soils would reduce the degree of difference between measured EC_e and EC_{sw} values.

While the results of this study do provide an indication of how salinity changes as water content fluctuates, it is important to acknowledge the limitations of the methods used. Similar to a saturated paste extract, the methods used to simulate unsaturated conditions assume that salinity in soil pore spaces is additive and that water and salts are evenly distributed throughout the pore spaces. These assumptions are not true for field soils. However, results of this study can still provide an indication of the interaction between salinity and water content. For example, exponential decline in EC_{sw} is highly plausible, as most studies typically assign an inverse relationship to soil water and salinity (Richards, 1954; Bernstein, 1975; Setia et al., 2011). However, it is likely that the range of salinity values observed as θ_v declines (Figure 24) is substantially higher than what would be observed in a particular pore under field conditions. The reasoning for this being that all the salts in 200 g of soil would not be concentrated in one pore, but rather unevenly distributed throughout multiple pores. Consequently, on an individual pore basis, salinity of EC_{sw} may still be exponential higher as θ_v declines, but maximum EC_{sw} in the pore would not be four times EC_e because all the salt present in 200 g for a saturated paste is likely not localized within one individual pore.

Conclusions

The methods employed in this research require measurement of ρ_B , ρ_S , M_m , θ_S , θ_{WP} , and the corresponding volumes of water associated with these water contents to estimate the salinity

during unsaturated soil water conditions. As salinity becomes increasingly more widespread, it is important to better understand spatial and temporal variation in soil salinity under field conditions. This is especially true for regions with arid and semi-arid climates. Soils under dryland conditions may not necessarily remain at field capacity throughout the growing season, which reduces the relevance of saturated paste extracts (Rengasamy, 2010) and may complicate responses of field crops to soil salinity in these regions.

Observations from this study indicated the importance of incorporating water content into salinity measurements, especially under field conditions when θ_v is highly variable. Most importantly, results from this study illustrate the intricate relationship between salinity management and water management. Even in low salinity samples, EC_{sw} reached values four times those measured by a saturated paste at θ_{WP} . Ultimately, management strategies that foster improved soil water dynamics will reduce the negative impacts of salinization.

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GENERAL CONCLUSIONS

Soil salinity is an increasingly problematic threat to soil health and land productivity. In order to encourage preventative and reclamation management of saline soils, it is necessary to study the effects of soil salinity on biological activity under field conditions. In the field component of this research, corn responded to sulfate salinity at higher thresholds than previously observed, whereas soybean responded at a lower threshold salinity. In the laboratory component of this research, microbial activity did not significantly respond to increasing EC_e up to 4.38 dS m^{-1} . Results from this study are contrary to most previously reported research assessing crop and microbial response to soil salinity. As a result, the findings from this study demonstrate the importance of assessing biological response under field conditions. Furthermore, results demonstrate that the chemical composition of salinity appears to play a pivotal role in biological response.

Despite the high variability among the results of this study, these results can still be used by producers to improve management of saline soils. Insight into the importance of texture in determining both crop and microbial response to increasing salinization is critical for understanding potential remediation strategies for salt-affected soils. Future research should address the spatial variability of salinity under field conditions, as this may provide an understanding of the high variation in crop yields and microbial respiration observed in this study.