THE EFFECT OF SALINITY ON SOIL MICROBIAL COMMUNITY STRUCTURE

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Soil salinity is a widespread problem that affects crop productivity. We expect that saline soils also have altered microbial community structure, soil food webs and related soil properties. To test this, we sampled field soils across four farms in eastern North Dakota that host salinity gradients. We evaluated microbial biomass carbon, phospholipid fatty acid analysis and nematode counts in moderately saline and low saline soils. Additionally, we measured soil properties that represent potential food sources and habitat characteristics that influence microbial communities. We found higher microbial group abundance in moderately saline soils than in the lower saline soils. In contrast, we found lower nematode abundances in the moderately saline soils. We also observed increased labile carbon, nitrogen, phosphorus, and water content in the moderately saline soils. Based on our results, saline soils appear to have unique soil biological characteristics, which have implications for overall soil function along salinity gradients.

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DEDICATION

To Brooke, may we always find the best of friends when we are not looking.

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INTRODUCTION

Soil salinity is the accumulation of an excessive amount of soluble salt ions, such as sodium, calcium, magnesium, carbonates, or sulfates (Keller et al., 1986). The most predominant salts in eastern North Dakota that contribute to salt-affected soils are sodium sulfate (Na_2SO_4) and magnesium sulfate ($MgSO_4$; Keller et al., 1986). Higher salt accumulation occurs in areas with shallow groundwater tables; a persistent wet cycle beginning in 1993 may have exacerbated the severity of salinity we currently see in eastern North Dakota (Franzen and Richardson, 2000; Rengasamy, 2006).

Soil salinity can reduce crop productivity as salt concentrations within the soil profile increase. Decreases in crop productivity can lead to salinity-induced yield losses, resulting in a yearly economic impact of over US $27 billion in crop losses (Qadir et al., 2014; Zörb et al., 2018). Crop growth and functionality are negatively impacted in salt-affected soils due to osmotic stress and specific ion toxicity (Munns and Tester, 2008). Osmotic stress is when high salt concentrations in the root zone limit water uptake, while specific ion toxicity is due to the excessive accumulation of salt ions in plant tissues which ultimately disrupts cell metabolism (Munns and Tester, 2008). Due to these disruptions in the physiology of crop plants, yield losses due to soil salinity can occur, although the growth-limiting effects are hard to quantify (Maas and Grattan, 1999).

Communities of soil microorganisms, including bacteria, fungi, and nematodes, help in maintaining soil quality in the ecosystem by regulating functions such as nutrient cycling, carbon storage, and decomposition (Bongers and Ferris, 1999; Egamberdieva et al., 2010). Microbes are sensitive indicators of change for soil quality in response to environmental stresses, such as salinity (Egamberdieva et al., 2010; Rietz and Haynes, 2003; Wong et al., 2008). Because of the

importance of soil microbes and nematodes in mediating soil function and their ability to reflect soil quality, these groups may be of research interest for indicating soil function and health in relation to salinity (Moura and Franzener, 2017).

Salinity not only impacts aboveground biomass but has variable effects on belowground organisms, depending on the concentration of the salts. Previous studies have shown that soils with high concentrations of chloride-based salts can be damaging to microorganisms by disrupting soil ecosystem processes they facilitate. Reitz and Haynes (2003) reported that salinity had adverse effects on the size and growth of microbial biomass, likely due to decreased rates of soil organic matter decomposition and nutrient mineralization as salinity increased. The relationship was best described as exponential between microbial biomass and electrical conductivity and was estimated that the salts leveled off the biomass at higher concentrations (EC_e = 17.5-24.8 dS/m).

Other research has shown similar results, with Yuan et al. (2007) having reported that microbial biomass carbon was strongly and negatively correlated with high levels of soil salinity. The analysis also revealed salinity to have a significant non-linear relationship, but microbial biomass carbon appeared to be adversely affected at approximately $EC_{1:5}$ = 20 dS/m. An earlier study concluded the same when it was observed that high salinity (EC_e = 24 dS/m) completely inhibited the growth of microbial biomass (Wichern et al., 2006). The results of these studies suggest that soils in salt-affected landscapes support less microbial biomass than non-saline soils which further perpetuates soil organic carbon losses (Wong et al., 2010).

Indicators of soil biological function and health often include microbial respiration and nitrogen mineralization (Knoepp et al., 2000). Microbial respiration is an important ecosystem process because it is coupled to decomposition, nutrient mineralization, and the global carbon

cycle, and is often used as a measurement for microbial activity. Respiration rates, accompanied by decreases in soil microbial biomass, were shown to decrease in moderately saline soils (EC_e = 8.6 dS/m; $EC_{1:5}$ = 10 dS/m), suggesting that salinity has significant negative effects on microbial activity (Muhammad et al., 2006; Wong et al., 2008). Nitrogen mineralization is another microbial-mediated process that is used as an indicator of soil biological activity (Knoepp et al., 2000). A number of investigations that have examined the effects of salinity on nitrogen mineralization have reported negative effects (Laura, 1974; Pathak and Rao, 1998; Singh et al., 1969; Yuan et al., 2007). Laura (1974) found that nitrification, the microbially-mediated conversion of ammonium to nitrate, was completely inhibited in the highly saline treatments (EC_e = 30.0-44.5 dS/m). Similarly, Pathak and Rao (1998) found high salinity (EC_e = 26 dS/m) to inhibit nitrification rates due to the decrease in microbial activity. Since microbial activity is typically more concentrated in the topsoil, salts near the soil surface may directly interfere with soil microbial processes that help control ecosystem function (Yuan et al., 2007).

Not only does soil salinity directly reduce microbial activity, it affects other microbial-related processes. Soil fertility represents nutrient availability and cycling. Microbial activity is vital for the maintenance of soil fertility, as these organisms play a key role in the decomposition of organic matter and various nutrient cycles (Valpassos et al., 2001). One study (Egamberdieva et al., 2010) found that irrigation-induced moderate salinity ($EC_{1:5}$ = 5.6-7.1 dS/m) was associated with higher concentrations of exchangeable ions, calcium, sodium, carbonate, and chlorine, and a decrease in carbon and nitrogen availability.

In addition to integrative measurements of microbial activity and abundance, measurements of specific biochemical processes may also indicate soil organism response to salinity. Soil enzymes catalyze essential biochemical processes that help regulate plant and

microbial metabolism and nutrient availability in soil (Singh, 2015). Research suggests that moderate to high salinity levels (EC_e = 28 dS/m; $EC_e \approx$ 12.5-24.8 dS/m) decreased soil enzymatic activity (dehydrogenase; beta-glucosidase, acid phosphatase, alkaline phosphatase and arylsulphatase), which regulate various nutrient cycles and soil organic matter pools (Batra and Manna, 1997; Rietz and Haynes, 2003; Wong et al., 2010). Decreased enzyme activity likely occurred because a smaller and less active microbial biomass was producing less enzymes, which resulted in a reduction in the cycling of nutrients through soil organic matter pools (Rietz and Haynes, 2003).

Soil microbial activity and biomass (especially bacteria and fungi) are commonly measured to assess soil communities and functions. However, soil organisms at higher trophic levels, such as nematodes, can provide unique insight into community dynamics (Briar et al., 2007). Additionally, nematodes are important for soil health because they help facilitate many ecological processes such as nutrient cycling and the decomposition of organic matter (Bongers and Ferris, 1999). Nematodes are thought to be excellent biological indicators of soil conditions because of their central position in the soil food web (Bongers and Bongers, 1998; Neher, 2001). Given their ecological significance and ability to be indicators of environmental conditions, they would be effective for assessing saline soil health, but there are not any research studies available that quantify that relationship within the soil environment.

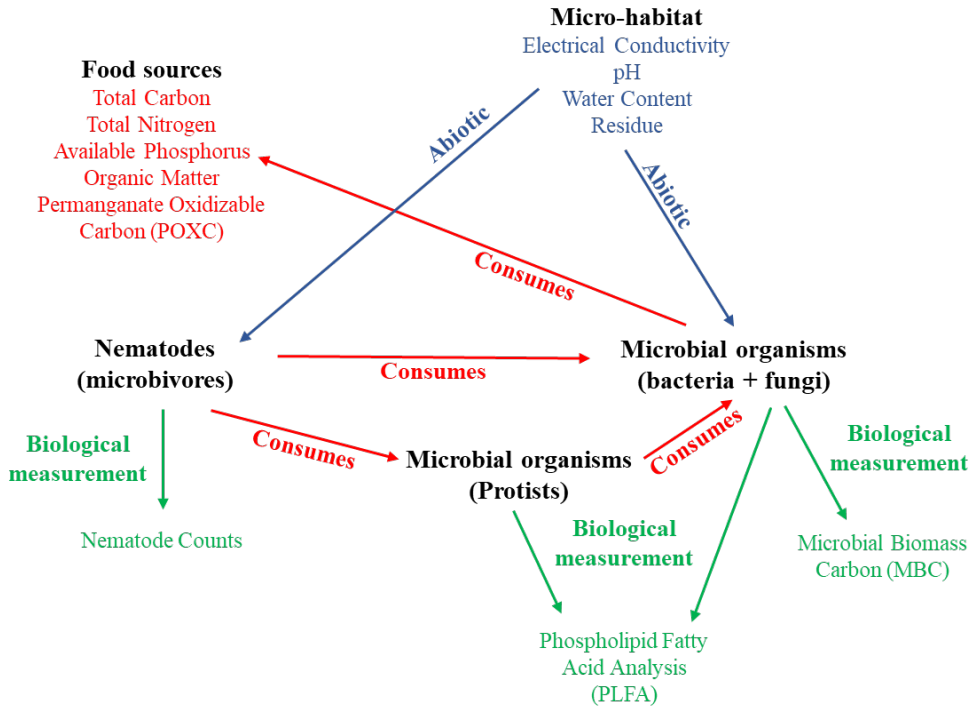
While there is evidence that salinity affects microbial communities and functions, the sulfate salts unique to eastern North Dakota may affect microbial communities differently than other types of salinity (such as chloride-based salts). There is a lack of research that examines the relationship between this type of regional salinity and the soil biological community. It is critical to understand these interactions, as it may help us better manage these problem soils and make

further management recommendations. This is especially of interest in soils that have moderate salinity ($EC_{1:1}$ > 2 dS/m and < 4 dS/m) that prohibit plant growth but may be candidate areas for remediation and management. While the effects of salinity on microbial organisms and their ecosystem roles are widely studied (Batra and Manna, 1997; Pathak and Rao, 1998; Reitz and Haynes, 2003; Wong et al., 2008), less research has focused on which microbial groups are specifically affected by the salts. Researching which microbial groups are directly affected by the salts will help us understand microbial community shifts in soils under salinity stress and how they might influence soil health and management success aimed at increasing plant productivity.

We designed a field survey study across eastern North Dakota to examine the relationship between soil salinity and soil biological properties, by measuring auxiliary soil properties (electrical conductivity, pH, water content, total carbon and nitrogen, available phosphorus, organic matter content, permanganate oxidizable carbon (POXC), and residue) and soil microbial communities (microbial biomass carbon, microbial group abundance, and nematode counts). The objective of this field study is to determine if field soils with moderate salt concentrations have different microbial community structure and nematode abundances compared to soils with lower salt concentrations. We also hope this study initiates further research and closes research gaps between the effects of regional sulfate salinity on the biological community, as well as the specific microbial groups directly affected by this type of salinity.

The soil ecosystem is a complex environment, and biological interactions within the soil rely on both abiotic and biotic components. Figure 1 illustrates how salinity may impact these interacting properties and their respective measurements.

(a)



(b)

Soil Property	Non-Saline ($EC_{1:1}$ = < 1 dS/m)	Saline ($EC_{1:1}$ = 2-4 dS/m)	Hypothesis
Electrical Conductivity (EC)	Lower	Higher	We would expect EC to be higher in the saline soils because this validates our field design
Total Carbon	Higher	Lower	We expect total carbon and nitrogen to be higher in the non-saline soils and associated with microbial measures and nematode counts
Total Nitrogen			
Microbial Biomass Carbon (MBC)	Higher	Lower	We expect higher microbial measures and nematode counts in the non-saline soils because of lower salinity and increased nutrients
Microbial Group Abundance (PLFA)			
Nematode Counts			

Figure 1. Conceptual representation of (a) the relationships between our measured auxiliary properties and biological properties and (b) associated expectations of how our measured soil properties will change between our salinity treatments.

Microbial organisms (bacteria + fungi) acquire nutrients (consumption dynamics are represented by red arrows and text, Figure 1a) from the soil, which are analytically represented by total carbon, total nitrogen, available phosphorus, and organic matter measurements. These

organisms use these food sources for their own growth and metabolism as well as for energy to continue to decompose organic residues and cycle carbon, nitrogen, and phosphorus. POXC is measured to provide us with an estimate of the amount of active organic carbon, which is also a readily-available food source for microbes. We expect carbon and nutrient content to be higher in the non-saline soils than the saline soils because of less salts inhibiting primary productivity and processes like decomposition of organic matter (Figure 1b).

Trophic interactions occur based on nutrient requirements, such as protists feeding on bacteria or nematodes feeding on microbial organisms (bacteria + fungi + protists) to acquire the necessary nutrients for sustaining growth and metabolic activities. Soil organisms release nitrogen and phosphorus in excess of their metabolic need, primarily in plant available forms such as ammonium and phosphate. The transferring of nutrients and energy across trophic levels and through the soil food web is the main driver of soil nutrient cycling.

Abiotic micro-habitat characteristics (indicated by blue arrows and text, Figure 1a) have direct effects on microbial organisms (bacteria + fungi) and nematodes. Electrical conductivity (EC) likely is inhibiting the growth and activity of soil biota by direct toxicity from the sulfate salt ions in the saline soils. We expect soil water to be higher in saline soils due to the topographic controls of water accumulation on the landscape, which coincides with salt accumulation (Figure 1b). Soil water is required for soil microbial and nematode growths, and allows nutrient solubilization and movement in soils. Nematodes live in the soil water layer, and use water for movement within pore space. Soil pH can inhibit growth and activity of microbes, such as bacteria and fungi, and each group of microbial organisms has its own optimum pH. Soil pH also controls behavior and availability of soil nutrients and solutes.

Residue is comprised of decomposed plant materials and was on the soil surface due to no-tillage management throughout the project. Residue on the soil surface is considered a habitat characteristic because it provides protection for soil biota by buffering the soil surface from evaporation and temperature fluxes, retaining soil water, and preventing soil erosion. Residue is chemically broken down by microbes through decomposition, wherein microbes produce energy and recycle essential nutrients.

Our biological measurements (represented by the green arrows and text, Figure 1a) were the focus of this study and were used to evaluate microbial community structure across salinity gradients. Microbial biomass carbon is the fraction of organic carbon that is within the living biomass, and is mostly comprised of bacteria and fungi. Microbial organisms (bacteria + fungi + protists) are the organisms that make up the five groups quantified by phospholipid fatty acid analysis (PLFA). The PLFA method identifies specific fractions of the microbial community that allows us to examine the microbial community composition, and compare them between our salinity treatments. Our final biological measurement are our nematode counts, which comes solely from the soil nematodes population. These counts are important to our research because they provide us with an idea of how many nematodes are present in our samples and how they differ between salinity treatments and across fields. We expect these biological measurements to be higher in the non-saline soils than the saline soils due to the lower concentrations of salts and increased nutrients for the continued energy renewal and nutrient acquisition (Figure 1b).

Existing literature has identified salinity impacts on soil life, so based on that and what we know about how it impacts plants, we expect to see reduced microbes and nematodes in soils with moderate salt concentrations. We also expect our microbial measures of microbial biomass carbon, microbial group abundance, and nematode counts to align with chemical properties such

as water content, total carbon and nitrogen, available phosphorus, and POXC based on what we know about soil characteristics.

METHODS

Study site description

Four in-field experiment sites were established on working farms in eastern North Dakota: Aneta, Eldridge, Midway, and Northwood. All sites were under no tillage management (1 to > 10 years) and were under a corn (*Zea mays*) and soybean (*Glycine max*) crop rotation. Table 1 lists site characteristics.

Four plots were established at each of the four field sites, each having four subplots, totaling 16 subplot locations that were distributed across each field. Each subplot was marked with a geo-referenced flag for location purposes. Plots were in a stratified split-plot design, with electrical conductivity as the basis for stratification. The subplots in each field span combinations of the following treatments: saline soil, non-saline soil, cover crop, and no cover crop. The saline soils were defined as having an electrical conductivity ($EC_{1:1}$) of 2.0-4.0 dS/m and the non-saline soils with an electrical conductivity ($EC_{1:1}$) of < 1.0 dS/m. These treatments will be referred to as "saline" and "non-saline" for simplicity.

Cereal rye (*Secale cereal*) cover crop was broadcast interseeded midseason (late June or early July for corn or late September for soybean) at 45 kg/ha (40 lbs/ac) in strips across the field. The strips were located in the same location every year and they were chemically terminated around planting the following spring. Establishment and growth of the cover crop in the field was sparse, and the biological soil properties of interest in this study did not differ between the cover crop and no cover crop treatments, so data were pooled across cover crop treatments and only the salinity treatment comparisons were made in this analysis (n = 8 samples per treatment per field).

Table 1. Field site location, soil background and climate information. Four in-field research sites were located in eastern North Dakota and were sampled in 2018 and 2019 for biological properties as part of a salinity research study.

Field Site	GPS Coordinate	Soil Type[†]	Crop 2018	Crop 2019	Drainage Class	Salinity Class	Average Temperature Range[§]	Average Temperature During Growing Season[§]	Total Precipitation[§]
Aneta	47°42'57" N 98°01'44" W	Aeric Calciaquolls	Soybean	Corn	Somewhat poorly drained	Slightly saline to moderately saline (4.0-8.0 mmhos/cm)	-19°C to 26°C	19°C	533 mm
Eldridge	46°56'46" N 98°50'27" W	Calcic Hapludolls	Corn	Soybean	Well-drained	Non-saline to very slightly saline (1.0-3.0 mmhos/cm)	-18°C to 28°C	20°C	508 mm
Midway	46°56'09" N 98°48'33" W	Aeric Calciaquolls	Corn	Soybean	Somewhat poorly drained	Slightly saline to moderately saline (4.0-8.0 mmhos/cm)	-18°C to 28°C	20°C	508 mm
Northwood	47°43'48" N 97°48'05" W	Pachic Hapludolls	Soybean	Corn	Moderately well-drained	Slightly saline (4.0-7.9 mmhos/cm)	-19°C to 27°C	19°C	533 mm

[†]Web Soil Survey, 2020.
[§]NDAWN, 2020. Based on 30-year averages.

Field sampling

In 2018 and 2019, soil samples were collected mid-growing season (June-August) from each subplot. Four soil cores from a 0-15 cm (0-6 inch) depth, two from within the inter row and one from each adjacent row of plants, were taken from within 1 m of each subplot flag using an auger and were composited. Soil cores were composited so we had one sample representative of each subplot. This sampling location was determined by haphazardly tossing a metal frame (0.30 m x 0.30 m). Soils were stored at 4°C until further analysis, except for a 15 g subsample, which was frozen (-20°C) immediately. Refrigeration and freezing of soil samples were critical to preserve the integrity of the microbial organisms and to prevent shifts in microbial community composition (Schnecker et al., 2012).

Crop residues on the soil surface provide habitat and protection for soil biota and are a source of food for soil heterotrophs. Non-living residue (dead plant biomass) samples were removed from the soil surface above where each corresponding soil sample was taken. One residue sample was collected for every one soil core taken, totaling four samples collected from each subplot. All samples were oven-dried at 60°C and weighed. The masses of residue were averaged within each subplot.

Physical and chemical analysis of soil

In order to understand the soil physical and chemical properties which indicate soil organism habitat conditions, soil properties in each site subplot were described in 2017 in detail to 122 cm depth (0-15 cm increments) for physical (texture, particle analysis, bulk density), and chemical properties (cation exchange capacity, total carbon and nitrogen, available phosphorus, and percent organic matter) by the NDSU Soil Testing Lab. Both soil pH and EC were measured on a 1:1 soil to water slurry of the samples taken in 2018 and 2019 as described by Thomas

(1996) and Rhoades (1996) using handheld probes (pH 150 Series Handheld Meter, Oakton Instruments, Vernon Hills, IL; Orion Star A112 Conductivity Meter, Thermo Scientific, Waltham, MA). Water content was determined using the gravimetric method described in Gardner (1986). A field-moist soil sample from each subplot was weighed, oven-dried, and weighed again to obtain the dry soil weight. Water content was calculated by subtracting the dry weight from the field-moist weight, then dividing by the dry weight.

Permanganate oxidizable carbon (POXC) analysis provided an estimate of the amount of active organic carbon, or a readily-available food source for soil microbes present in the soil. We measured POXC on triplicate subsamples (2.5 g) of each sample taken in 2018 and 2019, as described in Weil et al. (2003). In cases where triplicates had a coefficient of variation greater than 10 (24 samples), we increased analytical replications to six to increase statistical power in mean estimates per soil sample. These steps were taken as a quality control measure due to variability in soil POXC in 2.5 g samples. Subsamples were concentrated with 0.2 M $KMnO_4$, shaken for 2 minutes, and were to settle for 10 additional minutes. The supernatant was pipetted (0.5 mL) from the concentrated subsamples into deionized water, which formed diluted extractions ready to be analyzed. The POXC content in the extractions were estimated using a spectrophotometer and a standard curve. The POXC values for each sample (subplot) were calculated as a mean of all replicates.

Biological analyses

We used multiple measurements to examine microbial community structure to get a better idea of the belowground communities associated with the salinity levels.

Microbial biomass carbon (MBC) is the amount of carbon present in living biomass in the soil. MBC represents the active carbon pool, which is readily available to microbial

organisms as a food source (Islam and Weil, 2000; Weil et al., 2003). We used the chloroform fumigation direct extraction method described in Beck et al. (1997). A soil sample (20 g) from each subplot was split evenly – one sample was directly extracted (with 0.5 M K_2SO_4) and the other was placed into a dark vacuum dessicator with chloroform for 48 hours, and then extracted. Each 20 g sample was split to ensure both the unfumigated and fumigated extracts were analyzed from the same soil. Extracted samples were analyzed on a total organic carbon analyzer (TOC-V Series, Shimadzu, Columbia, MD) to determine total dissolved carbon. The microbial biomass carbon in a sample was estimated as the difference between the unfumigated and fumigated extracts and was adjusted for an extraction efficiency of 0.45. Extraction efficiency is soil-specific but is often estimated as 0.45 (Beck at al., 1997).

Phospholipid fatty acid (PLFA) analysis was used to determine the microbial community composition for each soil sample. PLFAs provide an overall snapshot of the microbial communities found in soils, and since PLFAs rapidly degrade upon cell death, they can be considered to be representative of the viable soil microbial community (Quideau et al., 2016). To determine microbial community composition, this analysis quantifies five microbial groups: bacteria (Gram-negative and Gram-positive), actinomycetes, fungi, arbuscular mycorrhizal fungi (AM fungi), and eukaryotes (protists).

Each microbial group that is identified using the PLFA method all have slightly different ecological functions in the soil. Bacteria are functionally-diverse single-celled organisms that decompose organic materials, which further contributes to nutrient cycling, and the building of soil aggregates (Brady and Weil, 2010). Actinomycetes are filamentous, Gram-positive bacteria that break down resistant compounds, like cellulose and chitin, into simpler forms (Brady and Weil, 2010). Fungi are decomposers that contribute to the recycling of organic matter and

nutrients (Parkinson, 1994). Arbuscular mycorrhizal (AM) fungi form symbiotic associations with plant roots and assist their host in capturing immobile nutrients from the soil, such as phosphorus (Sylvia, 1994). The final group that this method distinguishes are protists; eukaryotic, unicellular organisms that influence organic matter decay and nutrient release mostly through their bacterial feeding habits (Brady and Weil, 2010).

The frozen soil subsamples were freeze-dried, ground to pass a 2 mm sieve, and then analyzed for microbial community structure and abundance by Microbial Identification (MIDI) Labs, Inc. (Newark, DE). The MIDI lab follows lipid extraction procedures described by Buyer and Sasser (2012) and quantitative analysis with gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) was performed. Peaks were identified using Sherlock software version 6.2 (MIDI, Inc., Newark, DE) and identified using the PLFAD2 version 2.0 peak naming table. The abundances of each microbial group and their sum were expressed in terms of absolute abundance (nmol fatty acid/g soil).

In 2019, nematodes were extracted from the remaining stored (4°C) soil of the 64 samples using a centrifugal-flotation method (Jenkins, 1964). The soil samples were fresh and field-moist and were processed within two weeks of sampling. A subsample (57 g) was measured from each sample, was wet-sieved using 2 mm and 45 µm sieves, and the solution was transferred into centrifuge tubes. The tubes were placed into a centrifuge where they were spun at 1750 rpm. The supernatant was poured off, replaced with a 45% sucrose solution, and spun again at the same speed. The supernatant was poured onto a 45 µm sieve, the sucrose solution was rinsed off, and the nematodes were washed into a collection container. After extraction, the nematodes in a 10 mL subsample were counted, using a compound microscope. Nematode counts per 10 mL were multiplied by 14 to estimate total nematode counts based on the full 140

mL volume of the extract solution. The total nematode counts from the 140 mL were then divided by the 57 g soil sample to get nematode counts per g/soil.

A summary of our collected measurements is displayed in Table 2 with a brief description of the importance of the properties and how they are helpful for answering our research objectives.

Statistical data analysis

Statistical analysis of our field data was conducted using JMP version 13.2 for Windows (SAS Institute, 2016). To understand microbial communities across increasing salinity gradients and to determine if soils with moderate salt concentrations have different microbial community structure and nematode abundances than soils with lower salt concentrations, we used a two-sample Student's *t*-test. We used this *t*-test because we wanted to compare either chemical (electrical conductivity, pH, water content, total carbon and nitrogen, available phosphorus, organic matter content, POXC, residue) or biological (microbial biomass carbon, microbial group abundance, nematode counts) soil properties between the saline and non-saline treatments within a field.

To better explain the relationships between our chemical and biological soil properties, we used correlations to compare each pairwise relationship. The correlation coefficients yielded from this analysis were used to gauge the strength and direction of these associations. In addition to means comparisons, we calculated Bray-Curtis dissimilarity indices between salinity treatments for our microbial group abundance data, which quantified differences in both microbial abundance and composition.

Table 2. Summary of the chemical and biological measurements collected for this study. Measurements of electrical conductivity ($EC_{1:1}$), pH, water content, total carbon, total nitrogen, available phosphorus, percent organic matter, permanganate oxidizable carbon (POXC), residue, microbial biomass carbon (MBC), microbial group abundance (PLFA), and nematode counts are each accompanied by an explanation of how each variable fit into our research study.

Measurement	Measurement Purpose	Importance of Measurement to Our Study
Chemical		
Electrical Conductivity (EC)	Measures salt concentrations within the soil	EC validates our field design and helps us understand soil habitat
pH	Specifies if soil is acidic or alkaline	pH helps us understand the soil environment in which the microbial organisms live
Water Content	Measures soil moisture	Water content can be used to understand soil habitat but it is also required for growth of soil biota
Total Carbon	Measures total carbon in soil	These measurements provide us with knowledge on microbial food sources for growth/metabolism and cycling of carbon, nitrogen, and phosphorus
Total Nitrogen	Measures total nitrogen (inorganic + organic) in soil	
Available Phosphorus	Measures phosphorus in soil	
Organic Matter	Quantifies percent organic matter present in the soil	Microbes acquire nutrients and energy from organic matter
Permanganate Oxidizable Carbon (POXC)	Measures active carbon	POXC is a readily-available food source for microbial organisms

Table 2. Summary of the chemical and biological measurements collected for this study (continued).

Measurement	Measurement Purpose	Importance of Measurement to Our Study
Residue	Quantifies amount of soil cover	Residue provides habitat for soil biota and is a source of food for soil heterotrophs
Biological		
Microbial Biomass Carbon (MBC)	Measures the amount of carbon present in the living biomass fraction of the soil	MBC is part of the active carbon pool, which provides a food source for microbes
Microbial Group Abundance (PLFA)	Quantifies five different microbial groups that represent the soil microbial community	Determines microbial community composition for each soil sample
Nematode Counts	Extracts nematodes from the soil, allows a greater density of nematodes to be extracted	Help us assess nematode abundances in soils across two salinity treatments

RESULTS

Soil physical and chemical properties

Biological soil properties were the focus of this study, but we also measured soil physical and chemical properties to assess differences in our saline and non-saline soils at each of the four field sites. These additional properties were measured to aid in our understanding of the relationship between these biological properties and soil salinity.

Results for general soil properties of the field sites are reported in Table 3. General soil properties serve to describe each field site and were not analyzed for mean comparison statistics. Particle analysis varied slightly across all sites and treatments, but texture was consistently classified as loam across sites. Clay content and soil texture have been shown to positively impact microbial biomass carbon, and that in general, loam soils are considered to be suitable for microbial growth and activity (Gonzalez-Quiñones et al., 2011; Kasel and Bennett, 2007). Cation exchange capacity was noticeably higher in the saline soils than in the non-saline soils, a trend observed across all field sites. Cation exchange sites occur on the surfaces of clay minerals and organic matter, and are strongly correlated with microbial community composition. Soils high in exchangeable cations often have a higher soil pH and hold more nutrients. For these reasons, cation exchange capacity can enhance microbial habitat and metabolic activity as well as indicate favorable habitat conditions for soil organisms (Li et al., 2018; Zheng et al., 2019).

Table 3. General soil properties across field sites and treatments. Mean values of sand (%), silt (%), clay (%), bulk density and cation exchange capacity within a depth of 0-15 cm taken during the 2017 growing season (*n* =8 subplots) across four field sites and two salinity treatments.

Site	Texture	Sand	Silt	Clay	Bulk Density	Cation Exchange Capacity
		---------%----------			g/cm^3	mEq/100 g
Aneta						
Non-Saline	Loam	47	33	20	1.23	14.73
Saline	Loam	31	44	25	1.17	32.35
Eldridge						
Non-Saline	Loam	46	35	19	1.37	12.97
Saline	Loam	39	41	20	1.30	29.01
Midway						
Non-Saline	Loam	48	31	21	1.38	11.17
Saline	Loam	38	40	22	1.39	23.11
Northwood						
Non-Saline	Loam	39	39	22	1.30	19.95
Saline	Loam	31	44	26	1.21	28.21

Table 4 and Table 5 highlight the trend of the saline soils having mostly higher values in our chemical soil properties than the non-saline soils. We saw a trend of higher water content at the time of sampling in the saline soils across all sites except for Northwood, which had equal moisture in saline and non-saline soils in 2018 and 2019. At all sites we observed higher levels of total carbon, total nitrogen, and organic matter in the saline soils, but the Aneta site had the biggest differences between the two treatments, most notably a 1.52% increase in organic matter in the saline soils. Although Northwood hosted the smallest differences between the two salinity treatments for total carbon, total nitrogen, and organic matter, the salinity treatments at this field site had the largest increase in available phosphorus. Overall, we found that the differences of water content, several carbon sources (C, OM, and POXC), total nitrogen, and available phosphorus were the most substantial between the saline and non-saline treatments.

As expected, the amount of collected residue was higher in the non-saline soils than in the saline soils across all field sites (Table 4 and Table 5), likely due to the decreased plant growth in the saline patches. Average corn grain yield (a proxy for plant production) was reduced by 9-30%, and average soybean yield was reduced by 26-81%, in the saline plots compared to the non-saline plots across all four fields (data not presented). We found the Northwood field site had the largest accumulation of residue for both 2018 and 2019, however, all sites in 2019 had much lower amounts of residue in both salinity treatments than the previous year. The soil biological properties of interest will likely be influenced by the differences in these properties because they create the environment in which the microbial organisms live and provide nutrients that they require for growth and metabolism.

Table 4. Chemical soil properties across field sites and treatments for 2018. Means with standard deviation in parenthesis of electrical conductivity (EC), soil pH, soil water, total carbon, total nitrogen, available phosphorus, percent organic matter (OM), permanganate oxidizable carbon (POXC), and surface residue within a depth of 0-15 cm taken during the 2018 growing season (n =8 subplots) across four field sites and two salinity treatments. Superscripts indicate significant differences between properties within a site across saline and non-saline treatments ($p \leq 0.05$).

Site	Electrical Conductivity	pH	Water	Total C†	Total N†	P†	Organic Matter†	POXC	Residue
	µS/cm		g H_2O/g soil	-------g/kg-------		mg/kg	%	mg C/kg soil	g/m^2
Aneta									
Non-Saline	496.50^b (503.70)	7.34^b (0.45)	0.20^b (0.02)	25.83^b (4.03)	2.43^b (0.47)	8.38^a (2.50)	4.16^b (0.85)	631.91^b (128.45)	909.63^a (223.92)
Saline	2158.88^a (730.14)	7.75^a (0.17)	0.30^a (0.03)	34.33^a (3.45)	3.15^a (0.31)	12.25^a (11.40)	5.68^a (0.97)	791.64^a (72.97)	587.50^b (164.82)
Eldridge									
Non-Saline	267.13^b (63.97)	5.99^b (0.50)	0.13^b (0.02)	19.85^b (3.97)	2.21^b (0.35)	16.88^a (10.41)	3.84^b (0.43)	570.63^b (204.80)	119.38^a (204.13)
Saline	3501.25^a (880.69)	7.39^a (0.23)	0.24^a (0.03)	25.61^a (5.00)	2.68^a (0.48)	24.63^a (6.78)	4.71^a (0.61)	891.39^a (121.71)	36.00^a (23.71)
Midway									
Non-Saline	495.46^b (634.80)	6.54^b (0.77)	0.14^b (0.03)	17.35^b (3.15)	1.83^b (0.21)	5.00^a (3.59)	3.16^b (0.63)	512.53^b (73.08)	969.38^a (414.49)
Saline	2152.88^a (605.45)	7.53^a (0.42)	0.23^a (0.03)	23.21^a (4.14)	2.25^a (0.19)	7.88^a (4.73)	4.09^a (0.78)	720.99^a (115.82)	633.25^a (183.58)
Northwood									
Non-Saline	353.88^b (78.37)	7.77^a (0.37)	0.23^a (0.05)	24.15^a (5.15)	2.28^a (0.50)	7.50^b (4.47)	4.19^a (1.08)	678.43^a (128.98)	1088.88^a (286.67)
Saline	2498.25^a (1104.84)	7.93^a (0.27)	0.24^a (0.04)	27.41^a (4.66)	2.55^a (0.35)	35.38^a (11.20)	4.64^a (0.73)	718.57^a (98.87)	637.50^b (299.12)

†Measurements only collected in 2017

Table 5. Chemical soil properties across field sites and treatments for 2019. Means with standard deviation in parenthesis of electrical conductivity (EC), soil pH, soil water, total carbon, total nitrogen, available phosphorus, percent organic matter (OM), permanganate oxidizable carbon (POXC), and surface residue within a depth of 0-15 cm taken during the 2019 growing season (n =8 subplots) across four field sites and two salinity treatments. Superscripts indicate significant differences between properties within a site across saline and non-saline treatments ($p \leq 0.05$).

Site	Electrical Conductivity	pH	Water	Total C†	Total N†	P†	Organic Matter†	POXC	Residue
	µS/cm		g H_2O/g soil	-------g/kg-------		mg/kg	%	mg C/kg soil	g/m^2
Aneta									
Non-Saline	468.75^b (69.45)	7.10^b (0.57)	0.21^b (0.02)	25.83^b (4.03)	2.43^b (0.47)	8.38^a (2.50)	4.16^b (0.85)	625.57^b (161.55)	75.88^a (18.91)
Saline	2346.25^a (448.08)	7.88^a (0.15)	0.31^a (0.02)	34.33^a (3.45)	3.15^a (0.31)	12.25^a (11.40)	5.68^a (0.97)	773.99^a (103.27)	57.50^a (23.24)
Eldridge									
Non-Saline	274.08^b (78.25)	5.76^b (0.75)	0.22^b (0.03)	19.85^b (3.97)	2.21^b (0.35)	16.88^a (10.41)	3.84^b (0.43)	547.14^b (40.73)	92.00^a (26.98)
Saline	2353.88^a (521.71)	7.06^a (0.54)	0.31^a (0.04)	25.61^a (5.00)	2.68^a (0.48)	24.63^a (6.78)	4.71^a (0.61)	739.66^a (75.69)	89.13^a (28.70)
Midway									
Non-Saline	322.96^b (215.27)	6.19^b (0.93)	0.21^b (0.02)	17.35^b (3.15)	1.83^b (0.21)	5.00^a (3.59)	3.16^b (0.63)	466.66^b (75.14)	100.75^a (12.84)
Saline	2169.00^a (503.74)	7.42^a (0.57)	0.27^a (0.02)	23.21^a (4.14)	2.25^a (0.19)	7.88^a (4.73)	4.09^a (0.78)	637.88^a (95.72)	90.38^a (31.68)
Northwood									
Non-Saline	692.38^b (191.90)	7.20^a (0.60)	0.12^a (0.02)	24.15^a (5.15)	2.28^a (0.50)	7.50^b (4.47)	4.19^a (1.08)	734.91^a (78.32)	130.50^a (25.22)
Saline	2342.63^a (1036.13)	7.45^a (0.36)	0.12^a (0.03)	27.41^a (4.66)	2.55^a (0.35)	35.38^a (11.20)	4.64^a (0.73)	770.85^a (106.39)	52.75^b (24.16)

†Measurements only collected in 2017

Soil biological properties

We measured multiple soil biological properties that examined belowground microbial community structure associated with varying levels of salinity. In 2018, all four field sites leaned towards higher microbial biomass carbon in the saline soils than in the non-saline soils, with the Aneta and Eldridge sites being significantly different (Figure 2). In 2019, microbial biomass carbon was higher in the saline soil than in the non-saline soil at the Aneta site, the only site that was significantly different. Eldridge and Midway showed no differences in microbial biomass carbon between the salinity treatments, and Northwood reflected slightly lower biomass carbon in the saline soils.

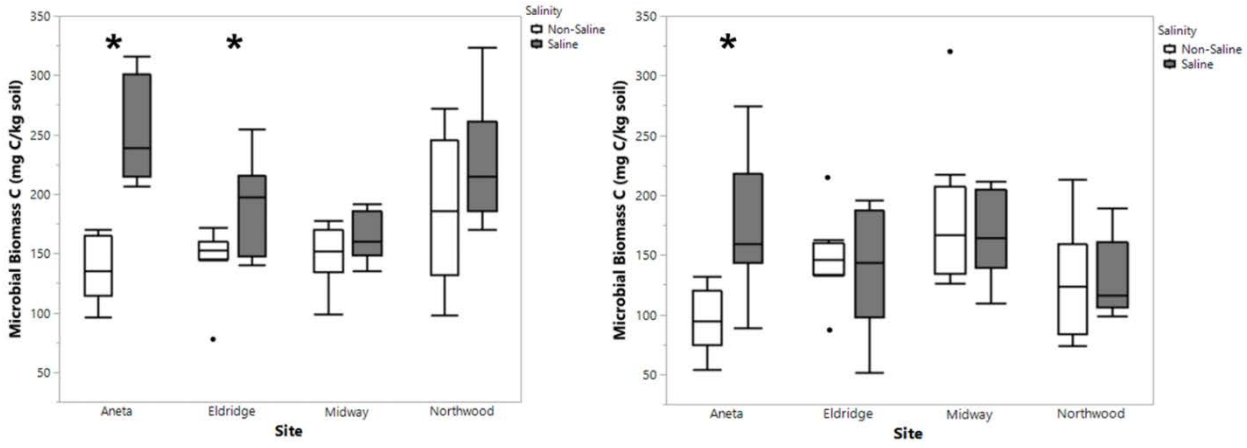


Figure 2. Microbial biomass carbon across sites and treatments in 2018 and 2019. Box-and-whisker plots of microbial biomass carbon across sites and two salinity treatments for 2018 and 2019. Asterisks indicate significant differences between saline and non-saline treatments within a field ($p \leq 0.05$).

Salinity had a more consistent effect on our soil microbial community composition data than what was reflected with our microbial biomass carbon results. We found this observation to be true in three out of the four field sites over the course of the 2018 and 2019 growing seasons. In general, the PLFA method is considered to be a more accurate estimation of biomass because it measures molecules rather than just carbon, so it is perhaps more precise and sensitive (Willers

et al., 2015). The difference in methodology between these two biological analyses could be a reason why a stronger pattern was indicated by our microbial community composition results compared to microbial biomass carbon.

Soil microbial community composition data from all four sites for the year of 2018 is shown in Figure 3. At the Aneta and Midway field sites, we observed more bacteria and actinomycete abundance in saline soils. At the Eldridge field site, we observed more abundance of all five groups in saline soils. At the Northwood field site, we only observed more AM fungi abundance in saline soils. All field sites except Northwood had significantly more total microbial group abundance in the saline soils for the 2018 growing season.

Our 2019 soil microbial community analysis results followed the same field-to-field trends as 2018 (Figure 4), with the bacteria and actinomycete microbial groups causing the largest difference between the saline and non-saline treatments. Similar to the previous year, in 2019, all sites except Northwood had significantly more total microbial group abundance in saline soils.

We also calculated the Bray-Curtis dissimilarity index to identify the percentage of how different the salinity treatments are based on both microbial abundance and composition. This index will help us further answer our question of how microbial community structure differs between varying salinity levels. According to the Bray-Curtis dissimilarity index, our 2018 data reflected that both treatments at all four sites were at least 77% similar (Figure 3). In 2019, the Bray-Curtis dissimilarity index indicated that both treatments across all sites were at least 86% similar (Figure 4). The indices calculated across the four sites for each year were relatively low, which demonstrated that microbial groups in the saline and non-saline treatments were more similar than different.

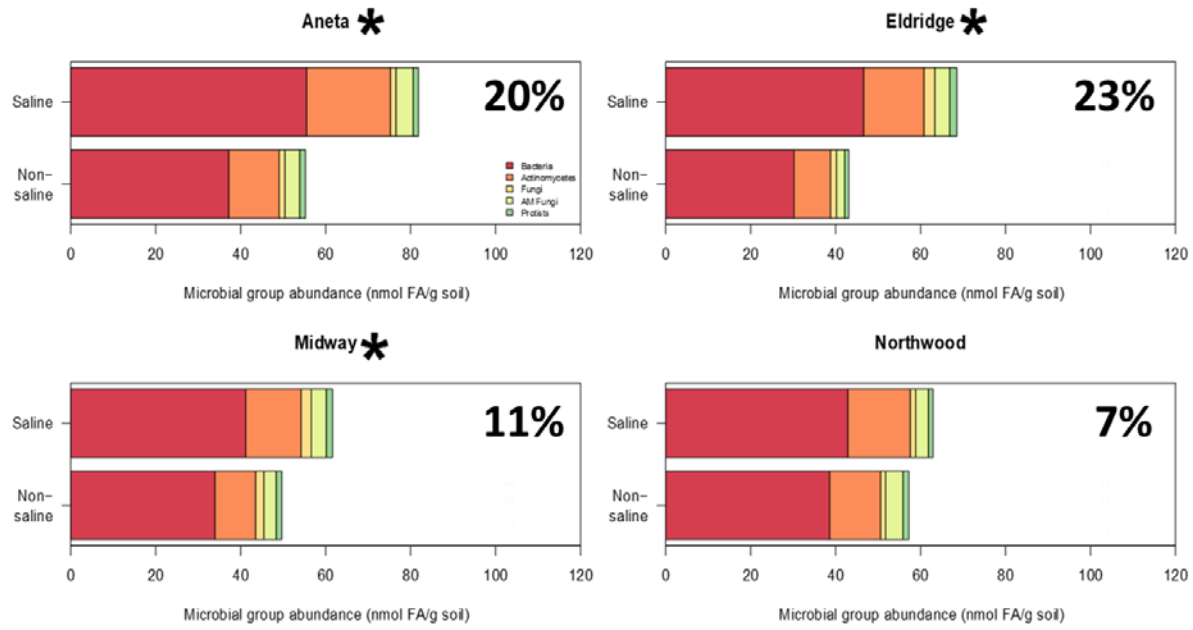


Figure 3. Absolute abundance of soil microbial community composition across treatments in 2018. Soil microbial community distribution of broad taxonomic groups in terms of absolute abundance (nmol/g). Microbial groups include bacteria (Gram-negative and Gram-positive), actinomycetes, fungi, arbuscular mycorrhizal fungi (AM fungi), and eukaryotes (protists). The values in the upper right corner of each graph represent the Bray-Curtis dissimilarity index. Asterisks indicate significant differences between saline and non-saline treatments within a field ($p \leq 0.05$).

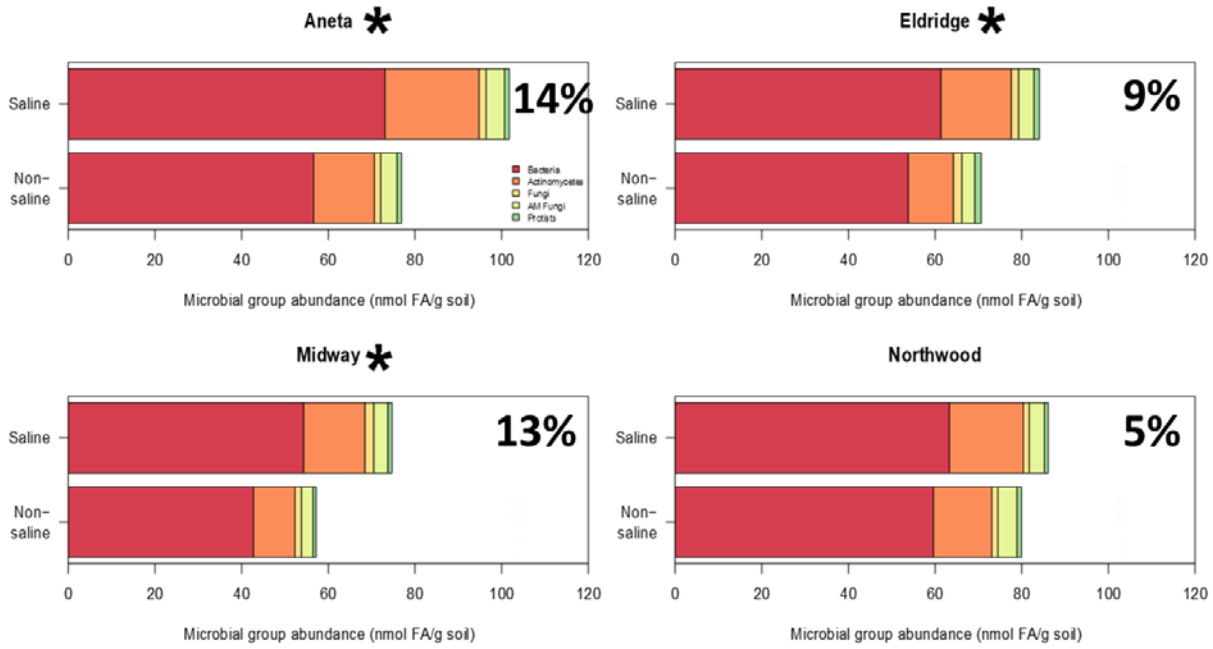


Figure 4. Absolute abundance of soil microbial community composition across treatments in 2019. Soil microbial community distribution of broad taxonomic groups in terms of absolute abundance (nmol/g). Microbial groups include bacteria (Gram-negative and Gram-positive), actinomycetes, fungi, arbuscular mycorrhizal fungi (AM fungi), and eukaryotes (protists). The values in the upper right corner of each graph represent the Bray-Curtis dissimilarity index. Asterisks indicate significant differences between saline and non-saline treatments within a field ($p \leq 0.05$).

In 2019, our nematode data reflected nematode counts tended to be higher in the non-saline soils than in the saline soils (Figure 5). A *t*-test comparison between salinity treatments indicated that Aneta was the only site out of the four to show a statistically lower mean count in the saline soils. The remaining three field sites had statistically similar means across treatments. The lowest number of nematode counts observed was 1 count/gram at the Aneta, Midway, and Northwood field sites, and the highest number of nematode counts observed was 10 counts/gram at the Midway site.

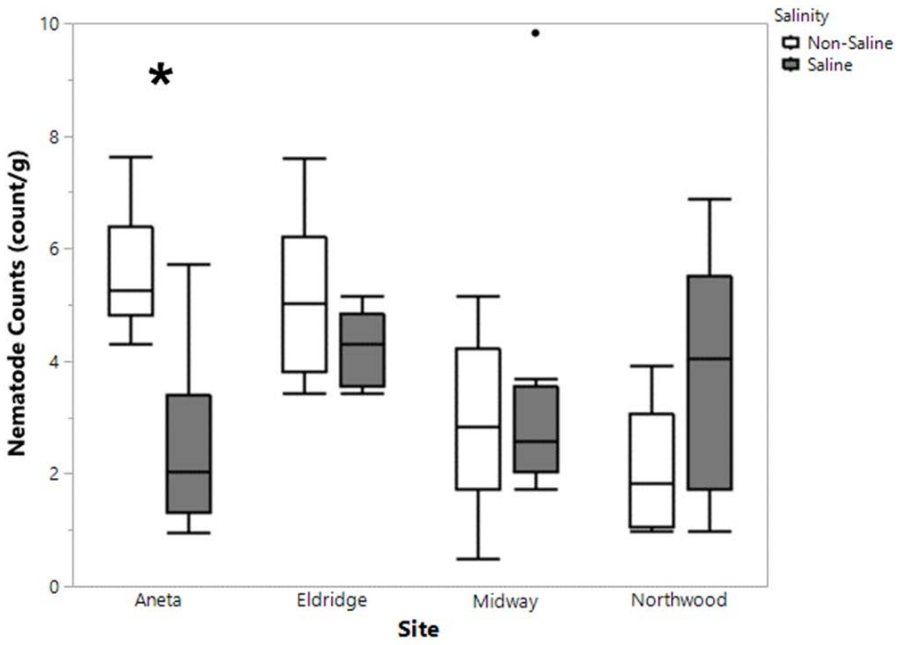


Figure 5. Nematode counts across sites and treatments in 2019. Box-and-whisker plots of nematode counts across sites and two salinity treatments for 2019. Asterisks indicate significant differences between saline and non-saline treatments within a field ($p \leq 0.05$).

Relationships between biological properties and other soil properties

We obtained correlations between soil biological properties and a set of soil properties that aimed to quantify potential microbial food sources and habitat characteristics. We consider a correlation coefficient greater than 0.40 or less than -0.40 to be a relationship of interest, which typically indicates a meaningful relationship in natural systems. Both statistical significance and the strength of the correlations were examined. A strong correlation that is not significant indicates that at least 40% of the variability between the biological properties of interest can be predicted by just one of the auxiliary properties, yet the likelihood of observing this relationship is low. A weak correlation that has significance indicates that there is an increased chance that the variability between the biological properties is unlikely to be explained by just one of the auxiliary properties. The correlation coefficients from this analysis are reported in Table 6.

In 2018, we observed water and organic matter as the only soil properties both having strong positive correlations between our microbial biomass carbon and microbial group abundance data across all four sites. Carbon sources (C and POXC) were also often strongly associated with both microbial biomass carbon and microbial group abundance, but there was variability between the field sites as Eldridge and Northwood were only strongly correlated with one or the other. All four sites had negative correlation results for the residue variable. Aneta and Eldridge resulted in negative correlations between residue and both microbial biomass carbon and microbial group abundance. At the Midway site, we observed residue having a negative correlation with only microbial group abundance, and at Northwood we observed residue negatively correlated with only microbial biomass carbon. Overall, Aneta had the strongest correlations between the microbial measures and auxiliary properties out of the four field sites.

In 2019, we observed no strong correlations at the Eldridge or Midway sites for microbial biomass carbon, however, we observed a trend of strong positive correlations between water, total carbon and nitrogen, and organic matter and microbial biomass carbon and microbial group abundance at the Aneta and Northwood sites. We observed negative correlations between microbial biomass carbon and phosphorus at three sites, and between microbial group abundance and phosphorus at one site. We also saw negative correlations between microbial biomass carbon and residue at Aneta and Northwood, and between microbial group abundance at Aneta, Midway, and Northwood.

Our 2019 data included our nematode count analysis as an additional biological property. We found negative correlations between nematode counts and all non-biological soil properties for the Eldridge site. At the Northwood site, all soil properties displayed positive correlations with nematode counts except with residue and POXC. Similar to 2018, we found the Aneta field

site to have the strongest negative correlations between nematode counts and the auxiliary properties. These results may have provided us with information that different variables influence the nematodes than the other microbial organisms.

Through our correlation analysis, we found that specific food and habitat characteristics were often strongly associated with our biological properties of interest. Water content, total carbon and nitrogen, and organic matter were often highly positively correlated with microbial biomass carbon and microbial group abundance. Our analysis also highlighted that our nematode counts were significantly and strongly negatively correlated with all auxiliary properties for two field sites. Although there was some variability with the strength and the direction of the correlations between both sampling years and the four field sites, this data will help us try to understand the relationships between microbial community structure and the surrounding soil environment.

Table 6. Correlation coefficients between measured variables. Correlation coefficients were calculated for data collected throughout the 2018 and 2019 growing seasons. Statistically significant correlations are indicated by * ($p \leq 0.05$) and ** ($p \leq 0.01$). Correlation coefficients ≥ 0.40 and ≤ - 0.40 are in bold. A strong correlation that is not significant indicates that at least 40% of the variability between the biological properties of interest can be predicted by just one of the auxiliary properties, yet the likelihood of observing this relationship is low. A weak correlation that has significance indicates that there is an increased chance that the variability between the biological properties is unlikely to be explained by just one of the auxiliary properties.

Aneta		--------2018--------		----------2019----------		
	Food/Habitat	**MBC**	**PLFA**	**MBC**	**PLFA**	**NEM**
	Water	**0.86**	**0.84**	**0.73**	**0.68**	**-0.72****
	pH	**0.57**	**0.45**	**0.56**	0.32	**-0.69****
	EC	**0.77**	**0.68**	**0.69**	**0.51**	**-0.80****
	C	**0.81**	**0.72**	**0.54**	**0.71**	**-0.68****
	N	**0.76**	**0.64**	**0.48**	**0.67**	**-0.59****
	P	**0.46**	0.20	-0.15**	-0.13**	-0.28**
	OM	**0.66**	**0.64**	**0.57**	**0.70**	**-0.44****
	Residue	**-0.52****	**-0.49****	-0.16**	-0.19**	**0.44**
	POXC	**0.58**	**0.64**	0.36	**0.58**	**-0.42****
Eldridge		--------2018--------		----------2019----------		
	Food/Habitat	**MBC**	**PLFA**	**MBC**	**PLFA**	**NEM**
	Water	**0.54**	**0.85**	-0.04**	**0.70**	**-0.46****
	pH	**0.64**	**0.85**	0.26	**0.47**	**-0.48****
	EC	**0.41**	**0.88**	-0.10**	**0.55**	**-0.41****
	C	0.28	**0.47**	-0.14**	**0.44**	**-0.42****
	N	0.24	**0.44**	-0.13**	**0.41**	-0.36**
	P	**0.44**	0.31	-0.28**	0.22	-0.33**
	OM	**0.46**	**0.58**	-0.24**	**0.59**	-0.07**
	Residue	-0.17**	-0.24**	0.29	0.25	-0.31**
	POXC	0.37	**0.64**	-0.37**	0.35	-0.34**
Midway		--------2018--------		----------2019----------		
	Food/Habitat	**MBC**	**PLFA**	**MBC**	**PLFA**	**NEM**
	Water	**0.57**	**0.69**	0.05	**0.68**	0.03*
	pH	0.07	0.28	0.34	0.12	0.35
	EC	0.25	**0.47**	-0.14**	**0.55**	0.01**
	C	**0.57**	**0.62**	0.13	**0.83**	-0.23**
	N	**0.59**	**0.65**	-0.19**	**0.70**	-0.18**
	P	**0.63**	0.37	-0.23**	0.30	**0.40**
	OM	**0.54**	**0.60**	-0.21**	**0.67**	-0.16**
	Residue	0.09	-0.16**	0.30	-0.32**	**0.41**
	POXC	**0.51**	**0.67**	-0.21**	**0.55**	0.04*
Northwood		--------2018--------		----------2019----------		
	Food/Habitat	**MBC**	**PLFA**	**MBC**	**PLFA**	**NEM**
	Water	**0.80**	**0.81**	**0.40**	**0.52**	0.08
	pH	0.06	0.03*	0.13	0.28	0.29
	EC	**0.40**	0.28	0.29	0.23	0.18
	C	**0.88**	**0.72**	**0.82**	**0.66**	0.05
	N	**0.89**	**0.77**	**0.83**	**0.65**	0.06
	P	**0.43**	0.11	0.10	0.17	**0.45**
	OM	**0.84**	**0.73**	**0.85**	**0.73**	0.00**
	Residue	-0.15**	0.24	-0.12**	-0.11**	**-0.49****
	POXC	**0.65**	0.38	0.14	0.04*	-0.35**

DISCUSSION

The objective of this study was to determine the effects of varying levels of salinity on soil microbial community structure and nematode abundance in field soils. Contrary to our expectations, we observed that microbial abundance tended to be higher in the saline soils compared to the non-saline soils, and this increase was positively associated with levels of soil water, nutrients, and organic substrates. However, nematode counts tended to be lower in the saline soils. These observations indicate that moderate levels of salinity, that prohibit plant growth, are also associated with drastic belowground differences. The unique set of characteristics in saline soils may have implications for how to best remediate these soils, since salt concentrations are not the only difference between saline and non-saline areas of the field. Results indicate that strategies for salinity remediation need to consider soil water and fertility management in addition to vegetation selection.

Contrary to results from previous studies with higher concentrations of salinity (Batra and Manna, 1997; Rietz and Haynes, 2003; Yuan et al., 2007) and contrary to our hypothesis, we found the moderately saline soils to have higher microbial biomass carbon. Our study found higher total carbon averages in the saline soils than in the non-saline soils across all sites, though only three of the four sites were statistically different (Table 4 and Table 5). The active carbon pool consists of readily oxidizable materials, including microbial biomass, which is largely controlled by climate and residue inputs (Schnurer et al., 1985). We also observed this trend with our organic matter data, with higher percentages of organic matter present in the saline soils across all field sites. Although we observed an opposite trend with our residue data, the increased active carbon and organic matter levels could be attributed to the dissolved forms of carbon, and their hydrological transport to, and accumulation in, the saline soils. Schnurer et al. (1985) found

that the amount of microbial biomass carbon and soil organic matter content are positively correlated with one another. This correlation may also explain why we observed that increase in microbial biomass carbon in the soils affected by salinity.

In this study, we mostly found higher microbial group abundance in the saline soils through our microbial community composition analysis. Out of all the microbial groups measured, the bacteria and actinomycete (a type of Gram-positive bacteria) groups caused the largest difference between the saline and non-saline treatments. Multiple studies have investigated bacterial communities within saline soils, and the likelihood that these increased bacterial populations are due to environmental adaptations (Chowdhury et al., 2011; Pankhurst et al., 2001; Zahran, 1997; Zheng et al., 2017). The higher accumulation of bacteria in the saline soils that we found in our study could also be due to the fact that these organisms have adapted to the moderate salt conditions. Unfortunately, the PLFA method does not allow us to identify specific traits in the microbial groups measured, but future research could investigate the microbial communities at a higher taxonomic resolution. Perhaps the reason we observed lower abundances of other microbial groups, such as fungi and AM fungi, is because the bacterial groups are out-competing the fungal organisms, rather than the fungi experiencing salinity intolerance. AM fungi form symbiotic associations with plants, and since plant growth is generally reduced in the saline soils, the AMF have fewer hosts to colonize.

We have noticed this trend of increased microbial abundance in both our microbial biomass carbon and PLFA data. A reason we could be seeing this trend could be due to increased nutrient availability in those saline areas for microorganisms to utilize as food sources. Salinity often has negative impacts on plant growth, which leads to a lack of plants in those areas and less nutrient uptake (Zörb et al., 2018). Our correlation analysis between our microbial measures and

food and habitat characteristics support that claim, as data reflected that carbon sources (C, OM, and POXC) were often strongly and positively correlated with both of these biological properties (Table 6). Another reason could be due to increased water content we are seeing in the soils affected by salinity. Soil moisture is a major abiotic factor that is essential for regulating microbial activity, and can influence the functional diversity of soil microbial communities (Liu et al., 2010). Our data support these speculations, as water content and carbon sources were strongly and positively correlated with these microbial results. Together the increased moisture and nutrients have likely created a thriving environment for the microbial organisms, especially bacteria and actinomycetes.

Interestingly, residue was mostly negatively and weakly correlated with microbial biomass carbon and microbial group abundance. Although decreased crop growth reduces residue accumulations, this relationship was unexpected, as residue inputs can increase the quantity and diversity of organic materials that can lead to higher primary decomposer abundances and viability, in addition to accelerated carbon and nutrient cycling (Zhong, 2017). Perhaps the surface residue is still contributing to the microbial habitat nutritionally and contributing to the organic matter content, but other food source (such as labile carbon and nutrients) and habitat characteristics support biological activity more strongly.

Nematode counts were higher in the non-saline soils than in the saline soils, a result that highlighted an opposite trend from our microbial biomass carbon data. Our microbial group abundance data reflected more bacterial and actinomycete abundance in the saline soils across all sites (Figure 3 and Figure 4), so perhaps there is a higher density of nematodes in those non-saline soils that have a different feeding preference than that of bacteria, such as fungal-feeders. Nematodes could also be more abundant in the non-saline soils due to the physical presence of

the salts in the saline soils. The salt ions in the soil may have created an unsuitable environment for the nematodes, which may be the reason why higher abundances of nematodes were observed in soils with no salts (Figure 5). It could be possible that when the salts are leached from the soil profile, the microbial community recovers, and we would observe higher abundances of nematodes in the moderately saline soils than what we observed in our study (Pankhurst et al., 2001; Zahran, 1997).

While there is a lack of literature that supports nematode tolerance to varying levels of soil salinity, Van Gundy (1965) reported soil nematodes are able to survive high osmotic pressures (15-20 atm). Since nematodes are able to survive these osmotic pressures, perhaps they are also able to withstand the osmotic effects that salinity may exert on them. However, there is variability in data regarding nematodes and their tolerance to individual ions (Van Gundy, 1965), so nematodes may still be experiencing intolerance to the types of salt ions present at our field sites. Our data reflected higher moisture and more bacteria in the saline soils, both of which can be critical in a habitat for a bacterial-feeding soil nematode. Despite the saline soils having these characteristics, we suspect that there are stronger selection pressures, such as the salts, that are preventing them from reaching the levels observed in the non-saline soils.

The ecological roles of nematodes are significant as they contribute to many soil processes and are a dominant player in the soil food web. We found the non-saline soils tended to have higher nematode abundances than the saline soils and this could be due to their feeding preferences, as they feed on microbial organisms such as bacteria and fungi. Nematode grazing on microbial populations is known to stimulate microbial growth and is assumed to maintain microbial populations in an active state, likely keeping populations managed (Neher, 2010). In the saline soils, microbial biomass carbon tended to be higher compared to the non-saline soils

which may indicate that the nematodes are driving food web dynamics in these soils. Nematode abundance is presumed to reflect the current or recent availability of their food sources, so it could be expected that if microbial biomass is increased in saline soils, nematode abundance is also increased (Ferris and Matute, 2003). However, since we do suspect the nematodes to be sensitive to the salts in our saline soils to some degree, the microbial organisms may be free from heavy predation pressure. This may be an alternative explanation to why we observed higher microbial biomass in the moderately saline soils.

The field site of Aneta had the strongest differences in soil properties across saline and non-saline soils. A reason for this may be attributed to its topography. Aneta is on a slope (3-9%; Soil Survey Staff, 2020), which makes this site unique from the other three. This increase in slope could also potentially cause Aneta to have stronger micro-climate and micro-topography gradients that result in greater differences between the saline and non-saline characteristics, including the biological communities. We also observed that the Northwood site was the only site that had decreased total microbial group abundance in the saline soils for both growing seasons. A reason for this may be that Northwood was generally wetter than the other three sites for the 2018 growing season. However, in 2019, despite receiving higher rainfall that year (NDAWN, 2020), Northwood was substantially drier than the other three sites. This drastic difference of soil water content between both years likely affected why we observed this decreased total microbial group abundance (Figure 3 and Figure 4). Although the season average was higher than the previous, the sampling time of Northwood may have been caught in a dry period. In 2019, the Northwood site also had some emergency vertical tillage throughout the field that helped with planting, due to a wet spring. The tillage was close in proximity to our plots, and may have contributed to the loss of moisture in both the non-saline and saline soils.

To have a more complete understanding of how salinity impacts microbial activity, our biological and auxiliary properties should be studied for a longer period of time. Designing a long-term study would help us continue to measure salinity impacts on microbial communities. Our data provided us with a snapshot of what microbial community structure and nematode abundances looked like in saline soils over the course of a growing season, and implementing a research study on a temporal scale will further help us examine the relationships between these dynamic properties. Another step for this research would be to perform feeding-group diversity analysis on our nematode data to give us a better idea of the nematode community structure across our treatments. This analysis could also potentially help us draw conclusions about the structure and function of the soil food web under soil salinity conditions.

Our hope is that this research will provide knowledge on the effects of sulfate-based salinity on soil microbial community structure and nematode abundance. We also hope that this study will initiate further research on this topic, as the scope of available literature is narrow. Understanding how soil organisms respond to salinity stress can help us better understand their role in soil health, and possibly allow us to make future land management recommendations for soil salinity. There is high value in researching these changes in microbial communities, as it aids in our better understanding of the impacts of salinity on soil communities and overall soil function.

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