

CAN SOIL MICROBIAL ACTIVITY BE IMPROVED WITH THE USE OF AMENDMENTS?

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Soil Science

June 2020

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Can Soil Microbial Activity Be Improved With The Use of Amendments?

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MASTER OF SCIENCE

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ABSTRACT

Low microbial activity and associated nutrient cycling are concerns in agricultural problem soils. The objectives of this study were to investigate microbial response on problem soils to amendments, drying-wetting cycles, and the interaction of amendments and drying-wetting cycles. In this laboratory study, soil carbon dioxide (CO₂) flux was measured from thermal desorption treated soils and saline soils in response to Proganics, spent lime, and composted beef manure applications. Microbial activity was measured through CO₂ flux and its rate of change, permanganate oxidizable C, and residual inorganic nitrogen. Proganics had the greatest ability to elevate and sustain microbial activity on problem soils, but spent lime and compost had the greatest potential to improve microbial mediated nitrogen mineralization. In conclusion, spent lime and compost can be effective amendments for improving soil quality of saline and thermal desorption treated problem soils to increase microbial activity and associated nitrogen cycling.

ACKNOWLEDGMENTS

I would like to thank Dr. Amitava Chatterjee for the privilege of being taken on as one of his grad students and for his trust and confidence in me to complete this project. I would also like to thank him for his generous assistance and guidance through the research for this project as well as his willingness to provide new laboratory equipment that was required for me to complete this research. I would also like to thank my supervisory committee including Dr. Thomas DeSutter, Dr. Caley Gasch, and Dr. Jason Harmon for their assistance in developing the research project and guidance throughout my work on it. Dr. DeSutter's assistance in providing soil samples and expertise on reclamation of soils was particularly helpful in designing and carrying out this research project. I would also like to thank graduate students and research specialists including Sailesh Sigdel, Dan Olson, Umesh Acharya, Mackenzie Ries, and Joel Bell for their guidance, advice, assistance, and support. Their generosity and kindness were a significant help while completing this research. Credit also needs to be given to my guide dog Anchor for faithfully guiding me every day through any obstacle weather presented while I conducted research. I couldn't have done it without him.

DEDICATION

This work is dedicated to my guide dog Anchor for the vital role he played in guiding me every day.

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INTRODUCTION

A problem soil is a soil with serious biological (Eswaran et al., 2001), physical or chemical limitations that requires special management to achieve satisfactory crop production (Osman, 2013). This can include soils where the soil characteristics themselves pose problems for their optimal use and soils where management interventions have created environmental and productivity problems (FAO, 2020). Physical land degradation leading to problem soils includes erosion, crusting, compaction, desertification, and environmental contamination. Chemical degradation leading to problem soils includes salinization, acidification, leaching, depletion of cation retention capacity, and depletion of fertility. Biological degradation includes reductions in total carbon (C), biomass C, and biodiversity (Eswaran et al., 2001). These processes can alter attributes of the soil including nutrient status, organic C content, labile C content, pH, texture, available water holding capacity, structure, and maximum rooting depth (National Research Council, 1993). World-wide, an estimated 52% of fertile agricultural land is degraded with an estimated annual cost of 6.3 to 10.6 trillion dollars (Young and Orsini, 2015) which has resulted in 20 to 40% yield reductions in row crops in parts of the Midwest of the United States (Eswaran et al., 2001). Just 11% of the earth's land is fertile agricultural soil making it imperative that this soil maintains production which requires sustainable soil management (Eswaran et al., 2001). Thus, problem soils are a concern world-wide and require research to recover agricultural production.

Problem soils in North Dakota include saline soils (Seelig, 2000) and soils treated with thermal desorption proceeding hydrocarbon contamination (O'Brien et al., 2016). Improvement of saline soils and reclamation of soils treated with thermal desorption is required to improve soil quality and restore ecosystem services. Recovery of microbial activity is a crucial step in

improvement of problem soils due to the ability of soil microbes to enhance decomposition (Acosta-Martinez et al., 2010), mediate nutrient cycling (Sardans, and Penuelas, 2010; Schjonning et al., 2003) and improves soil quality (Turco et al., 1994). Research is required to determine whether soil amendments are effective at increasing microbial activity on problem soils and which amendments would be most suitable for reclamation of problem soils. A soil amendment that enhances microbial activity could be beneficial to agricultural productivity (Cleveland et al., 2004) due to soil microbial ecosystem services.

Saline Soils

Soil salinity can have negative impacts on agricultural production (Ganjugunte et al., 2018; Corwin and Yemoto, 2017; Munns and Gilliam, 2015; Franzen, 2007; Seelig, 2000; Reeve and Fireman, 1967) with yield reductions of up to 50% reported in moderate to high saline soils which has a salinity over 4 dS m⁻¹ (Hadrich, 2011). The Red River valley of North Dakota has common shallow ground water tables resulting in saline soils (Hadrich, 2011). There is an estimated 0.5 million hectares of slightly saline soil (2-4 dS m⁻¹) and 0.1 million hectares of moderately saline soil (4-8 dS m⁻¹) in the Red River Valley (Hadrich, 2011). Annual revenue losses due to yield reduction of soybean, corn, wheat, and sugarbeet, have been estimated to be approximately 58, 48, 34 and 11 million dollars, respectively in the Red River valley (Hadrich, 2011). Thus, management of saline soils is required to maximize yield and prevent the loss of productive farm land (Hadrich, 2011).

Soil salinity can impact the physical (Rhoades, 1999), chemical (WenWen et al., 2019), and biological (Rath et al., 2019) characteristics of the soil. Despite the improved physical properties (Rhoades, 1999) and higher infiltration rates of saline soils (Oster et al., 1999), salinity can still have negative impacts on soil properties including increased pH (WenWen et al., 2019)

and decreased soil organic matter (SOM) (Rath et al., 2019; WenWen et al., 2019). Soil salinity inhibits plant growth and function (Keller et al., 1986) which is due in part to the salt's interference with nutrient uptake (Munns, 2002). Soil salinity can also negatively impact the soil microbial community by decreasing microbial growth, respiration (Rath et al., 2019), and biomass. The abundance and community composition of soil bacteria and fungi can both be significantly altered by saline conditions with shifts in the dominant genera observed (WenWen et al., 2019). Thus, saline soils require improved soil conditions to improve microbial activity and plant productivity on saline soils.

Thermal Desorption Treated Soils

Crude oil production has increased in regions sustained by agricultural economies in recent years including the Bakken and Three Forks Shale formations of the northern Great Plains and southern Canada. As a result, accidental releases of petroleum products associated with oil production are likely to occur on cropland and rangeland (O'Brien et al., 2016). The hydrocarbons released through crude oil spills can reduce plant germination and growth or have toxic effects on plants (Liste and Prutz, 2006) as well as result in deterioration of the soil biological community (Eom et al., 2007; Dorn et al., 1998). This creates a need for treatment processes such as thermal desorption.

Thermal desorption is a treatment process that involves vaporization of the contaminants from the soil (Lighty et al., 1990) at a temperature of 200 to 500 degrees C (Ritter et al., 2017). Thermal desorption allows for reuse of the treated soils, but physical, chemical, and hydraulic characteristics of the soil are a concern following thermal desorption treatment (O'Brien et al., 2018; Sierra et al., 2016). Other concerns for the productivity of treated soil include decreased SOM (O'Brien et al., 2016; Tatano et al., 2013) and decreased microbial activity (Cebon et al.,

2011). The low SOM levels of soils treated with thermal desorption due to mineralization of SOM at 220 degrees C make treated soil unsuitable for agriculture (Sierra et al., 2016). Thus, further reclamation is required on soils treated with thermal desorption to return the soils to pre-spill agricultural productivity.

Soil Amendments

Soil amendments are intended to improve soil physical (Zhao et al., 2019) and chemical (Badzmierowski et al., 2019) characteristics, increase SOM (Grandy et al., 2002) and biological activity (Brennan and Acosta-Martinez, 2019; Lupwayi et al., 2018; Basta et al., 2016).

Measuring CO₂ flux upon rewetting of dried soils can be used to measure the response of soil microbes to soil amendments (Dere and Stehouwer, 2011) as well as predict corn agronomic performance (Culman et al., 2013). The application of sugar beet processing lime (regionally referred to as spent lime), to sodic soils at a rate of 67.2 Mg ha⁻¹ resulted in more than 2 times higher cumulative microbial respiration relative to the control (Breker et al., 2018). A field study conducted over two years on a fallow compacted soil found that soil CO₂ flux was significantly higher for the two study years in response to poultry litter applied at a rate of 19.0 Mg ha⁻¹ when compared to the non-amended compact soil with soil CO₂ flux being 2.4 times higher in the first year and 1.4 times higher in the second year when compared to the control. The application of poultry litter created more favorable conditions for soil microbes including higher soil water content, higher soil temperature, and higher soil organic C (SOC) (Pengthamkeerati et al., 2005). A laboratory study found an increase in CO₂ flux with the application of pelletized poultry manure and green waste compost compared to the control with values of 85.0, 15.6 and 2.00 mg CO₂-C/kg respectively.

A significant increase in microbial biomass carbon (MBC) was also seen in the soil amended with pelletized poultry manure and green waste compost compared to the control. In soils amended with pelletized poultry manure, highest N mineralization rates were observed at the same time as the highest MBC values (Flavel and Murphy, 2006). Other research has found an increase in CO₂ flux in response to amendments including cattle manure (Lupwayi et al., 2018), poultry manure (Acosta-Martinez and Harmel, 2006), biosolids (Basta et al., 2016), crushed cotton gin (Tejada et al., 2007), straw (Flavel and Murphy, 2006), charcoal (Colb et al., 2009), hydrochar (Kammann et al., 2012), and biochar (Yoo and Kang, 2012) due to an increase in microbial activity (Dere and Stehouwer, 2011). CO₂ flux has increased in response to composted beef manure (Sadeghpour et al., 2016) and spent lime has increased cumulative microbial respiration (Breker et al., 2018), but the response of CO₂ flux to Proganics applications has not been evaluated. Research is required to examine the impact of composted beef manure, spent lime, and Proganics on soil quality through the response of CO₂ flux over drying-wetting cycles on saline soils and soils treated with thermal desorption.

Drying-Wetting Cycles

Drying-wetting events are common in most soil environments having important ecosystem level ramifications including an impact on soil microbial dynamics (Fierer and Schimel, 2002). In a process referred to as the Birch Effect, a flush of CO₂ is released upon rewetting of dried soils due to rapid mineralization of organic C (Barnard et al., 2020; Guo et al., 2014) with the size of the CO₂ pulse being dependent on the level of available C in the soil (Barnard et al., 2020) for microbial growth (Waring et al., 2016). The pulse of CO₂ is driven by microbial activity with a greater availability of labile organic C promoting a larger pulse upon rewetting (Waring et al., 2016; Jenerette and Chatterjee, 2012). The pulse in microbial activity

upon rewetting may be due to the release of SOM that was previously physically protected (Brangari et al., 2020; Waring et al., 2016; Fierer and Schimel, 2003). Increased diffusion of available C due to high soil water content may also be an explanation for the increase in CO₂ flux upon rewetting of dried soils (Brangari et al., 2020; Waring et al., 2016; Guo et al., 2012). Additional explanations are the accumulation of dissolved organic C during the dry soil conditions and osmoregulation in response to rewetting (Brangari et al., 2020), but osmolyte accumulation is not a major strategy of microbial drought resistance (Kakumanu et al., 2013). A 370 to 475% increase in soil respiration has been observed upon rewetting of dried soils (Fierer and Schimel, 2003). An increase in microbial respiration has also been observed following a precipitation event (Abagandura et al., 2019; McMullen et al., 2015). A strong relationship has been found between CO₂ flux upon rewetting of dried soils and nutrient cycling, total soil N (Ingram et al., 2005), N mineralization, microbial activity (Franzluebbbers et al., 2000), microbial biomass-C, C mineralization, and soc (Ingram et al., 2005; Franzluebbbers et al., 2000). Measuring CO₂ flux upon rewetting of dried soils responds rapidly to changes in management (Franzluebbbers et al., 2000) making this a valuable method for evaluating the recovery of problem soils (Ingram et al., 2005) and simulating natural microbial dynamics (Fierer and Schimel, 2002).

Soil Quality Indicators

Soil microbial communities and activity can be useful indicators of soil health (Pankhurst et al., 1995), and there are various methods for examining microbial communities and activity. For example, permanganate oxidizable C (POXC) and N mineralization can provide an assessment of sensitive soil functions and are additional soil quality indicators that can be measured to indicate changes in the soil microbial community. POXC is a measure of active C

that has been found to be significantly related to particulate organic C (POC), MBC, and SOC and it reflects a processed labile C pool that is sensitive to environmental and management changes which makes POXC an indicator of soil quality (Culman et al., 2012). A relationship has also been seen between POXC and CO₂ flux (Hurisso et al., 2016), and POXC has shown to increase in response to compost amendments (Culman et al., 2013). N mineralization is an indication of microbial activity (Hassett and Zak, 2005) due to the significant role in N mineralization that enzymes produced by soil microbes play in this process (Muruganandam et al., 2009). Nitrogen mineralization is related to substrate quality (Osterholz et al., 2017), MBC (Hassett and Zak, 2005), and CO₂ flux and can increase in response to soil amendments (Flavel and Murphy, 2006). Thus, POXC and N mineralization are additional parameters of microbial activity that can be measured in tandem with CO₂ flux to evaluate soil quality.

Soil management is required to improve saline soils and reclaim soils treated with thermal desorption to improve soil quality and restore agricultural productivity. A crucial step in successful improvement of soil quality on problem soils is recovering the microbial mediated carbon cycling and soil nutrient pools. The overarching research question of this experiment is can we recover the soil microbial activity of these problem soils using traditional amendments such as composted beef manure, sugarbeet spent lime, or commercial products such as Proganics Biotic soil Media? The objectives of this research are to (1) examine the effect of wetting-drying events on soil CO₂-efflux, (2) analyze the influence of amendments, spent lime, Proganics, and composted beef manure on the change of CO₂ flux rate and baseflux, (3) examine the interaction between amendments and wetting-drying events on soil CO₂ flux, and (4) analyze the relationship of CO₂ flux with POXC and soil inorganic N availability.

To achieve these objectives, a laboratory study was conducted where saline soil, non-saline soil, soils treated with thermal desorption, and non-treated soils were incubated over three drying-wetting cycles with four treatments. The treatments were a control with no amendments, and spent lime, composted beef manure, and Proganics applied at rates of 22.4 Mg/ha, 22.4 Mg/ha, and 5.61 Mg/ha respectively. Soils were wet to field capacity and the incubation continued until microbial activity stabilized where upon soils were rewet to field capacity. Soil CO₂ flux was measured over the drying-wetting cycles as an indicator of soil health. Inorganic N including ammonia and nitrate concentrations were determined for the control soil and the soils incubated with each amendment to determine available inorganic N as an indicator of soil quality. POXC was determined at the conclusion of each incubation as an additional soil health indicator. Other initial soil properties including SOM, total organic C, electrical conductivity (EC), and cation exchange capacity (CEC) were measured to quantify variables relating to soil quality.

LITERATURE REVIEW

This literature review establishes the concerns for agricultural production on saline soils and soils treated with thermal desorption as well as the scope of these problem soils. Current improvement strategies for saline soils and reclamation strategies for thermal desorption treated soils are discussed including benefits and limitations of these current strategies. Amendment characteristics are examined in relation to microbial response to specific characteristics. The review then transitions to an examination of soil moisture including the impact of soil amendments on soil moisture and the relationship between soil water content and parameters of microbial activity. This develops the discussion for an examination of the impact of drying-wetting cycles on microbial activity and the mechanisms responsible for the flush of CO₂ observed proceeding rewetting of dried soils. This is followed by an examination of the response of soil CO₂ flux and other soil quality indicators to amendment additions as well as a connection between soil CO₂ flux and soil quality.

Soil Salinity

Soil Salinization is the accumulation of excess soluble salts in the soil (Seelig, 2000; Volkmar et al., 1998) and it is most commonly seen in semiarid and arid regions (Munns and Gilliam, 2015; Reeve and Fireman, 1967). Salts that are more soluble than gypsum are considered to be soluble salts and result in soil salinity (Seelig, 2000). Constituents that are commonly seen in these soluble salts include calcium (Ca²⁺), magnesium (Mg²⁺), sodium (Na⁺), sulfates SO₄²⁻, chlorides (Cl⁻), and carbonate (CO₃²⁻) (DeSutter, 2008; Volkmar et al., 1998; Reeve and Fireman, 1967). Salts that are most commonly found in concentrations high enough to impact crop growth are sodium sulfate, calcium sulfate, magnesium sulfate, sodium chloride, calcium chloride, and magnesium chloride (Franzen, 2007). Sulfate salts make up a large proportion of saline soils in

North Dakota (Franzen, 2007; Seelig, 2000) with the most common salts being calcium sulfate, magnesium sulfate, and sodium sulfate.

Causes of Soil Salinity

Soil salinity can result from both natural and human-induced actions (Shahid et al., 2018; Munns and Gilliam, 2015) which is referred to as primary salinization and secondary salinization respectively. Primary salinization occurs on soils that favor discharge meaning evapotranspiration is favored over leaching (Seelig, 2000). Salinization can also occur naturally when local landscape features cause runoff to accumulate seasonally resulting in a water table that rises to a depth of 6 feet (Franzen, 2007). Secondary salinization refers to soils salinized as a result of human activity such as saline seeps, salinization along road ditches and lagoons, salinization from wetland drainage, and salinization from irrigation (Seelig, 2000). Thus, soil salinity can form problem soils due to both natural processes and human activities.

Extent of Soil Salinity

There are currently no statistics on the global extent of salinization. However, it has been estimated that 10 to 33% of total arable land is salt affected (Shahid et al., 2018; Eswaran et al., 2001). There is nearly 1 billion hectares of salt affected land (Shahid et al., 2018; Ghassemi et al., 1995) including 25 to 30% of irrigated soils rendering them commercially unproductive (Shahid et al., 2018). Eastern North Dakota's Red River Valley has approximately 162,000 ha of agricultural land that has a shallow water table sustained by upward flowing groundwater from the saline artesian aquifer (Doering et al., 1999). Globally, an estimated 2000 hectares of agricultural land has been lost per day to salinization over the last 20 years World-wide (Young and Orsini, 2015). It is expected that salinization will result in 50% of arable land lost by the year 2050 (Wang et al., 2003a). However, soil salinity is not a new problem, and dates back to early

civilizations in Mesopotamia that failed due to human-induced salinization (Shahid et al., 2018; Young and Orsini, 2015). Despite the extended time period that soil salinization has been an agricultural problem, salinization of arable land is expected to continue to increase with devastating effects worldwide (Wang et al., 2003a).

Economic Impact of Soil Salinity

Saline soils are an agricultural problem soil in the Northern Great Plains (Doering et al., 1999) as well as worldwide due to degradation of the soil resource base by decreasing soil quality (Shahid et al., 2018). Degradation of land due to soil salinity impacts the livelihoods of people both inside and outside farming communities (Qadir et al., 2014). Soil salinity can have negative impacts on agricultural production (Ganjegunte et al., 2018; Corwin and Yemoto, 2017) as well as financial losses to farmers due to loss of productive agricultural land (Hadrich, 2011) and increased operating costs (Doering et al., 1999). The global annual cost of land degradation from salinity in irrigated areas has been estimated at U.S. \$27.3 billion due to losses in crop production (Qadir et al., 2014) which is about 441 dollars per hectare (Shahid et al., 2018). There are other costs associated with soil salinity other than crop productivity including employment losses, property value losses of degraded farms, social costs of farm businesses, infrastructure deterioration, and increased human and animal health problems. The cost of no reclamation efforts on salt-affected land ranges from 15 to 69% of total profit depending on land degradation type and intensity, crop grown, quality of irrigation water, capacity of drainage systems, and on-farm soil management (Qadir et al., 2014). Due to the scarcity of uncultivated productive land and food security concerns, productivity enhancement of degraded lands has become an important issue. As a result, salt-affected lands are still a valuable resource that require reclamation efforts (Wang et al., 2003a).

Plant Response to Soil Salinity

A two-phase response to salts is typically seen in plants with the first phase being osmotic stress and the second phase being ion toxicity (Corwin and Yemoto, 2017; Munns, 2002). Salt stress results in similar plant responses as water stress because the plant's ability to take up water is reduced by salinity through osmotic stress (Munns, 2002; Seelig, 2000). Saline soils can result in a decrease in seed germination rate (Seelig, 2000) and plant growth rate (Volkmar et al., 1998; Keller et al., 1986) as well as metabolic changes (Volkmar et al., 1998). The constituents of soluble salts can inhibit plant function (Keller et al., 1986) and can result in declines in plant productivity (Munns, 2002). Salinity impacts on plants include loss of water in cells resulting in shrinkage of cells, reductions in cell elongation rates, lower rates of root and leaf growth, injury and death to leaves, inhibition of lateral shoots (Munns, 2002), and eventually, plant death. Excessive salt concentrations are toxic resulting in senescence in older leaves. This reduces the photosynthetic capacity of the plant which eventually causes plant growth to be unsustainable (Volkmar et al., 1998). Crop yields typically begin to decrease when salinity levels reach 4 dS m^{-1} , but nearly all crops yields are severely affected when salinity reaches a level of 16 dS m^{-1} based on EC measured by saturation extract (Seelig, 2000). Slightly saline and moderately saline soils result in a yield loss of up to 15% and 50% respectively (Hadrich, 2011). A plant's ability to take up nutrients can also be reduced on saline soils (Hadrich, 2011; Franzen, 2007) which upsets the nutritional balance of plants (Corwin and Yemoto, 2017; Reeve and Fireman, 1967) contributing to a loss of agricultural crop yield in both quantity and quality (Maas and Grattan, 1999). Thus, salt stress resulting from soil salinity negatively impacts plant growth and survival which is harmful to agricultural production.

Management of Saline Soils

Management of saline soils is required to maximize crop yield (Hadrich, 2011). Different management options currently exist for agricultural systems on saline soils. One management option is to grow salt tolerant cultivars which are typically drought resistant plants. A second option is to flush the salts from the surface soil prior to planting because many plants are most negatively impacted by soil salinity during germination and early growth stages (Seelig, 2000). One inch of rainfall can reduce salt concentrations by 50% in the seed bed which is the top 1-2 inches of soil (Franzen, 2007). A third option is implementing management practices to control evaporative discharge of soil moisture from the surface of the soil. One way of reducing evaporative discharge is by lowering the water table through drainage of the soil, but this is not always an economical management option. Evaporative discharge can also be reduced by applying surface mulch to the soil because mulch reduces evapotranspiration. A fourth option is leveling of irrigated fields which allows for more even water distribution. This avoids concentration of water in micro-lows and salts at micro-highs (Seelig, 2000). Thus, there is various strategies for managing salinity in agricultural systems, but all these strategies have limitations.

There are various management strategies for dealing with other forms of human induced salinity. When soil salinity results from saline seeps, soil salinity can be managed by controlling local groundwater recharge. This can be controlled by eliminating the summer fallow period (Franzen, 2007; Seelig, 2000) particularly in upland recharge positions due to the high-water tables resulting from summer fallow periods. This problem is compounded by the inefficient use of moisture by cropping systems when compared to native vegetation. The resulting high-water tables contribute to evaporative discharge which leads to salinization of croplands (Seelig, 2000).

Continuous cropping (Franzen, 2007; Seelig, 2000) and inclusion of hay or pasture in the rotation can reduce groundwater discharge which minimizes the water available to result in saline seeps (Seelig, 2000). Road ditch salinity can be reduced by preventing water from standing in drainage ditches. Ditches need to be designed as well as properly maintained to move water rapidly which minimizes standing water. Secondary salinization of soils through irrigation can be prevented by applying more water than is required for the crop provided subsurface drainage is adequate (Seelig, 2000). Thus, there are various strategies for dealing with different forms of human induced salinity, but many of these options are preventative and research is still required to manage salinity issues after the formation of this problem soil.

Additional options for managing soil salinity are tile drains or bands of deep-rooted crops. Tile drains or bands of deep-rooted crops can intercept the lateral flow of water to assist in prevention of salinity or reduction of salinity (Franzen, 2007; Seelig, 2000). These methods have been successful, but both have disadvantages. Tile drains can be expensive and create the problem of saline water disposal (Franzen, 2007; Seelig, 2000). Tile drains have an average cost of \$575 per acre and a range of \$400 to \$800 per acre in the Red River Valley with a useful life of 25 years. This results in a cost of \$23.75 per acre annually over the life of the tile drainage which is about 20% of total fixed costs for barley, corn, wheat, and soybeans. Installation of tile drainage results in a profit loss that ranges from 20% for soybeans up to 49% for barley (Hadrich, 2011). Soils that are silty clay loams, clay loams, and clay textures are the most expensive textures for tile drainage installation. Using bands of deep-rooted crops is often a short-term management option because salts can build up in the root zone leading to negative impacts to deep rooted crops (Seelig, 2000).

The application of a soil amendment to saline agricultural soils as a management strategy may be a cheaper method of improving soil quality including microbial activity resulting in improved yield. The input of organic matter is generally expected to enhance soil biological properties based on a study conducted to assess different agricultural management systems (Weyers et al., 2013). However, little research has been done on the impacts of soil amendments on saline soil particularly the microbial response to an organic amendment on a saline soil. In a study conducted in a sugar cane agricultural system (*Saccharum officinarum*) on saline soils, the application of an organic amendment of sour grass (*Andropogon intermedius* var. *acidulus* stapf) mulch at 10 Mg ha⁻¹ significantly increased crop yield when compared to a control plot that produced no marketable yield. The organic amendment also increased rainfall preservation and resulted in a volumetric moisture content 4% higher than the untreated control (Eavis and Cumberbatch, 1977). Mavi and Marschner (2013) found that the application of 2.50 mg C g⁻¹ of glucose increased soil cumulative respiration and MBC on saline soils indicating that the application of C to saline soils can improve the tolerance of soil microbes to salinity. This may be due to the ability of organic amendments to increase CEC and decrease the salt content and EC. The application of a mixture of green waste compost, sedge peat, and furful residue at a rate of 4.50 kg organic matter m⁻³ decreased the EC by 87% (Wang et al., 2014). Additionally, Ding et al., (2020) found that P applied with sewage sludge or farmyard manure to saline soils increased SOM, hydraulic conductivity, available water, and macronutrients while decreasing soil bulk density and salinity. Thus, soil amendments have the potential to improve soil quality of saline soils resulting in increased microbial activity, but limited research has been conducted regarding the response of microbes in saline soils to soil amendments.

Soils Contaminated by Hydrocarbons

Hydrocarbon contamination of soils from oil spills results in agricultural problem soils across North Dakota and the United States with petroleum contamination being the most common environmental contaminant (Truskewycz et al., 2019). From 1968 to 2015, there has been 10,810 inland oil spills of at least 1 gallon from pipelines in the United States (Etkin, 2017). These accidental releases of oil resulted in over 6.7 million barrels (bbl) of spilt oil (Etkin, 2017). One barrel is equal to 42 gallons. Only 37% of the oil spills were considered to be major spills (at least 10,000 gallons), but major spills account for 93% of spilt oil by volume (Etkin, 2017). It is expected that about 26 major pipeline spills will occur in the United States each year (Etkin, 2017). There is about 1 crude oil pipeline spill for every 3.3 million bbl. transported and 1 major pipeline spill for every 42 million bbl. transported (Etkin, 2017). This creates a need for reclamation strategies for hydrocarbon contaminated soils.

Impact of Soil Hydrocarbon Contamination on Soil

Hydrocarbon contamination of soil has a negative impact on the biological community of soils including plants (Liste and Prutz, 2006) and soil microbes (Eom et al., 2007; Dorn et al., 1998). Hydrocarbons released through crude oil spills can be toxic to plants as well as reduce germination and growth (Liste and Prutz, 2006). Low levels of soil hydrocarbon contamination negatively impact agricultural production with less than 2% by weight oil in the soil resulting in a yield reduction of plants. Oil also results in declines in plant available N and plant water uptake (De Jong, 1980). Additionally, it has a negative impact on the microbial community of the soil (Eom et al., 2007; Dorn et al., 1998) including inhibiting enzymes (Alrumman et al., 2015) and a toxic effect on soil microbes (Truskewycz et al., 2019; Alrumman et al., 2015). Hydrocarbon contamination reduces microbial diversity (Cheema et al., 2014; Nyman, 1999) which may be

due in part to soil conditions favoring hydrocarbon degrading microbes (Alrumman et al., 2015). Soil microbes are also prevented from obtaining essential building blocks for proliferation as a result of the ability of hydrocarbons to lock away nutrients and water. Soil microbes also have difficulty adjusting to the highly nonpolar environment with hydrocarbon contamination which can result in rupture of microbial cells due to the hydrocarbons dissolving the membrane lipids of the cytoplasm (Truskewycz et al., 2019). Due to the negative impact of hydro carbon contamination from oil spills on soils, remediation of the contaminated sites is required.

Thermal Desorption Process

Ex situ thermal desorption is one of the methods used for remediating soils contaminated by crude oil as part of the process of returning hydrocarbon contaminated soils to agricultural production (O'Brien et al., 2016). This treatment process is quicker than most other methods, reliable, and meets cleanup standards (Vidonish et al., 2016; Khan et al., 2004). It involves excavation of the contaminated soil which is then placed into a desorption unit (Lighty et al., 1990) and heated to a temperature of 200 to 500°C (Ritter et al., 2017) to physically separate petroleum from the soil (USEPA, 1994). The desorption unit enhances contaminant vaporization (Lighty et al., 1990), and the vaporized contaminants are passed through a thermal oxidation combustion chamber prior to being released to the atmosphere. At this point, the soil can be returned as subsoil or potentially topsoil (O'Brien et al., 2018; Ritter et al., 2017; O'Brien et al., 2016).

Impacts of Thermal Desorption

Even though thermal desorption allows for reuse of the treated soils (O'Brien et al., 2017), reclamation efforts are still required to return agricultural production to pre-spill levels (Sierra et al., 2016). Physical (O'Brien et al., 2017), chemical (Sierra et al., 2016), hydraulic

(O'Brien et al., 2016), and biological (Cebren et al., 2011) characteristics of the soil are concerns after thermal desorption. Physical characteristics of the soil that are a concern following thermal desorption include decreased SOM (Sierra et al., 2016; O'Brien et al., 2016), destruction of clays (Sierra et al., 2016), and textural shifts when heated above 500°C (O'Brien et al., 2018). Soils treated with thermal desorption have low SOM levels due to combustion of SOM at 220°C (Sierra et al., 2016). Decreases in soil organic carbon (SOC) of more than 25% have been seen in both topsoil and subsoil treated by thermal desorption as well as a 20% decrease in total aggregation of topsoil (O'Brien et al., 2017). In addition to low SOC levels on soils treated with thermal desorption, research has found that heating of soils can increase the recalcitrance of C in the soil making it more difficult for soil microbes to mineralize. Heating of soils can result in a decrease in the O-alkyl C to alkyl C ratio which indicates preferential denaturation of carbohydrates over aliphatic components such as waxes and cutins (Certinie, 2005) which are resistant to microbial decomposition (Filley et al., 2008). Plants suffer from nutrient and water stress under soils treated with thermal desorption due to the low SOC and microbial activity (O'Brien et al., 2017). Chemical characteristics of the soil that are concerns following thermal desorption treatment include decreased CEC (Ritter et al., 2017; Sierra et al., 2016), increased bioavailability of heavy metals (Bonnard et al., 2010) increased EC, pH alterations, sulfide oxidation, and transformation of iron hydrous oxides into maghemite (Sierra et al., 2016). Hydraulic characteristics of the soil that are concerns proceeding thermal desorption treatment include decreases in water retention at field capacity and permanent wilting point and up to a 400% increase in saturated hydraulic conductivity (O'Brien et al., 2016). One of the main biological concerns with soils treated with thermal desorption is decreased microbial activity (Cebren et al., 2011).

Despite the physical, chemical, hydraulic, and biological concerns with soils following thermal desorption treatment, it is expected that treated soils can still sustain vegetation, but productivity would likely be decreased which is a concern for agricultural production (O'Brien et al., 2016). The low SOM levels of soils treated with thermal desorption make treated soil unsuitable for agriculture (Sierra et al., 2016). Thus, reclamation is required to improve agricultural production to pre-spill levels proceeding thermal desorption treatment.

Long term agricultural productivity is a concern proceeding thermal desorption treatment indicating the need for reclamation of this problem soil. A greenhouse study was conducted by O'Brien et al., (2017) to examine the soil quality as well as the productivity and safety of a wheat crop grown on soils contaminated with hydrocarbons and treated with thermal desorption. The study found that grain yield and biomass was similar between soils treated with thermal desorption and native topsoils and there was no increase in levels of polycyclic aromatic hydrocarbon in the grain from the thermal desorption treated soils. Both these findings indicate that successful reclamation of this soil could lead to safe agricultural production on the soil. However, soil organic C was 84% lower and soil cumulative respiration was 66% lower in contaminated soils treated with thermal desorption which could lead to decreased long term production of agricultural systems on these soils. Contaminated soils treated with thermal desorption also had less N cycling and tended to have higher net immobilization. Thus, soils treated with thermal desorption have shown a potential to be agriculturally productive, but reclamation is still required to maintain agricultural production over the long term.

Reclamation of Soils Treated with Thermal Desorption

Mixing soil treated with thermal desorption with native topsoil has been investigated as a potential method to improve agricultural productivity of treated soils by O'Brien et al., (2017).

Mixing contaminated soils treated with thermal desorption with native topsoil resulted in increased SOC, total N, and soil respiration. However, this was not a successful method of restoring agricultural land on soils treated with thermal desorption because wheat productivity decreased when compared to unmixed soils. The addition of soil amendments to soils treated with thermal desorption has the potential as a reclamation method to improve long term agricultural productivity due to increased SOM and associated microbial activity. The input of organic matter is generally expected to enhance soil biological properties Based on a study conducted to assess different agricultural management systems (Weyers et al., 2013), but research is needed to examine the response of microbial activity on soils treated with thermal desorption to soil amendments.

Organic Amendments

SOM plays a crucial role in maintaining soil microbial communities. It is important to soil ecosystems because it determines soil water holding capacity, impacts macropore formation, affects micronutrient adsorption (Ingram et al., 2005), supplies available nutrients (Ingram et al., 2005; Franzluebbers et al., 2000), influences soil structure (Grandy et al., 2002; Franzluebbers et al., 2000), and impacts microbial activity (Franzluebbers et al., 2000). The improvement in soil stable aggregates seen with increased SOM can provide a habitat for soil microbes and soil microbe habitats benefit from SOM due to its function as a buffer against moisture and temperature changes (Kladvko and Clapperton, 2011; Doran and Smith, 1987). This buffering against soil moisture changes results in increased moisture retention which impacts microbial communities (Curtin et al., 2012). In addition to providing habitat, SOM provides energy for microbial metabolism (Kladvko and Clapperton, 2011; Doran and Smith, 1987). Higher levels of SOM increase the quality and quantity of the energy source for soil microbes (Kladvko and

Clapperton, 2011). Organic amendments can be applied to the soil to increase SOM (Larney et al., 2011; Carter, 2002; Stewart et al., 2000).

Both soil C and N can have various impacts on microbial communities. Soil microbes may be co-limited by C and N or limited by C depending on soil management (Danielson et al., 2017). C and N are required for microbial metabolism and to build cellular constituents. (Loynachan, 2012; Myrold and Bottomley, 2008). Soil microbes depend on both inorganic nutrients (Stotzky, 1986) and organic nutrients (Myers et al., 2001; Stotzky, 1986).

The quality of N and C in organic amendments has the greatest impact on microbial decomposition. Composted products tend to have less labile organic matter than amendments with less processing (Flavel and Murphy, 2006) and the response of CO₂ flux to amendments is related to the labile C and N constituents of the amendments (Zhang et al., 2014). Readily available C additions to soil causes a rapid increase in microbial metabolic activity which results in an increase in CO₂ flux (De Nobili et al., 2001) since labile fractions of organic matter are the most susceptible to mineralization (Mukherjee et al., 2014; Cook and Allan, 1992). Higher CO₂ flux has been seen with the application of less stable amendments (Flavel and Murphy, 2006). CO₂ flux has been positively correlated with the initial N content of organic amendments (Tejada et al., 2010). The level and stability of C and N vary with different soil amendments.

Nitrogen in Organic Amendments

Organic amendments vary in their nutrient composition including total N, NH₄ concentration, NO₃ concentration, and C:N ratio (Beegle et al., 2008). Animal-based organic amendments and animal-based compost amendments tend to have a higher N content than plant-based organic amendments. Solid dairy manure, beef manure, swine manure and horse manure have a low total N content relative to other manures whereas a high total N concentration relative

to other manures is seen in solid Broiler litter. Dairy manure, beef manure, and beef feedlot manure have similar concentrations of NH_4 which is low relative to other manures whereas the NH_4 concentration of broiler litter is much higher than other manures (Beegle et al., 2008). The concentration of NO_3 is nearly 10 times higher in poultry manure (1450 mg per kg) than in manure from cattle, horse, or swine (Wortman et al., 2017). Laying hen litter has a low C:N ratio with a value between 5 and 10. Beef feedlot manure, swine, dairy manure, and broiler litter have moderate low C:N ratios with values between 10 and 15. Sheep manure and beef manure have higher C:N ratios with values between 15 and 20. Horse manure has a C:N ratio with a value near 30 (Beegle et al., 2008). Thus, the inorganic N concentration of organic amendments can vary.

Available inorganic N has varying impacts on different aspects of microbial communities. The composition of soil microbe communities is associated with N availability (Stewart et al., 2018; Zhang et al., 2016; Chu et al., 2011). Enrichment of gram-negative bacteria, arbuscular mycorrhizal fungi, and saprotrophic fungi has been seen on soils with low or no N fertilizer inputs while enrichment of actinomycetes and gram-positive bacteria has been seen on soils with higher N fertilization rates (Stewart et al., 2018). On the other hand, significantly larger fungal communities have been observed in a soil receiving 140 kg N ha^{-1} of NH_4NO_3 fertilizer compared to a control with no fertilizer. Higher microbial enzyme activity was also observed in the fertilized soil (Kirchner et al., 1993). N mineralization rates tend to increase with additions of inorganic N (Sims and Stehouwer, 2008; Jansson and Persson, 1982) and lower C:N ratios tend to result in higher N mineralization (Schlegel and Grant, 2006). This is due to the priming effect which is a phenomenon where additions of soil amendments that contain inorganic N stimulate microbial activity (Jansson and Persson, 1982; Parr and Papendick, 1978).

A $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ ratio of greater than 0.16 indicates a less stable organic amendment (Bernal et al., 1998) and higher CO_2 flux has been observed with the application of less stable amendments (Flavel and Murphy, 2006). However, microbial activity can decrease with higher soil available N concentrations after long term inorganic N additions due to acidification of soil (Zhang et al., 2013). Thus, soil inorganic N concentrations tend to increase microbial activity, but can shift the composition of the microbial community and negatively impact soil microbes when applied excessively by changing microbial metabolism.

Organic N can also impact microbial communities (McCulley and Burke, 2004). A shift in the soil microbial community composition has been observed with a shift in the fungi to bacteria ratio with higher organic N content. However, variation in the direction of the ratio shift has been observed (Allison et al., 2005). Organic N applications to soil with crimson clover green manure had larger abundance of *Bacillus* spp., actinomycetes, and culturable bacteria with increases of 260%, 310%, and 120% respectively when compared to an inorganic fertilizer application of N (Kirchner et al., 1993). N mineralization rates (Sims and Stehouwer, 2008; Schlegel and Grant, 2006) and MBC (Kirchner et al., 1993) have shown increases with additions of organic N. Microbial enzyme activity has also been higher with organic N additions than inorganic N fertilizer additions (Kirchner et al., 1993). Thus, soil organic N content can increase microbial activity and shift the composition of the microbial community.

Carbon in Organic Amendments

Organic matter contains biochemically recalcitrant compounds. Organic matter derived from woody material contains higher amounts of lignin and aliphatic components than herbaceous organic matter (Filley et al., 2008) that are biochemically recalcitrant (Filley et al., 2008; Simpson et al., 2008). Lignin can be difficult for many microbes to break down due to the

complexity of the chemical composition (Baldock and Preston, 1995). Additionally, lignin condenses with organic N which forms highly stable organic N. Since this N is no longer easily available to soil microbes, this process basically results in a higher C:N ratio (Myrold and Bottomley, 2008). Aliphatic components in organic matter include cutins, suberins, and waxes (Filley et al., 2008; Simpson et al., 2008). Suberin is more resistant to microbial attack than cutin due to a higher concentration of phenolic compounds in suberin (Simpson et al., 2008). Higher ash (Wang et al., 2004a), polyphenol, aryl C, O-aryl (Wang et al., 2004b) amorphous and crystalline methylene carbon (Simpson et al., 2008), phenolic compounds, chlorogenic acid, and other recalcitrant C compounds (Zhang et al., 2014) content also indicates a more stable organic matter that will be more resistant to microbial attack. Amorphous and crystalline methylene carbon are recalcitrant compounds that are derived from the accumulation of plant cuticles (Simpson et al., 2008). A negative correlation has been seen between CO₂ flux and lignin, polyphenol, aryl C, and O-aryl contents of amendments (Wang et al., 2004b). Thus, some organic matter contains organic compounds that are biochemically recalcitrant making the organic amendments more stable.

Other organic matter contains more labile organic compounds. Herbaceous plants produce organic matter containing higher concentrations of cinnamyl phenols, which are easier for soil microbes to break down (Filley et al., 2008). However, grasses produce higher levels of Suberins than other plants (Simpson et al., 2008). Additionally, legume plant-based organic amendments have higher concentrations of organic N than other plant-based organic amendments such as municipal yard waste, woody-based, and straw-based organic amendments (Wortman et al., 2017). A higher alkyl to oalkyl ratio indicates that the organic amendment will have a higher degradation rate. Grassland organic matter has a higher alkyl to oalkyl ratio than

pine forest organic matter (Simpson et al., 2008). Fulvic acid concentrations indicate a less stable organic matter that is more susceptible to microbial attack. Higher CO₂ flux and MBC has been observed in amendments with higher fulvic acid concentrations (Tejada et al., 2010). Thus, the C compounds and biological availability of these C compounds varies with different organic amendments.

The concentration of labile C in the soil has various impacts on microbial communities (McCulley and Burke, 2004). Easily available labile C impacts the composition of soil microbial communities with a greater diversity of microbes seen with higher labile C (Mula-Michel and Williams, 2013). Higher levels of bacteria are seen with higher labile nutrients compared to higher levels of fungi with higher amounts of recalcitrant compounds (Myers et al., 2001). Higher microbial activity has been seen with SOM containing more labile C as opposed to SOM with more recalcitrant C (Osterholz et al, 2017). The vast majority of labile C measured in soils has been emitted as CO₂ flux over an incubation as a result of microbial respiration (Dere and Stehouwer, 2011). Additions of labile C to soil can result in microbial immobilization of N rather than mineralization (Sims and Stehouwer, 2008; Schlegel and Grant, 2006). Thus, the concentration of labile C in the soil can impact microbial community composition and activity.

The soil organic C content and total C content can impact microbial communities. Changes in microbial community composition have been seen with changes in levels of organic C (Blanco-Canqui et al., 2015; Allison et al., 2005) and total C with fungal to bacterial ratios decreasing with decreasing total C (Acosta-Martinez et al., 2010). Microbial respiration is dependent on the organic C content of soils (Stepniewski et al., 2011) and a decrease in soil respiration has been seen following a decrease in soil organic C (Danielson et al., 2017). Additionally, lower organic C concentrations is associated with lower MBC (Blanco-Canqui et

al., 2015; Stepniewski et al., 2011) and microbial activity (McCulley and Burke, 2004). Thus, the organic C content and total C content of soils can impact microbial community composition, activity, and biomass C.

Response of CO₂ Flux to Amendments

Additions of organic amendments, like manure increased soil CO₂ flux with an initial rapid mineralization of manure C (Rogovska et al., 2011). Pelletized poultry manure, green waste compost, and straw based compost all increased CO₂ flux in a laboratory incubation relative to a control when applied at rates of 30 m cubed ha⁻¹ (12 g dry material per 500 g soil). Compressed poultry manure resulted in the highest CO₂ flux followed by green waste compost, straw based compost and the control with the lowest CO₂ Flux. However, by day 142, only the compressed poultry manure and green waste compost had CO₂ -C flux rates significantly higher than the control (Flavel and Murphy, 2006). A laboratory study was conducted where spent coffee grounds, wood pellets, and horse bedding compost were applied at rates of 15 Mg ha⁻¹ and an increase in soil CO₂ flux was seen with all amendments relative to the control. Wood pellets resulted in the highest CO₂ flux followed by spent coffee grounds and horse bedding compost (Zhang et al., 2014). Unlabeled decomposable organic material was applied to soil in laboratory incubations and resulted in an increase in soil CO₂ flux over the first month when compared to a control without organic matter amendment. After the first month, the CO₂ flux of the amendment soil decreased to near the level of the control soil indicating that the increase in CO₂ flux was related to the increase in biological activity while decomposing the organic amendment (Sorensen, 1974). A study analyzing the response of CO₂ flux to humic acid additions found that the humic acid did not impact CO₂ flux (Mukherjee et al., 2014). Thus, the application of organic amendments has the potential to increase microbial activity and associated CO₂ flux.

Research has also examined the response of CO₂ flux to biochar and other organic amendments processed with heating. When biochar was formed from spent coffee grounds, wood pellets, and horse bedding compost at 700°C, there was no impact on soil CO₂ flux (Zhang et al., 2014). Similarly, biochar formed by heating at 650°C also had no impact on CO₂ flux (Mukherjee et al., 2014). Negative impacts on soil CO₂ flux has also been seen with the application of biochar produced by heating at 600-800°C with an 88% decline in CO₂ flux relative to the control with no amendment (Kammann et al., 2012). On the other hand, a significant increase in soil CO₂ flux has been observed with the application of biochar formed by heating at 400-500°C with the CO₂ flux increasing as biochar applications increased from 5 to 20 g kg⁻¹ (Rogovska et al., 2011). Therefore, the temperature that biochar was formed at may impact the properties of the amendment which influences CO₂ flux (Zhang et al., 2014). A minimal increase in CO₂ flux was observed with black carbon applications to soil when compared to a control (Major et al., 2010). The application of hydrochar to soil resulted in a significant increase in CO₂ flux relative to a control (Kammann et al., 2012). Thus, organic amendments formed by the heating of organic matter at high temperatures has variable impacts on CO₂ flux and the response of CO₂ flux may be dependent on the temperature that the amendment was formed at.

Relationship Between Microbial Activity and Soil Moisture Content

Soil water holding capacity can be increased by the addition of organic amendments to increase SOM. This benefits microbial habitat (Kladivko and Clapperton, 2011; Doran and Smith, 1987) and can result in an increase in microbial activity (Rochette et al., 1999; Sommers et al., 1981). Organic amendments generally increase moisture retention due to improved soil structure, increased water infiltration, and decreased crusting (Unger and Stewart, 1983). A 1% increase in SOM can result in a 1.8% increase in available water retention. However, a 1%

increase in SOM to a depth of 30 cm requires the addition of about 45 Mg ha⁻¹ of dry organic material, but repeated applications may be required due to not all additions becoming stabilized SOM (Unger and Stewart, 1983). Feedlot manure applied at rates of 22 and 67 Mg ha⁻¹ increased the water intake by 10 to 15% as well as the water holding capacity of soils when compared to soils amended with inorganic fertilizers (Mathers et al., 1977). Thus, organic amendments have the potential to increase the water holding capacity of soils which can benefit microbial habitat.

Drought can have negative impacts on microbial activity. Drought can result in a prolonged dormancy period or direct mortality of soil microbes due to dehydration in severe cases (Schimel et al., 2007). A field study in a Mediterranean forest conducted with 6 years of drought manipulation found that microbial activity was negatively impacted by drought. Urease activity was 25% lower in autumn, winter, and spring with drought conditions when compared to regular precipitation. Drought conditions resulted in a 33% decline in protease activity in winter and spring. Glucosidase activity was decreased by 25-30% in summer and spring with the drought conditions when compared to regular precipitation. N mineralization rates were also lower in the soil under drought conditions (Sardans and Penuelas, 2010). A field study conducted over a growing season in Quebec comparing tillage to no-till observed soil respiration rates well below the respiration-temperature curve during a drought indicating that soil respiration was limited by soil moisture (Rochette et al., 1999). An additional field study that was conducted in South Carolina observed a significant increase in MBC following a rain event that ended a drought indicating that the drought conditions may have been negatively impacting MBC. During the drought, high topographic landscapes had lower N mineralization than low topographic landscapes which was attributed to higher soil moisture in the low topographic landscapes (Ricker and Lockaby, 2014). Thus, negative impacts on microbial activity measured

through enzyme activity, N mineralization, and MBC have been seen in response to drought, but soil microbial activity has been found to continue over the full range of soil moisture content (Sommers et al., 1981).

On the other hand, soil moisture above optimum levels can have a negative impact on microbial activity. Microbial growth is limited at high soil moisture content by low oxygen supplies or toxic gas build up due to slow diffusion of air through the water (Parr and Papendick 1978). Decreases in decomposition rates has been seen with an increasing length of time that a soil is anaerobic (Reddy and Patrick, 1975). A laboratory study found that minimal N mineralization took place in a saturated soil (Sindhu and Cornfield, 1967). A decrease in N mineralized was also seen by Stanford and Epstein (1974), when soil moisture content was above optimum. However, this may have been due to denitrification rather than alterations in microbial activity. A soil incubated under anaerobic conditions for 128 d showed more than a 50% decrease in soil respiration relative to soils incubated under aerobic conditions (Reddy and Patrick, 1975). Similar results were seen in a laboratory study where soil was incubated for 1 year with sludge. Soil respiration was 50% lower under the saturated conditions when compared to a soil with a water potential of 0.3 bars (Sommers et al., 1979). Thus, research has found that saturated soil conditions have the potential to have a significant negative impact on microbial activity.

A relationship has also been seen between MBC and soil moisture content. A field study examined MBC across a 500 mm regional precipitation gradient in the central Great Plains and found that MBC increased across the gradient as precipitation increased (McCulley and Burke, 2004). In a study using undisturbed topsoil, MBC was found to be highest in moist winter soils and was found to be lowest after prolonged dry hot periods in summer (Van Gestel et al., 1992).

A field study conducted in South Carolina found a significant positive correlation between MBC and precipitation in wetter transitional and back-swamp landscapes as well as a significant positive correlation between MBC and soil water holding capacity on the flat and transitional landscapes (Ricker and Lockaby, 2014). On the other hand, a laboratory study found no consistent trend between MBC and soil water potential for the range of soil water potentials of -0.05 bars to -12 bars but found the relationship between MBC and temperature to be linear (Curtin et al., 2012). Thus, research has indicated that there may be a relationship between soil moisture content and MBC.

Soil moisture content has a significant influence on N mineralization (Sommers et al., 1981). Several studies have found a linear relationship between N mineralization and soil moisture content with N mineralization increasing as soil moisture increases. A laboratory study was conducted that examined the relationship between soil water content, matric suction, and N mineralization on nine different soils that had different chemical and physical properties. A nearly linear relationship was observed between soil moisture content and N mineralized with N mineralization declining from field capacity to wilting point (Stanford and Epstein, 1974). A second laboratory study also observed a linear relationship between N mineralization and soil gravimetric water content with Ammonifiers being more tolerant of moisture stress than nitrifiers (Curtin et al., 2012). A laboratory study found that N mineralization decreased continuously with decreasing soil water content from a soil water potential of -0.30 bars to 15.0 bars. Microbial activity was found to be more sensitive to soil moisture content than C availability (Sierra and Marban, 2000). Thus, several laboratory studies have found a linear relationship between N mineralization and soil moisture content.

Other studies have found the relationship between N mineralization and soil moisture content to be non-linear. The ideal moisture content for net N mineralization was found to be between a soil water potential of -0.10 bars and -0.30 bars in a laboratory study. No net N mineralization occurred at a soil water potential near -40.0 bars. Some of the soils analyzed in this study showed a curvilinear relationship between net N mineralization and soil moisture content (Myers, 1982). An additional laboratory study found that the ideal soil water potential for N mineralization was from -0.30 bars to -3.00 bars. N mineralization began decreasing at -5.00 bars (Sindhu and Cornfield, 1967). Thus, research has been very consistent in finding that there is a relationship between N mineralization and soil moisture content, but whether this relationship was linear or non-linear varies.

Soil CO₂ flux is also strongly dependent on soil moisture content (Rochette et al., 1999; Sommers et al., 1981). Similar to the findings on N mineralization, research found a linear relationship between CO₂ flux and soil moisture content with CO₂ flux increasing with increases in soil moisture content. In a laboratory study, a linear relationship was seen between CO₂ flux and gravimetric moisture content. Minimal decreases in soil water potential below -0.05 bars resulted in relatively large decreases in CO₂ flux. CO₂ flux decreased by 13% on average with a decrease in soil water potential from -0.05 bars to -0.20 bars (Curtin et al., 2012). In another laboratory study, a linear relationship was seen between soil moisture content and CO₂ flux over four wetting-drying cycles for a range of water potentials from -0.10 bars to -85.0 bars as long as microbial activity was not limited by the availability of substrate in the soil. A 10% decrease in CO₂ flux was seen as soil moisture decreased from -0.10 bars to -0.20 bars (Orchard and Cook, 1983). Thus, research has found a linear relationship between CO₂ flux and soil moisture content.

Other research has found a non-linear relationship between soil CO₂ flux and soil moisture content. A field study conducted over a growing season in Quebec observed increased soil CO₂ flux following a rain event and subsequent rewetting of dry soil (Rochette et al., 1999). In a study using undisturbed field soil, microbial activity measured through soil CO₂ flux increased significantly with increasing soil moisture content, but CO₂ flux reached a plateau where there was no significant difference in CO₂ flux between different soil water potentials (Schjonning et al., 2003). A field study conducted in Southern Finland found that soil CO₂ flux was strongly dependent on soil moisture content (Pumpanen et al., 2003). A field study was also conducted on a grassland soil of the arid shrub-step that found that temperature and soil moisture content are interdependent in their effects on soil CO₂ flux. Microbial activity was impacted by a 1 to 2% change in soil moisture content. Higher soil moisture resulted in higher soil CO₂ flux, but this relationship was non-linear. This study determined that the interaction of soil moisture content and temperature accounts for 70% of the variation in soil CO₂ flux rate. From -0.30 bars to -15.0 bars, soil CO₂ flux declined rapidly. After this rapid decrease, a linear relationship was seen between soil moisture content and soil CO₂ flux down to a soil water potential of -80.0 bars (Wildung et al., 1975). A similar relationship was seen in a laboratory study conducted by Laura, 1974 where a rapid decrease in CO₂ flux was observed from -0.05 bars to -10.0 bars. This was followed by a linear decrease in CO₂ flux to a soil water potential of -100 bars. A laboratory study found that decreasing soil water potential from -0.10 bars to -100 bars resulted in a 100 to 1000-fold decline in CO₂ flux (Nyhan, 1976). Thus, research has found a relationship between soil CO₂ flux and soil moisture content, but findings vary on whether this relationship is linear or non-linear.

Additionally, a study examined the sources of soil respiration over different soil water potentials. Bacterial respiration was found to rapidly decrease from -3.00 bars to -6.00 bars and it continued to decline until reaching a soil water potential of -20.0 bars where bacterial respiration made up a very slight part of total respiration. Soil respiration was still maintained by other soil microorganisms until a soil water potential of -30.0 bars. At this point, soil respiration began to decline until it was negligible at -50.0 bars. Amending the soil with glucose did not improve soil respiration when the soil water potential was under -50.0 bars (Wilson and Griffin, 1975). Thus, even though microbial activity may continue into lower soil moisture levels, there may be shifts in the type of soil microorganisms that are active as the soil transitions to a lower soil water potential.

Soil water content is one of the most important factors in decomposition rate (Zak et al., 1999; Sommers et al., 1981; Parr and Papendick 1978). A qualitative indication of the importance of soil water content to decomposition rate can be seen with the high organic matter content of poorly drained soils relative to well-drained soils (Sommers et al., 1981). This is a result of declines in decomposition as water content nears saturation (Reddy and Patrick, 1975). However, decomposition occurs over the full range of soil moisture content (Sommers et al., 1981). In a study examining decomposition of rice straw, decomposition was maximized at a soil water potential of -0.30 bars (Pal and Broadbent, 1975). Based on a review of research examining the relationship between decomposition and soil water potential, decomposition has a two-phase process as soil moisture declines. Decomposition declines rapidly from a soil water potential of -0.30 bars to -10.0 bars which is followed by a range of soil water potentials where decomposition has a linear relationship with soil water potential (Sommers et al., 1981). Thus,

decomposition declines in saturated soil, but decomposition takes place over the full range of soil moisture levels.

Measuring CO₂ Flux Over Drying-Wetting Cycles

A characteristic decomposition pattern has been observed upon rewetting of dried soils with rapid decomposition that lasts for a few days followed by a slow and steady decomposition rate. This pattern continues over consecutive drying-wetting cycles. Similar patterns of decomposition have been observed under field conditions with dry-wet seasons. Therefore, regions with distinct dry-wet seasons have similar conditions and responses to simulated drying-wetting cycles in laboratories (Birch, 1958) with a significant portion of annual CO₂ flux in arid, semi-arid, and Mediterranean environments being seen after a rain event. Surface soils experience large fluctuations in moisture content particularly with shifts in seasons (Fierer and Schimel, 2003); Franzluebbers et al., 2000). Thus, drying-wetting cycles in laboratories can simulate soil conditions in regions with dry-wet seasons and natural fluctuations in surface soil.

Drying-wetting cycles can impact CO₂ flux from soils. A 2-6 d pulse in CO₂ production is often observed upon rewetting of a dry soil. A 370 to 475% increase in CO₂ flux has been observed on rewet soils compared to the CO₂ flux levels prior to soil drying with a total of 36 to 71 micrograms CO₂-C per g soil released over 6 days (Fierer and Schimel, 2003). CO₂ flux was increased by 16 to 121% over incubations of 260 to 500 d with rewetting every 30 days compared to controls maintained at a continuous moisture content (Sorensen, 1974). A sharp increase in microbial activity was observed upon rewetting of soils with a 40-fold increase in soil CO₂ seen when the change in water potential after rewetting of the soils was greater than 50 bars (Orchard and Cook, 1983). A laboratory study that measured CO₂ flux over 60 rewetting events found that 31.2, 18.0, and 17.0% of C was lost as CO₂ with soils dried at 80 degrees C, 30

degrees C, and un-dried respectively (Jager, 1975). A field study conducted over a growing season in Quebec comparing tillage to no-till observed increased soil CO₂ flux following a rain event and subsequent rewetting of dry soil (Rochette et al., 1999). A laboratory study with alternating flooding and drying found that soil CO₂ flux spiked after draining compared to a soil with no flooding and draining. However, within 3 d after draining, there was no significant difference between the soil with alternating flooding and draining and the soil with no flooding. The soil without alternated flooding and draining had the lowest MBC, but the soils with the lowest water table after draining had the highest MBC among the soils with alternated flooding and draining (Morris et al., 2004). Thus, drying-wetting cycles can impact microbial activity as seen in the increase in CO₂ flux proceeding rewetting of dried soils.

Different explanations have been proposed for the increase in CO₂ flux upon rewetting of dried soils. It has been proposed that the magnitude of decomposition depends on the % C in the soil as well as the degree of drying that the soil underwent. The decomposition is a result of direct microbial attack of the solid soil substrate. The pattern of decomposition with drying-wetting cycles is a result of the state of the microbial community following drying and the behavior of the microbial community upon rewetting (Birch, 1958). The pulse of microbial activity is due to rapid mineralization of organic C (Barnard et al., 2020; Guo et al., 2014) with the size of the CO₂ pulse being dependent on the level of available C in the soil (Barnard et al., 2020; Waring et al., 2016) and a larger pulse promoted by greater availability of labile organic C (Waring et al., 2016; Jenerette and Chatterjee, 2012). Another explanation for the pulse in CO₂ flux upon rewetting of dried soils is that the drying-wetting process releases SOM that was previously physically protected (Brangari et al., 2020; Waring et al., 2016) with increases in extractable SOM-C of up to 200% (Fierer and Schimel, 2003). Bonds between soil particles or

clay leaves can be broken over drying-wetting cycles resulting in a release of physically protected SOM, but this SOM released may be stable and therefore resistant to decomposition particularly with consecutive drying-wetting cycles (Degens, 1995). Increased diffusion of available C due to high soil water content may also be an explanation for the increase in CO₂ flux upon rewetting of dried soils (Brangari et al., 2020; Waring et al., 2016; Guo et al., 2012). The increase in CO₂ flux upon rewetting of dried soils may also be due in part to reestablishment of the microbial community proceeding microbial death resulting from the drying process (Haney et al., 2004). An additional explanation is that the pulse of CO₂ is generated by the rapid mineralization of highly enriched intracellular compounds (cytoplasmic solutes) which is a response of microbial biomass to the rapid increase in soil water potential. However, frequent drying-wetting cycles may select for soil microbial communities that are capable of adjusting to rapid increases in water potential without rapidly mineralizing their accumulated cytoplasmic solutes. Research has found that drying-wetting cycles do not result in significant cell lysis contributing to the increase in CO₂ flux with increases in MBC being observed after rewetting (Fierer and Schimel, 2003), but osmotic shock and osmoregulation in response to rewetting has been proposed as a potential explanation for the increase in CO₂ flux (Brangari et al., 2020; Haney et al., 2004). However, Kakumanu et al., (2013) found that osmolyte accumulation is not a major strategy of microbial drought resistance. Brangari et al., (2020) found that microbial synthesis of biomass, osmoregulation, mineralization by cell residues, and dormancy accounted for 75%, 21%, 4%, and less than 1% of respiration upon rewetting of soils respectively. An additional benefit of air-drying soils prior to analysis is that it can avoid biochemical artifacts that can be seen in soils that are kept moist (Haney et al., 2004). Thus, various explanations have been proposed for the increase in CO₂ flux upon rewetting of dried soils.

A method of evaluating soil microbial activity is measuring CO₂ flux upon rewetting of dried soils (Franzluebbbers et al., 2000) since CO₂ flux released from soil is due in part to microbial respiration as soil microbes decompose SOM (Tonitto et al., 2016). It is possible to predict N availability based on CO₂ flux (Franzluebbbers et al., 2018) because increased microbial activity implies increased nutrient cycling (Laird et al., 2010), and increased nutrient cycling has the potential to lead to increased primary production (Major et al., 2010). Microbial activity is a key component of soil quality (Franzluebbbers et al., 2000) due to soil microbes ecosystem functions such as decomposition (Acosta-Martinez et al., 2010) and nutrient cycling (Acosta-Martinez et al., 2010; Sardans and Penuelas, 2010) and higher CO₂ flux indicates higher soil quality (Franzluebbbers et al., 2000). CO₂ flux proceeding rewetting of dried soils is a rapid and robust indicator of soil-test biological activity (Franzluebbbers et al., 2018). Measuring CO₂ flux upon rewetting of dried soils responds rapidly to changes in soil management (Haney et al., 2004; Franzluebbbers et al., 2000) and can be useful when evaluating the impact of organic and inorganic amendments on soil function (Haney et al., 2004). It is also considered to be an effective method of evaluating the recovery of reclaimed soils (Ingram et al., 2005) as well as useful for assessing soil C stabilization and nutrient mineralization in agroecosystems (Hurisso et al., 2016).

CO₂ flux is related to N mineralization (Franzluebbbers et al., 2018; Haney et al., 2001), MBC (Franzluebbbers et al., 2000); Haney et al., 1999; Anderson, 1982), and POXC (Hurisso et al., 2016). CO₂ flux upon rewetting of dried soils explains 67% of the variability in net N mineralization and 86% of the variability in MBC (Franzluebbbers et al., 2000). In a study with compressed poultry manure, green waste compost, and straw based compost, gross N mineralization, MBC and CO₂ flux increased in response to amendment applications relative to

the control (Flavel and Murphy, 2006). In a study with spent coffee grounds, wood pellets, and horse bedding compost, both MBC and CO₂ flux increased in response to these amendments, but neither MBC or CO₂ flux was impacted by these amendments when heated to form biochar (Zhang et al., 2014). A relationship has been seen between POXC and CO₂ flux in 13 studies over 76 total sites with a range of soil types, management histories, and geographic locations (Hurisso et al., 2016). Wang et al., (2003b) found a close relationship between POXC and CO₂ flux upon rewetting of dried soils but found a weaker correlation between MBC and CO₂ flux. Thus, relationships have been seen between CO₂ flux, N mineralization, MBC, and POXC.

MATERIALS AND METHODS

Site Description

For this laboratory study, four soils were sampled from two different locations in North Dakota. Site 1, saline- and site 2, non-saline-samples were collected within 0-15 cm depth from an agricultural field near Grand Forks, North Dakota, (-97.2196, 47.8746) (Fig. 1a). Site 3, A previously Bakken oil contaminated subsoil treated using thermal desorption was sampled from an oil spill site located in western North Dakota (-102.8759, 48.5224) (Fig. 1b) and site 4, same subsoil material from site 3 but non-contaminated and non-treated using thermal desorption.

Table 1. Initial soil properties of four experimental sites

Soil Properties	Site 1: Saline	Site 2: Non-Saline	Site 3: Treated	Site 4: Non-Treated
Field Capacity g g ⁻¹	0.311	0.376	0.233	0.234
Total C g kg ⁻¹	47.0	49.0	20.0	21.0
Inorganic C g kg ⁻¹	13.0	2.00	11.0	11.0
Organic C g kg ⁻¹	34.0	47.0	9.00	10.0
%OM	5.08	7.62	0.66	0.89
Total N g kg ⁻¹	3.12	4.22	0.05	0.01
Available P g kg ⁻¹	0.018	0.005	0.006	0.002
pH _{1:1}	7.51	7.63	7.78	8.00
EC dS m ⁻¹	2.70	0.54	1.19	0.96
CEC cmol _c kg ⁻¹	27.8	n/a	15.7	n/a
Carbonates g kg ⁻¹	109	15.0	94.0	93.0

C: Carbon; OM: organic matter; N: nitrogen; P: phosphorus, EC: electrical conductivity; CEC: cation exchange capacity

For Site 3, the subsoil material was treated using thermal desorption to achieve a total petroleum hydrocarbon (TPH) concentration of less than 500 mg/kg. Concentration of TPH was not determined for this experiment. Site 1 (saline) was topsoil from an agricultural wheat-soybean rotation on the saline phase of the Bearden series. The Bearden series has a silty clay loam A horizon and is classified as a Fine-Silty, Mixed, Superactive, Frigid Aeric Calciaquoll. This series is somewhat poorly drained and has a seasonal high-water table in April through June

that is at a depth of 1.5 to 3.5 feet (National Cooperative Soil Survey,) (Table 1). Site 2 was topsoil from an agricultural wheat-soybean rotation on the Bearden series.

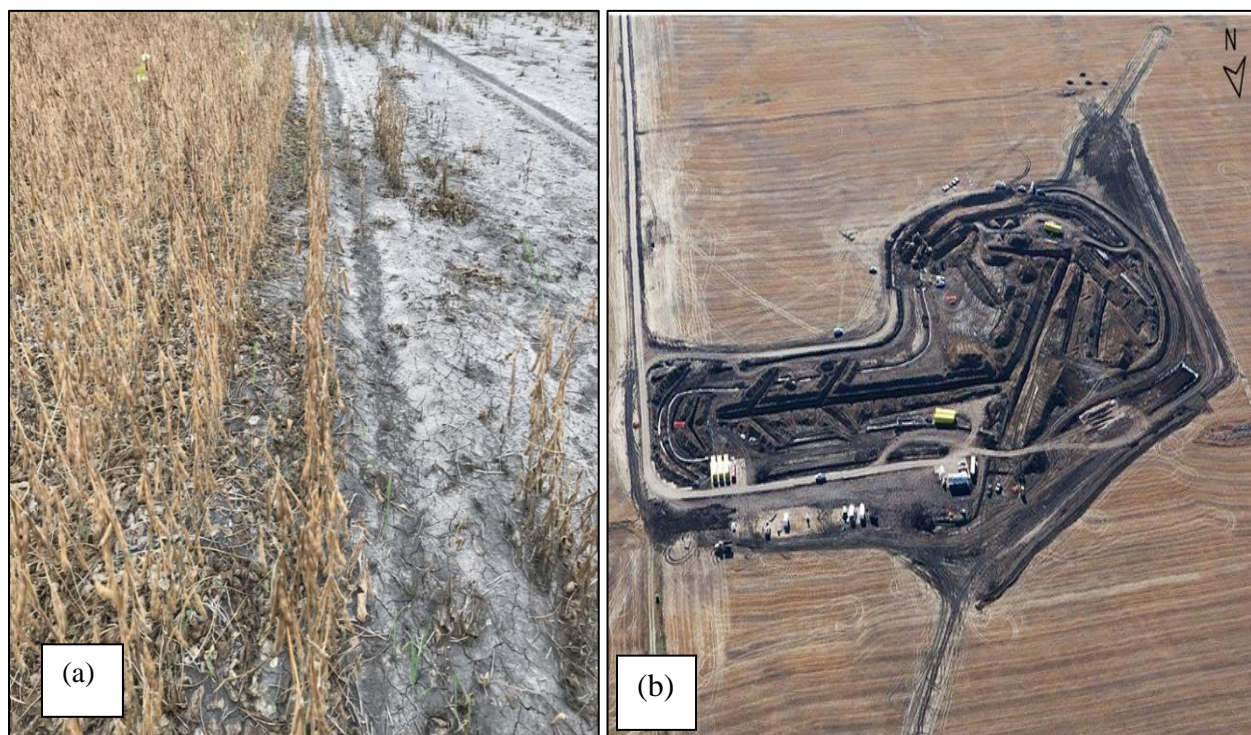


Figure 1. (a) Saline site at Grand Forks, ND, and (b) Tioga-Tesoro oil spill site located in western North Dakota

Sites 1 and 2 are moderately to slowly permeable soils that formed in calcareous silt loam and silty clay loam lacustrine sediments. They are located on glacial lake plains with slopes of 0 to 3%. The mean annual precipitation is 45.7 cm and the mean annual air temperature is 3.89°C. The topsoil is typically very hard, friable, slightly sticky, and slightly plastic with many fine pores. It has a strong to violent effervescence and is slightly alkaline. This soil series is nearly all cropped with small grains and row crops such as sugar beet (National Cooperative Soil Survey).

Site 1 had a lower field capacity than Site 2 with a difference of 0.065 g g⁻¹. The total C was very similar between Site 1 and Site 2. Site 1 had higher inorganic C and lower organic C (Table 1). Site 2 had higher organic matter than Site 1. Total nitrogen (N) was similar between Site 1 and Site 2, but Site 1 had higher P than Site 2. Thus, despite Site 1 and Site 2 being the

same soil series, there were large differences seen in some of the soil properties due in part to the salinity. Site 3 was hydrocarbon contaminated subsoil that was treated with thermal desorption from the Tioga-Tesoro oil spill site. The subsoil at this site was clay loam. Site 4 was non-contaminated and non-treated native subsoil. Sites 3 and 4 consist of surface soils having soils that are moderately slow to slowly permeable and they formed in calcareous glacial till. They are located on glacial till plains and moraines. The mean annual precipitation is 35.6 cm and the mean annual air temperature is 4.44°C.

Many of the soil properties were very similar between Site 3 Treated and Site 4 Non-Treated (Table 1). Total N and available phosphorus (P) were slightly higher in Site 3 Treated. Thus, the thermal desorption process had little impact on the subsoil properties relative to non-treated subsoils.



Figure 2. Biochamber used for the incubation of soil samples and CO₂ flux measurement for the study

In this laboratory study, 200 g of air-dried soil sieved to 2 mm were placed into 2 L biochambers from Vernier Software and Technology. Each biochamber was covered with a lid

that had two holes in the top. One hole was left open for the entire incubation. This allowed the soils to air-dry over the duration of the incubation. It also allowed for gas exchange, so CO₂ and other gasses released from the soil were not accumulating in the biochamber between readings. The CO₂ gas sensor was inserted into this open hole for measurements. The other hole in the top of the biochamber was permanently plugged for the duration of the incubation. This was to create a closed system for all CO₂ readings (Fig. 2). The biochambers were not in controlled temperature for the incubation, but the temperature of the laboratory was approximately 25°C.

Table 2. Nutrient levels of selected amendments for the incubation study

Amendment	Total N g/kg	Total C g/kg	C:N ratio
Compost	20.0	190	9.50:1
Spent lime	3.20	130	40.6:1
Proganics	44.0	460	10.5:1

Treatments

Incubations were carried out on all 4 sampled soils with 4 treatments. The treatments were (i) a control with no amendment, (ii) spent lime, (iii) composted beef manure, and (iv) Proganics Biotic Soil Media. Spent lime was applied at a rate of 22.4 Mg ha⁻¹ which was 57 g for each biochamber. Similar rates have been applied by both farmers and researchers with sugar beet farmers applying 8.97 to 17.93 Mg ha⁻¹ and researchers applying 6.73 to 56.0 Mg ha⁻¹ (Windelsl, 2017). Composted beef manure was also applied at a rate of 22.4 Mg ha⁻¹ which was 57 g for each biochamber. The application rate of composted beef manure varies widely in previous research from 14.5 to 58 Mg ha⁻¹ on a dry weight basis (Helton et al., 2008). In a study that examined soil CO₂ emissions in response to beef manure at the North Dakota State University research farm in Fargo, ND, beef manure was applied at a rate of 34 and 20.2 Mg ha⁻¹ in consecutive years (Niraula et al., 2019). Other research measuring soil CO₂ flux applied composted beef manure at rates of 46 and 76 Mg ha⁻¹ on a wet weight basis (Sadeghpour et al.,

2016). Proganics was applied at a rate of 5.61 Mg ha⁻¹ which was 15 g per biochamber. The company has recommended application rates for various SOM ranges listed on their web site (<https://www.profileevs.com/products/engineered-soil-media/proganics-biotic-soil-media-bsm>).

Spent Lime is a by-product of the purification process of sugar beet sugar (Sims et al., 2018; Windels, 2017). It is formed by heating mined calcium carbonate limestone to form calcium oxide and CO₂. These products are then injected into the product produced from sugar beet processing which is a thick juice (Sims et al., 2018). Calcium carbonate precipitates adsorbing many impurities from the juice in the process. This results in about 453,592 Mg of spent lime generated as a byproduct annually from the seven sugar beet processing plants in North Dakota and Minnesota (Windels, 2017; Sims et al., 2018). The spent lime has traditionally been stockpiled on site, but sugar beet processing plants are running out of space to store this byproduct. As a result, it is available as a free or inexpensive soil amendment (Sims et al., 2018).

The absorption of impurities by the calcium carbonate results in spent lime containing various macro and micronutrients. However, the chemical composition of spent lime can vary widely and the chemical form of nutrients in spent lime is not known. The lack of knowledge regarding the chemical composition of spent lime stems in part from variation in the impurities absorbed by the calcium carbonate based on different impurities in the sugar beet plant with differences in regions, environments, and soils. Total N content in spent lime varies from 2.60 g kg⁻¹ to 5.10 g kg⁻¹ with the most common range being from 3.00 g kg⁻¹ to 3.60 g kg⁻¹ (Sims et al., 2018). The spent lime used in this research fell within the common range for total N content (Table 3). Based on spent lime analysis by the sugar beet processing plants, organic N makes up part of the total N in spent lime (Sims et al., 2018). The spent lime used in this research contained 130 g/kg total C (Table 2). The C:N ratio was 40.6:1 (Table 2). Total P content of

spent lime ranges from 3.47 g/kg to 7.20 g/kg. However, the concentration of plant-available P in spent lime is unknown. K concentrations range from 0.528 g/kg to 4.31 g/kg.

Ca and Mg concentrations vary in spent lime, but spent lime contains large amounts of both elements in the form of carbonates. The level of Ca tends to be about 20 times higher than the level of Mg. The concentration of Na ranges from 0.191 g/kg to 1.19 g/kg with the most common range from 0.250 to 0.350 g/kg (Sims et al., 2018). Spent lime has 86% of the acid neutralizing power of fresh lime (Windels, 2017; Sims et al., 2018). Thus, the knowledge regarding the chemical composition of spent lime is still limited, and variation in spent lime composition adds to this difficulty, but it is known that spent lime contains various macro and micronutrients.

The composting process can alter the nutrient content of beef manure, but the application of composted beef manure to soil can significantly increase NO_3 and P concentrations in the soil (Ferguson et al., 2005). Studies have found that composted beef manure has a lower C content (161 g/kg) compared to stock piled beef manure or fresh manure with C levels of 248 g/kg and 314 g/kg respectively (Larney et al., 2006). The total C content of the composted beef manure used in this study was slightly higher (190 g/kg) (Table 2). A significant amount of the labile C of beef manure is mineralized during composting leaving a humus like substance (Larney et al., 2006; Hao et al., 2004). Beef manure contains about 6 g/kg of total N (UNL Water, 2018) with various impacts on total N and inorganic N reported from the composting process. However, the composted beef manure used in this study contained higher total N than the reported value (20.0 g/kg) (Table 2). Even after the composting process, composted beef manure typically has a higher N content than plant-based organic amendments (Wortman et al., 2017). Some research has found that total N content is not significantly different between composted, stockpiled, and

fresh beef manure (Larney et al., 2006) while other studies have found that the composting process decreases the total N content of beef manure (Miller et al., 2009). However, most of the inorganic N in manure is either immobilized or lost to denitrification, ammonia volatilization or leaching (Miller et al., 2009). Some research has found a decrease in inorganic N from the composting process with only 5.1% inorganic N in composted beef manure compared to stockpiled beef manure with 28.9% inorganic N and fresh beef manure with 24.7% inorganic N (Larney et al., 2006). Other research has found between 5% (Miller et al., 2009) and 25% of total N in inorganic forms (UNL Water), 2018). Research has found that composted beef manure has a lower C:N ratio than fresh manure with a ratio near 11:1 (Larney et al., 2006) which is similar to the C:N ratio of 9.5:1 for the composted beef manure used in this study (Table 2). Composted beef manure has been reported to have a P concentration of 5.5 g/kg (Larney et al., 2006). Manure contains other constituents that may include Ca, Mg, Mo, Cu, Zn, and soluble salts, (Sims and Stehouwer, 2008). The benefit of composted manure over fresh manure is that composted manure doesn't contain pathogens and parasites (Larney et al., 2011).

Proganics is a commercial product that was designed as a topsoil alternative for disturbed soils including soils disturbed by construction, mining, and energy development. Peer reviewed published literature regarding this amendment was unable to be found, so the information about this amendment was obtained from the producer's web site. It was designed to be beneficial for soils that have difficulties sustaining vegetative establishment due to low organic matter, low nutrients, or low biological activity. This product is not a direct replacement for topsoil. Rather, it was designed to provide a source of organic matter and soil building components to modify soil chemistry to favor growth establishment and development of O and A horizons. It was engineered to optimize establishment and growth of vegetation as well as moisture retention.

Proganics contains various components that are derived from renewable resources and natural products and is similar to compost. This product is composed of thermally processed bark and wood fibers (89%) and a blend of polysaccharide polymers, biochar, seaweed extract, humic acid, endomycorrhizae, and bacteria (11%). The bark and wood fibers were phytosanitized with the purpose of eliminating weed seeds and pathogens. Proganics is made up of 94% organic matter and has a water holding capacity of greater than or equal to 900%. Proganics has a pH of 6.0 (+ or – 1). It contains 44.0 g/kg total N and 460 g/kg total C (Table 2). The C:N ratio is reported as 50:1 (+ or – 10). However, the C:N ratio determined for the Proganics used in this study was 10.5:1 (Table 2). It is biodegradable in the environment and passed the EPA's metal limits as well as the pathogen reduction based on 40 CFR 503 Class A Compost.

Soil Properties

Total C, inorganic C, organic C, total N, P, and carbonates were analyzed by Agvise Laboratories following the standard methods of Agvise Laboratories. Field capacity for the soils was determined using the standard pressure plate method which was described by Barasa, (2014). SOM was estimated using the weight loss-on-ignition method which is a modification of the method described by Ben-Dor and Banin (1989) and later described by Nelson and Sommers (1996) which has been considered one of the most effective methods for evaluating SOM (Roper et al., 2019). Soil was ignited in a muffle furnace at 400 degrees C for 16 hours and the SOM was determined by the difference in weight from an oven-dried sample and an ignited sample. The pH was determined with a 1:1 soil to water ratio w/v with 10 g of soil and 10 ml of distilled water which was described by Van Lierop (1990) and is one of the most common soil to water ratios recommended for pH determination (Van Lierop, 1990). The pH was measured using a Beckman Coulter 340 pH meter. The electrical conductivity (EC) was measured using a 1:1 soil

water ratio with 10 g of soil and 10 ml of distilled water as described by Smith and Doran (1996). EC is a surrogate measure of salinity, and this has been the standard measure of salinity that has been used in plant salt-tolerance studies (Corwin and Yemoto, 2017).

Cation exchange capacity (CEC) was determined using a sequential extraction of soil by 20 ml of 0.05 M calcium acetate, followed by 50 ml of 0.05 M sodium oxalate. The EC of the suspension was determined and a regression equation from the calibration curve was used to calculate CEC from the EC. The regression equation was $CEC = EC * -14.95 + 113.2$ and the r squared value on the calibration curve was 0.996.

Soil CO₂ Flux

Soil CO₂ flux was measured over incubations with three drying-wetting cycles. To begin the incubation, 200 g of air-dried soil sieved to 2 mm was taken from each of the four sample sites and placed into separate 2 L plastic biochambers which were the biochamber 2000 from Vernier software and technology (order code BC-2000). Each biochamber had a lid with two sensor ports. A size six rubber stopper was placed into one of the sensor ports.

There were four treatments, a control with no amendment, spent lime applied at a rate of 22.4 Mg/ha, composted beef manure applied at a rate of 22.4 Mg/ha, and Proganics applied at a rate of 5.61 Mg/ha. These amendment rates when applied to 200 g of soil were 57.0 g, 57.0 g, and 15.0 g per biochamber for spent lime, composted beef manure, and Proganics respectively. Each treatment was replicated five times. Soils with amendments were mixed with the applied amendment and all soils were wet to field capacity allowing the drying cycle to encompass the moisture conditions commonly observed in soil. To wet the soils to field capacity, 62.0 g, 75.0 g, 47.0 g, and 47.0 g were added to the 200 g of soil for Site 1 Saline, Site 2 Non-Saline, Site 3 Treated, and Site 4 Non-Treated respectively. Other studies that measured CO₂ flux over drying-

wetting cycles wet the soils to 10 bar (Fierer and Schimel, 2003), 50 percent of field capacity (Haney et al., 2004), 50 percent water-filled pore space (Franzluebbers et al., 2000), 75 percent water-filled pore space, and 90 percent water-filled pore space (Guo et al., 2014). Research has also been conducted where soils were maintained at a range of 30 to 90 percent water filled pore space (Guo et al., 2014; (Guo et al., 2012), but this loses the benefit of simulating natural wetting-drying cycles in the environment. Microbial cell lysis is a potential concern upon rewetting of dried soils due to an environmental shock on the soil microbial community. However, this concern was examined by Fierer and Schimel (2003), and they found no evidence of substantial microbial cell lysis impacting CO₂ flux upon rewetting of dried soils. The biochambers were placed in the laboratory which was approximately 25 degrees C for the duration of the incubations. Other studies have also measured CO₂ flux from soils incubated at 25 degrees C (Franzluebbers et al., 2018; Guo et al., 2012).

Proceeding rewetting of the soil, CO₂ flux was measured 3 times per day with a CO₂ gas sensor (order code CO2-BTA), labquest Mini interface (order code LQ-Mini), and Logger Pro 3.15 software (order code LP-E) all from Vernier Software and Technology. The frequency of CO₂ measurements provides an advantage over other studies such as Guo et al. (2012) who measured CO₂ just six times over a 10-day incubation. The CO₂ gas sensor was set to the low range to measure CO₂ from 0 to 10000 ppm. The CO₂ gas sensor obtains 95% of full-scale reading in 2 minutes (Vernier, 2019a). However, each reading was taken for 5 minutes to increase the consistency of readings. The sensor requires a warmup time of 1.5 minutes (Vernier, 2019a). The CO₂ readings are very sporadic if the sensor is not fully warmed up, so a 3-minute warm up period was aloud for this study to ensure that the sensor was fully warmed up and connecting to Logger Pro 3 Software through the LabQuest mini interface.

The CO₂ gas sensor was set to take the first reading at time 0 with a sampling rate of 4 samples per second. The pressure effect on the CO₂ gas sensor is 0.19% of reading in mm/Hg from standard pressure output. The gas sampling mode is diffusion (Vernier, 2019a). The CO₂ gas sensor measures gaseous CO₂ levels by monitoring the amount of infrared radiation that is absorbed by CO₂ molecules. The sensor has 20 vent holes in the sensor tube to allow for diffusion of CO₂. A small incandescent light bulb is used to generate infrared radiation at one end of the shaft. There is an infrared detector at the other end of the shaft that measures how much radiation gets through the sample without being absorbed by the CO₂ molecules. The infrared detector measures infrared radiation at 4260 nm. The higher the CO₂ concentration in the sampling tube, the less radiation that makes it to the infrared detector. The detector produces a voltage which is converted to ppm by Logger Pro 3.15 software (Vernier, 2019b). The normal operating temperature range is 25 degrees C (+ or - 5 degrees C). The operating humidity range is 5 to 95% (non-condensing). The typical accuracy for the low range setting on the CO₂ gas sensor at standard pressure of 1 atm is plus or minus 100 ppm for 0 to 1000 ppm and plus or minus 10 percent of reading for 1000 to 10000 ppm. The resolution for the CO₂ gas sensor is 3 ppm at 0 when set to the low range (Vernier, 2019a). The output signal range is 0 to 4.0 V. The input potential is 5 V (plus or minus 0.25 V) (Vernier, 2019b).

The CO₂ gas sensor has a calibration stored on the sensor prior to being sold, so no calibration is required to start use with the CO₂ gas sensor. The default calibration has a slope of 2500 ppm and intercept of 20 ppm on the low range. However, when a calibration was needed, the calibration was based on a sample of outside air having a CO₂ level of 400 ppm. To calibrate, the CO₂ gas sensor, LabQuest Mini interface, and laptop with Logger Pro 3 software were taken

outside. The sensor was allowed to warm up for 3 minutes and then the sensor was set to 400 ppm (Vernier, 2019b).

A USB cable was used to connect the CO₂ gas sensor to the LabQuest Mini and the LabQuest Mini to the windows laptop with the LabQuest Mini acting as an interface between the sensor and Logger Pro 3.15 software. This interface is hardware that allows for the interaction between Vernier sensors and a computer in addition to a physical adapter (Vernier, 2019c). Logger Pro 3.15 was used to import the data from the LabQuest Mini interface. A linear slope was fit to the data using Logger pro to obtain the slope of CO₂ over the 5-minute reading. The value in ppm per second was converted to micrograms of CO₂-C/se and the ideal gas law was used to adjust the temperature and pressure of the CO₂ flux readings using the following equation. Micrograms CO₂-C/day = (C*P*V)/(R*T*200 g*100 cm³/L)*86400 sec/day. Where C = CO₂ in ppm/day, P = the pressure in Fargo ND which is 101900 pa, V = volume of biochamber which is 2 L, r = the ideal gas constant which is 8.3144 pa*m³/K/mol, T = 298.15 degrees K, and 200 g = the mass of soil in each biochamber.

Since the CO₂ gas sensor was in one port and the rubber stopper was in the other port, the reading was taken from a closed system and the slope was the change in CO₂ flux over that 5-minute period. This was done to eliminate any additional CO₂ sources in the laboratory from influencing the CO₂ reading in the biochamber. This was similar to the closed system used by Borken et al. (2003). Measuring the change in CO₂ over 5 minutes provides advantages over research conducted by Guo et al. (2014) where the CO₂ reading was taken at a single point in time. When a CO₂ flux reading was not actively being taken, the sensor port for the CO₂ gas sensor was left open to allow for evaporation and normal gas flow. Guo et al. (2014) also

prepared a system with holes left open in the top for the duration of the incubation when CO₂ measurements were not being taken.

The CO₂ flux readings continued until the CO₂ flux reading was less than 2.00 micrograms CO₂-C/day for at least two consecutive CO₂ readings. When all five replications had completed, the soil was rewet to field capacity for a second cycle. For each drying-rewetting cycle, slope and intercept or base flux were determined using a linear regression fit of changes in soil CO₂ flux (micrograms CO₂-C/day) over time (hr).

Other studies that measured CO₂ flux over drying-wetting cycles end the incubations after a set time such as 3-6 days (Fierer and Schimel, 2003), 7 days (Haney et al., 2004), or 10 days (Guo et al., 2012), but this can result in a lack of knowledge of the full impact of an amendment on soil microbial activity over drying-wetting cycles and is less valuable for application to semi-arid and arid regions. The methods above were repeated with CO₂ flux readings beginning immediately after rewetting and being taken 3 times per day until the CO₂ flux reading was less than 2.00 micrograms CO₂-C/day for at least two consecutive CO₂ readings. At this point, the soil was rewet to field capacity for a third cycle and readings repeated. After the CO₂ flux reading was less than 2.00 micrograms CO₂-C/day for at least two consecutive CO₂ readings in the third cycle, the incubation was concluded. The purpose of three drying-wetting cycles was to simulate natural rain events particularly in semi-arid to arid regions that have distinct dry and wet seasons. Other incubations that measured CO₂ flux over drying-wetting cycles used 2 drying-wetting cycles (Fierer and Schimel, 2003) or 5 drying-wetting cycles (Guo et al., 2012), so the number of drying-wetting cycles used in this study falls in the middle of other research. Similar to other studies such as Fierer and Schimel (2003), the soil was

never removed from the biochamber or agitated over the duration of the incubation. After the conclusion of the third cycle, soil was bagged and refrigerated for further analysis.

Residual N

Residual N was determined by analyzing the inorganic N content of the sampled soils prior to incubation and the inorganic N content of the soils incubated with the 4 treatments following the incubation. Inorganic N Was extracted with 2M KCl based on the findings of Bremner and Keeney (1966), and later described by Mulvaney (1996). N extraction with 2.00 M KCl is preferred because it extracts NH_4 , NO_3 , and NO_2 . The high salt concentration also flocculates soil solids which simplifies the filtration due to the physical changes to the soil. The filtrates can be stored in a refrigerator without the formation of precipitates (Mulvaney, 1996). This method also doesn't lead to any chemical or biological reactions that would alter the NH_4 , NO_3 , or NO_2 concentrations (Keeney and Nelson, 1982).

Triplicate 10 g subsamples were taken from the soils sampled from the four sites after soils were air-dried and sieved and these subsamples were placed into a 250 ml bottle. The gravimetric soil moisture content was determined for each sample. Next, 100 ml of 2.00 M KCl were added to each bottle. The samples were then shaken for 1 hour using a reciprocating shaker. proceeding shaking, the suspension was allowed to settle until the supernatant was clear which was 15-30 minutes. A small amount of the supernatant was decanted to moisten the filter paper. Next, the funnel was brought under vacuum, the solution was decanted into the funnel, and the filtration was carried out using a 5.5 diameter funnel, Whatman 42 filter paper, and a filter stand. The filtrate was stored in plastic bottles and placed in a refrigerator for later N analysis.

Soil available NH_4 and NO_3 concentrations were determined on a TL2800 ammonia/nitrate analyzer system (Timberline Instruments, Co). The TL2800 ammonia/nitrate

analyzer is based on the principle of diffusion across a gas diffusion membrane along with EC measurements. The NH_4 in the sample is converted to NH_3 ammonia by mixing the sample with a caustic solution (reagent grade NaOH pH 11-13). The sample is passed through a membrane that is permeable to gasses allowing the dissolved ammonia gas to pass through the membrane. After passing through the membrane, a buffer solution (reagent grade boric acid) absorbs the ammonia gas. Then the thermal equilibrium is established and the change in electrical conductance is measured which is proportional to the NH_4 concentration in the sample. This method was described by Carlson (1978). The sample slash caustic mixture is also passed through a zinc reduction cartridge which reduces NO_3 to NH_4 allowing for a measurement of inorganic N. This method was described by Carlson (1986). Inorganic N mineralized was calculated as the difference between the inorganic N content of the four sampled soils and the inorganic N content of the soils after incubation with the four treatments.

Permanganate Oxidizable Carbon (POXC)

Table 3. Methods to prepare standard stock KMnO_4 solution

Concentration	Volume of KMnO_4 stock solution	Volume of deionized water
0.005 M	0.25 mL: (1 x 0.25 mL draw)	9.75 mL: (1 x 8 mL & 7 x 0.25 mL draw)
0.010 M	0.50 mL: (2 x 0.25 mL draw)	9.50 mL: (1 x 8 mL & 6 x 0.25 mL draw)
0.015 M	0.75 mL: (3 x 0.25 mL draw)	9.25 mL: (1 x 8 mL & 5 x 0.25 mL draw)
0.020 M	1.00 mL: (4 x 0.25 mL draw)	9.00 mL: (1 x 8 mL & 4 x 0.25 mL draw)
0.025 M	1.25 mL: (5 x 0.25 mL draw)	8.75 mL: (1 x 8 mL & 3 x 0.25 mL draw)

Soil POXC was determined based on the method described by Weil et al. 2003. POXC is a quick and useful method for analyzing changes in the labile soil C pool. It can be used for routine evaluations of biologically active soil C (Culman, et al., 2012). The incubated soils were removed from the biochamber and air-dried for 3 days. The dried soil was ground and passed

through a 2 mm sieve. All visible organic matter was removed during the sieving process. The ground soil was placed into plastic bags. KMnO_4 stock solution (0.2 M) was made and standard stock KMnO_4 solution was prepared (Table 3).

The concentrate stage was performed in batches of twelve with ten soil samples, one lab standard, and one blank tube. 2.5 g of soil were weighed out into disposable centrifuge tubes and 18 ml of deionized water was dispensed into each tube using an adjustable dispenser. 2 ml of KMnO_4 stock solution was pipetted into each tube and the tubes were capped and shook by hand to agitate the soil prior to oscillation. The samples were agitated for 2 minutes with the oscillator on high. Following shaking, the samples were allowed to stand for 10 minutes. The dilution stage was performed with a second set of centrifuge tubes which was prepared by filling with 49.4 ml of deionized water using an adjustable dispenser. 0.5 ml of supernatant was pipetted from the concentrated samples into the corresponding tubes filled with deionized water. The 0-100 transmittance bounds were set, and Absorbance was measured at 550 nm for the standard samples and desired samples. The mass of POXC was calculated using the following equation determined by Weil et al. (2003). $\text{POXC (mg kg}^{-1} \text{ soil)} = [0.02 \text{ mol/L} - (a + b * \text{Abs})] * (9000 \text{ mg C/mol}) * (0.02 \text{ L solution/Wt})$. Where 0.02 mol/L = initial solution concentration, a = intercept of the standard curve, b = slope of the standard curve, Abs = absorbance of unknown, 9000 = milligrams of carbon oxidized by 1 mole of MnO_4 changing from $\text{Mn}^{7+} \rightarrow \text{Mn}^{4+}$, 0.02 L = volume of stock solution reacted, and Wt = weight of air-dried soil sampled in kg.

Statistical Analysis

Statistical analyses were conducted using SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC). A factorial complete randomized design (CRD) was used to determine the effect of amendment, and drying-rewetting cycle on the slope and base flux, POXC, and N mineralization

rate between sites one and two, and sites 3 and 4. Mean separation was conducted using Fisher's least significant difference at 95% significance level. Pearson correlation coefficients were studied to determine associations among slope and base flux, POXC, and N mineralization rate were conducted using Proc CORR method at 95% significance level.

RESULTS AND DISCUSSION

The results and discussion section is divided into two sections, (i) soils sampled from Grand Forks, ND, including saline and non-saline soils, and (ii) soils sampled from Tioga, ND, including both soils treated with thermal desorption and non-treated soils. Both the Grand Forks section and Tioga section begin with tables presenting data of baseflux, rate of change, POXC, and mineralized N. The results for each section are preceded by the discussion for each section which contains subsections of impact of soil, impact of amendment, impact of drying-wetting (DW) cycles, and impact of amendment \times cycle interaction.

Table 4. Main and interaction effects of soil types, amendment, and cycle on Base flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) and rate of change in flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) for non-saline and saline Soils in response to amendment additions for three drying-wetting cycles using laboratory incubation

Source	Baseflux (Pr>F)	Rate of change in flux (Pr>F)
Soil	<0.001*	<0.001*
Amendment	<0.001*	0.004*
Soil* Amendment	<0.001*	0.025*
DW Cycle	<0.001*	0.045*
Soil* DW cycle	<0.001*	0.159
Amendment*Cycle	<0.001*	0.073
Soil*Amendment* DW cycle	0.007*	0.163

“ * ” indicates significant at the $p \leq 0.05$

Table 5. Permanganate oxidizable carbon (POXC) (mg kg^{-1}) and N mineralized (mg kg^{-1}) for non-saline and saline soils in response to amendment additions at the end of three drying-wetting cycles using laboratory incubation

Amendments	POXC		N mineralized	
	Saline	Non- saline	Saline	Non- saline
	-----(mg kg^{-1})-----		-----(mg kg^{-1})-----	
Proganics	1134 a†	1289 a	2.94 c	6.47 c
Spent lime	792 c	963 d	117 a	96.3 a
Compost	782 c	1034 c	110 a	92.4 a
Control	865 b	1110 b	56.1 b	71.1 b
Site mean	894 B	1099 A	71.3 A	66.6 B

† Different lowercase and uppercase letters indicate significant difference ($\text{LSD}_{0.05}$) between amendments for the same soil and the different soils respectively.

Grand Forks Site

Type of Soil Effect

Baseflux was significantly higher on non-saline soils compared to saline soils when all treatments were considered together (P less 0.01) Table 4). Saline soils had a significantly higher rate of change compared to non-saline soils when all treatments were considered together (P less 0.05) (Table 5). The concentration of POXC was significantly higher on non-saline soils compared to saline soils when all treatments were considered together (P less 0.01) (Table 5). When all treatments were considered together, saline soils had significantly higher N mineralized at the conclusion of the incubation than non-saline soils (P less 0.01) (Table 5).

Amendment Effect

Baseflux was significantly impacted by amendment on saline soils (P less 0.01) (Table 4). Proganics showed a significantly higher baseflux than spent lime and Compost (Table 6). Spent lime and compost were not significantly different from each other, but they were both significantly higher than the control. The impact of amendments on baseflux differed from saline soils when considered for both non-saline and saline soils together (P less 0.01) with a significantly elevated baseflux relative to the control only observed with Proganics and spent lime. Amendment had significant influence on the rate of change of CO₂ flux with compost resulting in a significantly lower rate of change relative to the control soil as well as soils amended with Proganics and spent lime (Table 6).

Amendment had a significant impact on POXC concentrations on the saline soil (P less 0.01) (Table 5). Proganics was significantly higher than all other treatments. The control soil had significantly higher POXC concentrations than spent lime and compost but spent lime and compost were not significantly different than each other.

Amendments significantly impacted the concentration of N mineralized on saline soils (P less 0.01) (Table 5). Soils amended with compost and spent lime had the highest mineralized N concentrations at the conclusion of the incubation and were not significantly different than each other. Soils amended with Proganics had significantly lower N mineralized than the control soil.

Table 6. Base flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) and rate of change in flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) for non-saline and saline soils in response to amendments additions for three drying-wetting cycles using laboratory incubation

Amendments	DW Cycle	Baseflux		Rate of change in flux	
		Saline	Non- saline	Saline	Non- saline
		---- ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) ---		--- ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) ---	
Proganics	1st	48.0 a†	46.6 a	-0.161 bc	-0.082 c
	2nd	23.0 b	24.2 b	-0.070 ab	-0.028 ab
	3rd	16.0 cd	10.8 def	-0.051 ab	-0.009 ab
Spent lime	1st	16.8 c	25.7 b	-0.115 abc	-0.038 abc
	2nd	8.58 e	13.1 de	-0.121 abc	-0.018 ab
	3rd	4.39 fg	5.80 g	-0.152 bc	-0.005 a
Compost	1st	13.1 d	13.8 d	-0.051 ab	-0.022 ab
	2nd	5.58 efg	7.30 fg	-0.011 a	-0.005 a
	3rd	6.00 efg	5.80 g	-0.020 a	-0.011 ab
Control	1st	7.24 ef	18.7 c	-0.089 ab	-0.059 bc
	2nd	2.92 g	9.91 ef	-0.061 ab	-0.009 ab
	3rd	2.88 g	5.75 g	-0.210 c	-0.003 a
Amendments					
		29.0 a	27.2 a	-0.094 b	-0.040 b
		9.93 b	14.9 b	-0.129 b	-0.020 ab
		8.23 b	8.76 c	-0.027 a	-0.013 a
		4.35 c	11.4 d	-0.120 b	-0.024 ab
DW Cycle					
	1st	21.3 a	26.2 a	-0.104 a	-0.050 b
	2nd	10.0 b	13.1 b	-0.066 a	-0.015 a
	3rd	7.32 c	7.41 c	-0.108 a	-0.007 a

† Different letters indicate significant difference ($\text{LSD}_{0.05}$) between amendments for the same soil

Interaction Effect of Soil \times Amendment

The soil \times amendment interaction significantly impacted baseflux (P less 0.01) (Table 4). Spent lime resulted in a significantly higher baseflux on non-saline soils compared to saline soils. The non-saline control soil showed significantly higher baseflux than the saline control

soil. The rate of change was significantly influenced by the soil \times amendment interaction (P less 0.05) (Table 6). Spent lime amended saline soils showed a significantly higher rate of change than non-saline soils.

POXC concentrations were significantly impacted by the soil \times amendment interaction (P less 0.01) (Table 5). For non-saline soils, Proganics had significantly higher POXC than all other soil \times treatment interactions. Proganics successfully improved POXC on saline soils to a level that was not significantly different than the non-saline control. The control had significantly higher POXC than compost and spent lime on both the saline and non-saline soil.

Mineralized N concentrations were significantly impacted by the soil \times amendment interaction (P less 0.01). Both spent lime and compost resulted in significantly higher N mineralized on saline soils compared to non-saline soils. The non-saline control soil resulted in significantly higher N mineralized than the saline control soil.

Effect of DW Cycles

Baseflux was significantly impacted by DW cycle (P less 0.01) (Table 4) with a significant decrease in baseflux with each consecutive DW cycle. DW cycles had no influence on the rate of change of CO₂ flux on saline soils which differed from when both non-saline and saline soils were considered together. DW cycle 1 had a significantly higher rate of change than DW cycle 2 (P less 0.05) (Table 6).

Interaction Effect of Soil \times Cycle

The soil \times cycle interaction significantly impacted baseflux (P less 0.01) (Table 4). Non-saline soils had significantly higher baseflux than saline soils in DW cycle 1 and DW cycle 2. This difference in baseflux did not persist into the third DW cycle, but the rate of change was significantly higher for saline soils compared to non-saline soils.

Interaction Effect of Amendment × Cycle

Baseflux was significantly impacted by the amendment × cycle interaction (P less 0.01) (Table 4). Proganics maintained elevated baseflux relative to the control through all three DW cycles. Spent lime additions maintained elevated baseflux relative to the control through the first two DW cycles. Compost additions only showed a significantly higher baseflux than the control in the first DW cycle. The amendment × cycle interaction differed for both non-saline and saline soils considered together compared to saline soils in that only Proganics and spent lime showed a response of baseflux relative to the control. The saline soil with compost additions had a significantly lower rate of change than the control soil in the third DW cycle (P less 0.05) (Table 6).

Interaction Effect of Soil × Amendment × Cycle

The soil × amendment × cycle interaction significantly impacted baseflux (P less 0.01) (Table 4). Spent lime resulted in significantly higher baseflux on non-saline soils compared to saline soils in DW cycle 1. The non-saline control soil had significantly higher baseflux than the saline control soil in the first and second DW cycle.

Impact of Non-Saline vs Saline Soils

Soil C release as indicated by baseflux, rate of change, and POXC concentration was not improved on saline soils to the same level of non-saline soils when all amendments were considered together. The lower baseflux with higher rate of change on saline soils reflects lower microbial activity that declines more rapidly than non-saline soils. The lower POXC of saline soils reflects a lower-processed labile C pool (Culman et al., 2012) that is easily degradable by soil microbes (Calderon et al., 2017) compared to non-saline soils. Application of C to saline soils can improve the tolerance of soil microbes to salinity (Mavi and Marschner 2013), but an

improved tolerance does not indicate that negative impacts from salinity are eliminated. Soil salinity induces osmotic stress (Chambers et al., 2011) and osmotic desiccation (Frankenberger and Bingham, 1982) on soil microbes which impacts biogeochemical cycles (Chambers et al., 2011), decreases microbial enzyme activity (Pereira et al., 2019; Frankenberger and Bingham, 1982), N mineralization (Pereira et al., 2019), MBC (Mavi and Marschner 2013), and microbial activity measured through CO₂ flux (Baumann and Marschner, 2013; Mavi and Marschner 2013). Additionally, there is a set population of microbes in the soil that are adapted to the saline environment which limits the potential of improved microbial activity. Thus, despite improved microbial activity on saline soils with amendment applications, microbial activity was not improved to the same level of non-saline soils as indicated by the baseflux, rate of change, and POXC concentrations which is likely due to the amendments not actually impacting salt concentrations in the soil or limitations to the microbial community due to salinity.

The concentration of mineralized N at the conclusion of the incubation showed different results than the microbial results based on baseflux, rate of change, and POXC concentration with a higher mineralized N concentration on saline soils when all amendments were considered together. This indicates that amendments successfully improved nutrient cycling on saline soils, and saline soils actually showed a better response to amendments than non-saline soils. The EC of the saline soils of 2.70 dS m⁻¹ (Table 1) may have been a salinity level that slowed microbial activity but did not cause significant damage to the microbial population allowing microbes to respond to substrate additions. In addition, soil microbes may have been adapted to this salinity level allowing this adapted microbial population to respond to substrate additions. Thus, the mineralized N results showed different results than the other measures of microbial activity and

indicated that amendments could successfully improve microbial mediated nutrient cycling on saline soils.

Impact of Amendment

All three amendments significantly increased the baseflux relative to the control which indicates that the addition of soil amendments initially improved microbial substrate resulting in improved microbial activity despite the soil salinity. Increased microbial activity implies increased nutrient cycling (Laird et al., 2010), and increased nutrient cycling has the potential to lead to increased primary production (Major et al., 2010). Thus, all three amendments significantly improved microbial activity relative to the control as indicated by the baseflux suggesting that amendments have the potential to improve microbial activity and associated nutrient cycling on saline soils.

Proganics resulted in a significantly higher baseflux and POXC concentration than all other treatments which may be due to a larger labile substrate pool and improved microbial habitat. Proganics may have resulted in a higher baseflux than spent lime and compost due to a higher C substrate with a total C content of 460 g kg^{-1} (Table 3). Substrate supply is a control over microbial activity (Wang et al., 2003b) and readily available C additions to soil causes a rapid increase in microbial metabolic activity which results in an increase in CO_2 flux (De Nobili et al., 2001) provided other nutrients aren't limiting factors for microbial activity with additions of high C soil amendments (Kolb, et al., 2009). Proganics has a C:N ratio of 10.5:1 which is lower than spent lime and similar to compost (Table 3). A low C:N ratio can indicate that a soil amendment is more susceptible to microbial decomposition (Johnson et al., 2007). Microbial activity has shown an increase with increasing charcoal application, but the greatest increase in microbial activity was observed on the soil with the highest extractable N and P (Kolb et al., 2009). Other

research has found that the quantity of C substrate can impact microbial activity provided N is not a limitation (Sparrow et al., 2011) like in the case of Proganics. Similarly, in mine reclamation, the material used to rebuild topsoil with the highest organic C content resulted in the highest microbial activity in reclaimed soils measured by enzyme activity (Quideau et al., 2013). Higher SOC can result in higher microbial activity (McCulley and Burke, 2004), and associated CO₂ flux (Stepniewski et al., 2011; Danielson et al., 2017; Franzluebbers et al., 2000).

The chemical composition of bioavailable C can impact CO₂ flux upon wetting of dried soils (Laffely et al., 2020) with CO₂ flux showing a greater response to labile C and N constituents of amendments (Zhang et al., 2014). Hypothesized components of Proganics that may have contributed to high microbial activity include polysaccharide polymers, seaweed extract, and biochar. Polysaccharides are organic C that is easily degradable and associated with less mature composts. Higher microbial activity has been observed with higher polysaccharide content (Annabi et al., 2007). Components of seaweed extract include complex polysaccharides and fatty acids (Bhattacharyya et al., 2016). Biochar has resulted in mixed results on microbial activity (Zhang et al., 2014; Mukherjee et al., 2014; Kammann et al., 2012; Yoo and Kang, 2012; Rogovska et al., 2011), but biochar can improve microbial habitat by increasing soil surface area (Rogovska et al., 2011). The POXC results indicate that Proganics increased the processed labile C pool (Culman et al., 2012) that is easily degradable by soil microbes (Calderon et al., 2017). Labile organic matter pools as measured by POXC can be increased by additions of labile substrate as well as through introduced microbial populations with organic amendments (Bongiornoab et al., 2019). Thus, proganics had a significantly higher baseflux and POXC concentration than all other treatments which may be due to high labile substrate and improved microbial habitat.

Spent lime and compost additions elevated baseflux relative to the control, but not to the level of Proganics which probably resulted from a smaller labile substrate contribution from spent lime and compost compared to Proganics. Spent lime and compost contain 130 and 190 g kg⁻¹ total C respectively (Table 3). In addition to the difference in total C, spent lime and compost may have resulted in lower microbial activity due to the C:N ratio and availability of C. Proganics has a lower C:N ratio when compared to spent lime (Table 3), so more of the C substrate can be utilized before N limitations become a concern. The response of CO₂ flux to amendments is related to the labile C and N constituents of the amendments (Zhang et al., 2014). Spent lime has a significant inorganic C component in the form of calcium carbonate (Sims et al., 2018) that cannot be mineralized by soil microbes (Wang et al., 2016). Composted products tend to have less available organic matter than amendments with less processing (Flavel and Murphy, 2006) because compost is partially decomposed (Tirol-Padre et al., 2007). The compost has a similar C:N ratio as Proganics, but research has found that composted manure can retain 75 to 80% of added N over 1 year (Dere and Stehouwer, 2011) indicating that a majority of N in composted manure is stable (Zvomuya et al., 2007). This may be a result of microbial transformation of N to more recalcitrant forms (Dere and Stehouwer, 2011) which could result in N limitations for microbial decomposition of C substrate. Research has found that composted manure results in a similar level of C retained in the soil as alfalfa even though twice as much C was applied with alfalfa indicating the high stability of C in composted manure (Zvomuya et al., 2007). Thus, spent lime and compost had lower microbial activity than Proganics which was probably a result of lower substrate quantity as well as a higher C:N ratio for spent lime and less labile C for compost.

The lack of significant difference between spent lime and compost with the lower rate of change on compost indicates that spent lime may have resulted in a greater priming effect, but compost may have resulted in the accumulation of organic matter in the soil. Compost contains slightly higher total C than spent lime (Table 3), so the lack of significant difference in baseflux indicates more C from spent lime was utilized relative to the total amount of C in the substrate which is supported by the lower rate of change observed with compost additions to the soil. This indicates that spent lime may contain impurities that are more labile substrate than compost substrate or spent lime resulted in a greater priming effect of SOM. Spent lime contains N (Sims et al., 2018) and is hypothesized to contain sucrose and both N (Jansson and Persson, 1982; Parr and Papendick, 1978) and sucrose (Hopkins et al., 2014; Nottingham et al., 2009). Microbial activity continued into drier soil conditions for compost when compared to amended with spent lime based on the lack of significant difference in baseflux and significant difference in rate of change. This could be a result of improved microbial habitat (Kladivko and Clapperton, 2011; Rogovska et al., 2011; Kolb, et al., 2009; Doran and Smith, 1987). The lower rate of change of compost is likely due to the recalcitrant substrate of compost (Wilson et al., 2018). Organic amendments have the potential to improve microbial habitat (Kladivko and Clapperton, 2011; Rogovska et al., 2011; Kolb, et al., 2009; Doran and Smith, 1987) which may have resulted in microbial activity extending into drier soil conditions. Thus, even though there was no significant difference in baseflux between spent lime and compost, compost resulted in a lower rate of change which is likely due to higher labile substrate or a greater priming effect with spent lime and indicates a potential for improved microbial habitat with compost.

Interestingly, the POXC results indicate that the control soil had a larger pool of processed labile C (Culman et al., 2012) that could be easily degraded by soil microbes

(Calderon et al., 2017) than spent lime or compost after 3 DW cycles. The lower POXC of soils amended with spent lime and compost compared to soils amended with Proganics is likely due to smaller labile substrate pools in both these amendments compared to Proganics. These results did not differ when both non-saline and saline soils were considered together indicating that the POXC response was due to amendment characteristics rather than soil salinity. The smaller labile substrate pool of spent lime and compost is likely due to the inorganic C component of spent lime (Sims et al., 2018) and the recalcitrance of substrate in compost (Wilson et al., 2018; Tirol-Padre et al., 2007; Zvomuya et al., 2007; Flavel and Murphy, 2006). The relative abundance of easily degradable carbohydrates of cattle manure decreases with increasing composting time while aromatic C and aromatic C-O increase. These findings indicate that the recalcitrance of compost increases over the composting process which is supported by research that has found that the chemical structure of composted cattle manure remains unchanged from day 36 of composting to day 70 of composting. The low C:N ratio of the compost used in this study may reflect a mature compost (Huang et al., 2017) with a greater proportion of recalcitrant substrate (Wilson et al., 2018; Flavel and Murphy, 2006) that tends to result in lower microbial activity (Dere and Stehouwer, 2011). Other research has found that compost additions increase POXC (White et al., 2020), but characteristics of compost vary with the source of compost (Huang et al., 2017). Thus, spent lime and compost had significantly lower POXC than proganics and the control which was likely due to limitations in labile substrate and a priming effect.

The concentration of mineralized N preceding the incubations differs from the baseflux and POXC concentration results with compost and spent lime additions to the soil both resulting in a higher N concentration mineralized relative to the control whereas Proganics resulted in a lower mineralized N concentration relative to the control. The C:N ratio of compost used in this

study was 9.5:1 (Table 1) which indicates that N mineralization would be expected (Sullivan et al., 2002). A majority of N in compost can be stable (Dere and Stehouwer, 2011; Sullivan et al., 2002), but characteristics of compost may vary with the source of the compost (Huang et al., 2017), and maturity of compost (Wilson et al., 2018). The concentration of mineralized N at the conclusion of the incubation indicates that the compost used in this research did not contain a majority of stable N. Spent lime has a C:N ratio of 40.6:1 which typically indicates immobilization of N in the soil (Sullivan et al., 2002). However, the inorganic C contained in calcium carbonate is not available for microbial mineralization (Wang et al., 2016) which may have led to a lower effective C:N ratio resulting in mineralization of N. Additionally, the sucrose that may exist in spent lime as an impurity has the potential to lead to a priming effect resulting in mineralization of SOM derived N (Hopkins et al., 2014; Nottingham et al., 2009) which may have contributed to the mineralized N concentration. Proganics showed N immobilization relative to the control despite showing higher microbial activity based on the baseflux and POXC results. The C:N ratio found for Proganics in this study was 10.5:1 (Table 3) which indicates that N mineralization would be expected to occur (Sullivan et al., 2002). However, the producing company reports the C:N ratio of Proganics as between 40:1 and 60:1 which would indicate that immobilization of N would occur (Sullivan et al., 2002). Proganics contains biochar which has the potential to absorb nutrients including inorganic N (Mukherjee et al., 2014; Zheng et al., 2013) and enhance N immobilization (Zheng et al., 2013). Additionally, the stability of N in Proganics is unknown, so it is possible that Proganics contains a significant portion of N that is not easily available to soil microbes. Thus, compost and spent lime both increased the N mineralized over the incubation period, whereas Proganics additions resulted in immobilization of N which may be a result of the biochar component of Proganics.

The response of baseflux to amendment additions differed from saline soils when considered for both non-saline and saline soils with no difference seen between the baseflux of soils amended with compost and control soils which is likely due to the SOM of non-saline soils and the substrate characteristics of compost. The non-saline soils contain higher SOM levels than saline soils with values of 7.62 and 5.08% respectively. Additionally, compost contains recalcitrant substrate (Wilson et al., 2018). These factors indicate a greater potential that non-saline soils contain SOM that is more labile substrate than compost leading to microbial utilization of SOM rather than compost. Thus, the lack of response of baseflux to compost additions when both non-saline and saline soils were considered together may be due to microbial utilization of substrate from SOM rather than compost on non-saline soil.

The baseflux results for the soil \times amendment interaction showed a greater microbial response to spent lime on non-saline soils compared to saline soils which may be due to higher SOM and a larger microbial population on non-saline soils. Spent lime has a large inorganic C pool (Sims et al., 2018) that is not mineralized by soil microbes (wang et al., 2016). However, spent lime contains nutrient impurities including N (Sims et al., 2018) and is hypothesized to include sucrose and a priming effect can result from both N (Jansson and Persson, 1982; Parr and Papendick, 1978) and sucrose (Hopkins et al., 2014; Nottingham et al., 2009). Based on this, the non-saline soil with higher SOM (Table 1) would have a greater potential to support a larger priming effect. Additionally, given the inorganic composition of spent lime, soil microbes would be responsible for utilization of nutrient impurities and the priming effect rather than microbes in the amendment which differs from compost (Tejada et al., 2010; Flavel and Murphy, 2006) and is hypothesized to differ from Proganics. Soil salinity negatively impacts MBC (Mavi and Marschner 2013) and microbial activity (Pereira et al., 2019; Baumann and Marschner, 2013;

Frankenberger and Bingham, 1982). Based on this, non-saline soils would be more likely to have a greater microbial response to nutrients in spent lime and lead to a priming effect.

The POXC concentrations for the soil × amendment interaction indicates that Proganics applications would improve microbial activity while spent lime and compost applications would decrease microbial activity. Proganics applications to non-saline soil had significantly higher POXC concentrations than all other soil × treatment interactions. Proganics applications to the saline soil showed significantly lower POXC concentrations than Proganics on the non-saline soil, but it was not significantly different than the non-saline control. This indicates that Proganics improved soil quality of saline soils because POXC has been correlated with TOC, total N, cation exchange capacity, water stable aggregates, water holding capacity, bulk density, MBC, microbial biomass N, and CO₂ flux (Bongiornoab et al., 2019). This was likely due to the labile substrate contained in Proganics. The POXC concentrations indicate that compost and spent lime either did not significantly increase labile substrate or did not significantly introduce soil microbes with amendments (Bongiornoab et al., 2019) which is likely due to the inorganic C component of spent lime (Sims et al., 2018) and the recalcitrant substrate of compost (Wilson et al., 2018). Non-saline soils showed a similar response of POXC concentrations to spent lime and compost additions. Therefore, based on POXC measures alone, Proganics has the potential to improve microbial activity on saline soils whereas spent lime and compost don't which may be due to the levels of labile substrate in amendments or microbes contained in amendments.

POXC concentrations were significantly higher for compost than spent lime for the non-saline soil whereas the saline soil showed no significant difference between compost and spent lime. The significantly higher POXC for compost relative to spent lime on the non-saline soil is probably due to the large organic component of compost compared to the large inorganic

component of spent lime. Compost also may have had a significantly higher POXC than spent lime in the non-saline soil due to additions of soil microbes in the compost as concluded to occur with compost in other research (Tejada et al., 2010; Flavel and Murphy, 2006). This may have only shown a significant difference on the non-saline soil because the microbes added to the soil with the compost amendment wouldn't particularly be tolerant to soil salinity whereas soil microbes in a saline soil would have some tolerance to soil salinity. Therefore, the microbes contained in compost may have been impacted by osmotic stress (Chambers et al., 2011) and osmotic desiccation (Frankenberger and Bingham, 1982) upon application to the saline soil. Thus, POXC for compost showed different results between the saline and non-saline soils which was likely due to the negative impact of soil salinity on microbes contained in compost.

The soil \times amendment interaction results showed that the mineralized N concentration was higher for saline soils amended with spent lime and compost than non-saline soils amended with spent lime and compost. Spent lime contains a large inorganic C component (Sims et al., 2018) that is not mineralized by soil microbes (wang et al., 2016), so the improved N cycling resulting from spent lime applications is likely due to impurities in spent lime that can result in a priming effect such as N (Jansson and Persson, 1982; Parr and Papendick, 1978) and hypothesized sucrose (Hopkins et al., 2014; Nottingham et al., 2009). Provided the elevated N cycling with spent lime additions is due to a priming effect, this indicates that the saline soil contains higher labile substrate despite the lower total SOM (Table 1). This is a potential given lower microbial activity on saline soils that may have utilized less labile components of the SOM prior to amendment additions. The difference in N mineralized with compost additions between non-saline and saline soils may be due to the difference in P content between the soils. The saline soils had 3.6 times higher P concentrations, and P concentrations in soil can be a limiting factor

for microbial activity particularly in soils without P additions (Griffiths et al., 2012). Therefore, the higher P of the saline soils may have allowed for higher microbial activity resulting in higher N mineralization. Thus, spent lime and compost successfully increased the mineralized N concentration of saline soils to a level higher than non-saline soils with the same amendments.

Impact of DW Cycle

There was a decrease in baseflux which each consecutive DW cycle on the saline soil indicating that labile substrate was not depleted after the second DW cycle with all amendments considered together. Decomposition upon rewetting of dried soils is a result of direct microbial attack of the solid soil substrate, and the magnitude of decomposition depends on the percent C in the soil and degree of drying (Birch, 1958). Therefore, as the C content of the substrate decreased, microbial activity could decrease. . A second potential explanation is the release of SOM that was previously physically protected over the DW cycles. A release of physically protected SOM can occur over DW cycles due to breaking bonds between soil particles or clay leaves (Degens, 1995). The silty clay loam texture of the A horizon seen on the saline soil (National Cooperative Soil Survey, 2020) particularly has the potential to contain physically protected SOM. This soil has a moderately high SOM content of 5.08% (Table 1), so there is a potential for a high quantity of substrate to be released. Fierer and Schimel, (2003) observed a 200% increase in extractable SOM-C in rewet soils. However, this SOM released may be stable and therefore resistant to decomposition particularly with consecutive drying-wetting cycles (Degens, 1995) which is a potential explanation for the declines in microbial activity with consecutive DW cycles. Similarly, the amendments applied to the soil would get more stable with time through DW cycles due to utilization of labile substrate. The increase in CO₂ flux seen in cycle 1 may also be due in part to reestablishment of the microbial population proceeding

microbial death from the drying process (Haney et al., 2004). Frequent drying-wetting cycles may select for soil microbial communities that are capable of adjusting to rapid increases in water potential without rapidly mineralizing their accumulated cytoplasmic solutes (Fierer and Schimel, 2003). This is a potential explanation for the high baseflux in cycle 1 followed by a sharp decline to cycle 2 and a slight decline to cycle 3. Thus, the significant difference in baseflux seen between all three DW cycles with microbial activity declining with consecutive DW cycles may be due to reduced C substrate or a microbial community that has adjusted to DW cycles.

The rate of change when both non-saline and saline soils were considered together differed from saline soils in that a significant difference was observed between DW cycle 1 and DW cycle 2 with both soils whereas no significant difference was seen with saline soils. Given that non-saline soils had higher baseflux compared to saline soils in the first DW cycle, a higher rate of change reflects a higher C utilization rate. This difference is likely due to the negative impact of salinity on soil microbes (Pereira et al., 2019; Baumann and Marschner, 2013; Mavi and Marschner 2013; Frankenberger and Bingham, 1982) that hindered the C utilization rate in saline soils. This difference could also be due to the difference in SOM between non-saline and saline soils (table 1) because the higher SOM of the non-saline soil may have contained more labile substrate. Thus, the rate of change results differed for both non-saline and saline soils considered together compared to saline soils with a significantly higher rate of change in DW cycle 1 compared to DW cycle 2.

The soil \times cycle interaction for baseflux showed that non-saline soils have significantly higher microbial activity in the first two DW cycles, but there was no significant difference in baseflux in the third cycle which may be due to a larger microbial population and larger SOM

pool in non-saline soils. However, the significantly higher rate of change with the saline soil compared to the non-saline soil in DW cycle 3 indicates that microbial activity was not equal despite the lack of significant difference in baseflux because microbial activity declined more rapidly on saline soils. The larger SOM of non-saline soils reflects a potential for a greater release of physically protected SOM upon rewetting (Fierer and Schimel, 2003) and a potential for a larger labile substrate content in SOM. Soil salinity negatively impacts soil microbes (Pereira et al., 2019; (Baumann and Marschner, 2013; Mavi and Marschner 2013; Chambers et al., 2011; Frankenberger and Bingham, 1982), so the higher microbial population of non-saline soils may have depleted labile substrate to a similar level of saline soils by the third DW cycle despite higher SOM substrate. The sharper decline in microbial activity on the saline soil in the third DW cycle as well as the lower baseflux in the first two DW cycles may be due to an increased concentration of salts in the soil water as the level of water in the soil decreases (Yan et al., 2015) resulting in osmotic stress (Chambers et al., 2011). Thus, baseflux was significantly higher on non-saline soils compared to saline soils over the first two DW cycles and the rate of change indicates that microbial activity declined more rapidly on saline soils in the third DW cycle which may be due to a combination of a larger microbial community and more SOM substrate on non-saline soils.

Impact of Amendment × Cycle Interaction

The baseflux and rate of change results for the saline control soil indicate that labile substrate may have limited microbial activity and salinity may have negatively impacted soil microbes. The first DW cycle with the control soil showed a lower baseflux than all other treatments in DW cycle 1. Even though this soil has a high SOM content, the labile substrate may have been rapidly utilized in the first DW cycle which is supported by the lack of significant

difference in baseflux between the second and third DW cycle. The labile substrate limitations were probably compounded by the soil salinity. Despite the lack of significant difference in baseflux between the second and third DW cycle with the control soil, the third DW cycle had a significantly higher rate of change than the second DW cycle and all other amendment \times cycle interactions in the third DW cycle. This indicates that microbial activity declined more rapidly in the third DW cycle with the control soil. These results reflect an increased concentration of salts in the soil water (Yan et al., 2015) as the level of water in the soil decreases resulting in osmotic stress (Chambers et al., 2011) and negative impacts on microbial activity from salinity (Pereira et al., 2019; Baumann and Marschner, 2013; Mavi and Marschner 2013; Frankenberger and Bingham, 1982). Thus, the baseflux and rate of change results indicate that microbial activity may have been negatively impacted on saline soils due to limited labile substrate compared to amended soils and the negative impact of salinity on soil microbes.

Proganics maintained higher microbial activity in the first two DW cycles than any other amendment. Proganics cycle 3 was not significantly different than spent lime and compost in the first DW cycle, but it was significantly higher than all other amendment \times cycle interactions other than the previous two DW cycles with Proganics. This reflects that after two previous DW cycles, Proganics contained similar labile substrate to spent lime and compost at their initial application in the first DW cycle. This is probably due to the combination of high labile substrate, high total C content, and low C:N ratio. The high microbial activity in DW cycle 3 indicates a strong potential that microbial activity would still be elevated with additional DW cycles. This suggests that Proganics has the potential to improve microbial activity on saline soils in regions with distinct dry-wet seasons or surface soils that experience natural moisture fluctuations. Thus, proganics showed a greater potential to improve and sustain microbial

activity on saline soils than all other amendments as indicated by the baseflux.

The first 2 DW cycles with spent lime on the saline soil improved microbial activity relative to the control as indicated by the baseflux. However, microbial activity declined in spent lime cycle 3 with no significant difference between spent lime cycle 3 and either control cycle 2 or control cycle 3. This indicates that the substrate contributed to the soil from spent lime application was depleted after two DW cycles which supports the hypothesis that sucrose from sugar beets was one of the impurities in spent lime. Since sucrose is a labile C substrate (Wang et al., 2015) the sucrose was rapidly utilized in DW cycle 1 and DW cycle 2 leaving little labile substrate for DW cycle 3. This may also indicate that other nutrient impurities in spent lime that may have contributed to a priming effect such as N were depleted after 2 DW cycles. Based on this, spent lime may be a beneficial soil amendment on saline soils to initially improve microbial activity and nutrient cycling, but the increased microbial activity would be short-lived when applied at a rate of 22.4 Mg ha⁻¹. The increase in microbial activity in DW cycle 1 and DW cycle 2 reflect a potential that spent lime could improve microbial activity over a longer time span on saline soils when applied at a higher rate. Other researchers have applied spent lime at rates of 6.73 to 56 Mg ha⁻¹ (Windelsl, 2017), but additional research is needed to determine the response of soil microbes to higher spent lime application rates over DW cycles. Thus, spent lime improved microbial activity in DW cycle 1 and DW cycle 2, but microbial activity was not improved relative to the control in DW cycle 3 indicating that the beneficial impacts of spent lime on microbial activity are short lived on saline soils with an application rate of 22.4 Mg ha⁻¹.

The response of baseflux to compost additions indicate that compost can initially improve microbial activity on saline soils, but increased microbial activity is short-lived which is likely due to the recalcitrant substrate in compost. Baseflux for Compost cycle 1 was significantly

higher than control cycle 1, but microbial activity declined after DW cycle 1 with no significant difference between the baseflux of the latter two cycles with compost or the control soil. This may be a result of microbial utilization of the labile components of compost in DW cycle 1 due to the high recalcitrant substrate in compost (Wilson et al., 2018) resulting in low labile substrate for DW cycle 2 and DW cycle 3. Similar results were seen by Tirol-Padre et al., (2007) where labile C pools of composted manure were rapidly consumed by soil microbes leaving a compost with more resistant lignin and humic substances. Additionally, compost in the third DW cycle showed a lower rate of change than the control in the third DW cycle. This leads to an accumulation of C and N in the soil which has been observed in other studies with composted manure (Sadeghpour et al., 2016; Dere and Stehouwer, 2011; Tirol-Padre et al., 2007; Zvomuya et al., 2007). A higher application rate could potentially extend the period of time that microbial activity is increased. An application rate of 58 Mg ha⁻¹ on a dry weight basis of composted manure has been used in other research (Helton et al., 2008), but high manure application rates can lead to water quality concerns while microbes rapidly utilize the labile portion of compost (Small et al., 2019). An alternative option would be smaller but more frequent applications of compost which may provide repeated short term increases in microbial activity with more frequent applications and provide the additional benefit of building SOM. A minimum compost application rate may be required to increase SOM (Sadeghpour et al., 2016), but a composted manure application rate of 3 Mg ha⁻¹ has increased SOC by nearly 50% over 20 yr (Mpeketula and Snapp, 2019). However, small but more frequent applications of compost require additional labor and time for farmers attempting to reclaim saline agricultural soils which may pose a limitation for this strategy. Thus, compost resulted in an initial increase in microbial activity as indicated by the baseflux, but this increase did not persist through DW cycle 2 and DW cycle 3

indicating that the potential of compost to improve microbial activity on saline soils may be limited to the short term.

The amendment \times cycle interaction results when considered for both non-saline and saline soils together differed from saline soils in that Compost did not elevate baseflux over the control in the first DW cycle. This is likely due to higher SOM levels of non-saline soils (Table 1) and the recalcitrant substrate of compost (Wilson et al., 2018) leading to utilization of substrate from SOM rather than compost.

The only soil \times amendment \times cycle interaction that was significantly different for baseflux was spent lime which had higher baseflux on non-saline soils compared to saline soils. This is likely due to the large inorganic C component of spent lime (Sims et al., 2018) that is not microbial substrate or contain an active microbial population. Therefore, the response of soil microbes to spent lime would largely be based on a priming effect from impurities in spent lime which would have a greater response to the higher SOM on non-saline soils. The negative impact of salinity on soil microbes (Pereira et al., 2019; Baumann and Marschner, 2013; Mavi and Marschner 2013; Frankenberger and Bingham, 1982) would result in microbes contained in non-saline soils more likely to facilitate a larger priming effect. Thus, the lower microbial response to spent lime on saline soils relative to non-saline soils as indicated by baseflux reflects the lower SOM of saline soils as well as potential negative impacts on the microbial community from salinity.

Tioga Site

Table 7. Main and interaction effects of soil types, amendment, and cycle on base flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) and rate of change in flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) for soils treated with thermal desorption and non-treated soils from Tioga in response to amendments additions for three drying-wetting cycles using laboratory incubation

Source	Baseflux (Pr>F)	Rate of change in flux (Pr>F)
Soil	<0.001*	0.010*
Amendment	<0.001*	<0.001*
Soil* Amendment	0.015*	0.004*
DW Cycle	<0.001*	<0.001*
Soil* DW cycle	0.097	0.088
Amendment*Cycle	<0.001*	0.001*
Soil*Amendment* DW cycle	0.289	0.222

“ * ” indicates significant at the $p \leq 0.05$

Table 8. Permanganate oxidizable carbon (POXC) (mg kg^{-1}) and N mineralized (mg kg^{-1}) for soils treated with thermal desorption and non-treated soils from Tioga in response to amendment additions at the end of three drying-wetting cycles using laboratory incubation

Amendments	POXC		N mineralized	
	Treated	Non- treated	Treated	Non- treated
	-----(mg kg^{-1})-----		-----(mg kg^{-1})-----	
Proganics	770 a†	683 a	8.82 b	6.02 c
Spent lime	380 b	237 c	82.7 a	66.6 b
Compost	373 b	395 b	102 a	80.8 a
Control	260 c	160 c	1.12 b	5.40 c
Avg	446 A	384 B	48.7 A	39.7 B

† Different lowercase and uppercase letters indicate significant difference ($\text{LSD}_{0.05}$) between amendments for the same soil and the different soils respectively.

Soil Type Effect

Non-treated soils had significantly higher baseflux than the treated soil when all treatments were considered together ($P < 0.01$) (Table 7). When all treatments were considered together, the rate of change was significantly higher for treated soils compared to non-treated soils ($P < 0.01$) (Table 7). Treated soils had significantly higher POXC concentrations than non-treated soils when all treatments were considered together ($P < 0.01$) (Table 8). Treated soils had significantly higher mineralized N concentrations at the conclusion of the incubation than non-treated soils when all treatments were considered together ($P < 0.01$) (Table 8).

Table 9. Base flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) and rate of change in flux ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) for soils treated with thermal desorption and non-treated soils from Tioga in response to amendments additions for three drying-wetting cycles using laboratory incubation

Amendments	DW Cycle	Baseflux		Rate of change in flux	
		Treated	Non-treated	Treated	Non-treated
		--- ($\mu\text{g CO}_2\text{-C/m}^2\text{/day}$) ---		--- ($\mu\text{g CO}_2\text{-C/m}^2\text{/day/hr}$) ---	
Proganics	1st	33.6 a†	39.9 a	-0.250 f	-0.162 e
	2nd	21.8 b	32.5 b	-0.162 e	-0.134 de
	3rd	18.4 bc	19.7 c	-0.113 de	-0.078 abcd
Spent lime	1st	19.0 b	22.6 c	-0.112 cde	-0.121 cde
	2nd	8.24 d	12.8 d	-0.081 abcd	-0.078 abcd
	3rd	8.13 d	9.11 de	-0.076 abcd	-0.055 abc
Compost	1st	10.3 cd	10.5 de	-0.047 abc	-0.035 ab
	2nd	7.86 d	8.96 de	-0.023 ab	-0.027 ab
	3rd	5.61 d	6.86 ef	-0.011 a	-0.019 a
Control	1st	1.87 d	4.92 ef	-0.062 abcd	-0.062 abc
	2nd	2.55 d	3.33 f	-0.061 abcd	-0.098 bcde
	3rd	2.24 d	2.99 f	-0.097 bcde	-0.050 abc
Amendments					
Proganics		24.6 a	30.7 a	-0.181 c	-0.125 c
Spent lime		11.8 b	14.8 b	-0.090 b	-0.084 b
Compost		7.93 b	8.76 c	-0.027 a	-0.027 a
Control		2.22 c	3.75 d	-0.073 b	-0.070 b
DW Cycle					
1 st		16.2 a	19.5 a	-0.118 b	-0.095 b
2 nd		10.1 b	14.4 b	-0.081 a	-0.084 b
3 rd		8.60 b	9.68 c	-0.079 a	-0.050 a

† Different letters indicate significant difference ($\text{LSD}_{0.05}$) between treatments for the same soil

Amendment Effect

Amendment application significantly increased baseflux on treated soils (P less 0.01) (Table 7) with Proganics showing a significantly higher baseflux than all other treatments. Spent lime and compost had a significantly higher baseflux than the control but they were not significantly different than each other. Amendment application significantly increased baseflux when both treated and non-treated soils were considered together (P less 0.01) (Table 7). The

baseflux results differed from the treated soil results with a significantly higher baseflux for spent lime than compost. The rate of change of CO₂ flux was significantly impacted by amendment on treated soils (P less 0.01) and both non-treated and treated soils considered together (P less 0.01) (Table 9). Compost resulted in a significantly lower rate of change and Proganics resulted in a significantly higher rate of change relative to the control.

POXC was significantly impacted by amendment (P less 0.01) (Table 9) with all amendments significantly increasing POXC relative to the control. Proganics had significantly higher POXC concentrations than all other treatments. Spent lime and compost were significantly higher than the control but not significantly different than each other. Both non-treated and treated soils considered together differed from treated soils considered alone in that compost had significantly higher POXC concentrations than spent lime (P less 0.01) (Table 8).

Amendments significantly impacted the concentration of N mineralized (P less 0.01) (Table 8). Soils amended with compost and spent lime had the highest mineralized N concentrations at the conclusion of the incubation and were not significantly different than each other. Soils amended with Proganics did not have significantly different mineralized N concentrations than the control soil. The results differed when both soils were considered together in that compost had a significantly higher mineralized N concentration compared to spent lime (P less 0.01) (Table 11).

Interaction Effect of Soil × Amendment

Baseflux was significantly impacted by the soil × amendment interaction (P less 0.05) (Table 9). Proganics was the only amendment that resulted in a significant difference between non-treated and treated soils. Proganics application to non-treated soils had a significantly higher baseflux than all other soil × amendment interactions, but this was followed by the treated soil

with Proganics application. The rate of change of CO₂ flux was significantly impacted by the soil × amendment interaction (P less 0.01) (Table 9). Non-treated compost and treated compost had a significantly lower rate of change than all other soil amendment interactions and treated Proganics resulted in a significantly higher rate of change than all other amendment cycle interactions. Non-treated Proganics also resulted in a significantly higher rate of change relative to the control for either soil.

The soil × amendment interaction significantly impacted POXC concentrations (P less 0.01) (Table 8). The only amendment that showed a significant difference between non-treated and treated soils was spent lime which showed significantly lower POXC concentrations on non-treated soils compared to treated soils.

Mineralized N concentrations were significantly impacted by the soil × amendment interaction (P less 0.01) (Table 8), but compost was the only amendment that showed a significant difference between soils with significantly higher mineralized N on treated soils compared to non-treated soils.

Effect of DW Cycles

DW cycles significantly impacted baseflux on treated soils (P less 0.01) (Table 7). With baseflux significantly decreased from the first DW cycle to the second DW cycle, but not from the second DW cycle to the third DW cycle. Both non-treated and treated soils considered together differed from treated soils with a significant difference seen in baseflux between DW cycle 2 and DW cycle 3 (P less 0.01) (Table 9). DW cycle 1 had a significantly higher rate of change than DW cycle 2 and DW cycle 3 on treated soils (P less 0.01) (Table 9). The results for rate of change differed when both non-treated and treated soils were considered together with a significant difference observed between DW cycle 2 and DW cycle 3 (P less 0.01) (Table 9).

Interaction Effect of Soil × Cycle

Baseflux in non-treated DW cycle 1 was significantly higher than treated DW cycle 1 in the soil × cycle interaction (P less 0.05) (Table 9). Non-treated Tioga DW cycle 2 was significantly higher than treated DW cycle 2 (P less 0.05) (Table 9).

Interaction Effect of Amendment × Cycle

Baseflux was significantly impacted by the amendment × cycle interaction on treated soils (P less 0.01) (Table 9). Proganics showed a significantly higher baseflux in each of the three DW cycles relative to the control while spent lime significantly increased baseflux in the first DW cycle relative to the control. Baseflux was significant for the amendment × cycle interaction when both treated and non-treated soils were considered together (P less 0.01) (Table 9). The results differed from treated soils in that the second and third DW cycle significantly increased baseflux relative to the control and the first cycle with compost significantly increased baseflux relative to the control. The amendment × cycle interaction had significant influence on rate of change on treated soils (P less 0.01) (Table 9). Proganics in the first DW cycle was the only amendment that significantly increased the rate of change of CO₂ flux over all control cycles and no amendments significantly decreased the rate of change of CO₂ flux under all control cycles. However, compost cycle 3 had a significantly lower rate of change than the third DW cycle with the control soil. The results for rate of change when both non-treated and treated soils were considered together differed from treated soils in that Proganics cycle 2 also significantly increased the rate of change relative to all DW cycles with the control soil and compost cycle 2 showed a significantly lower rate of change than DW cycle 2 with the control soil (P less 0.01) (Table 9).

Interaction Effect of Soil × Amendment × Cycle

The only significant soil × amendment × cycle interaction for baseflux was observed with Proganics in DW cycle 2 with the non-treated soil showing significantly higher microbial activity than the treated soil (P less 0.05) (Table 9). The only individual soil amendment cycle interaction that was significantly different for rate of change was a higher rate of change in treated Proganics cycle 1 relative to non-treated Proganics cycle 1 (P less 0.05) (Table 9).

Impact of Non-treated Verses Treated Soil

Microbial activity was not improved on the treated soil to the same level of the non-treated soil when all amendments were considered together as indicated by the baseflux and rate of change results. This is likely due to differences in SOM and the impact of thermal desorption on the microbial community. The lower baseflux reflects lower microbial activity while the higher rate of change reflects a sharper decline in microbial activity proceeding the peak of microbial activity. The non-treated soil was subsoil and contained low SOM (0.89%) but the treated soil had lower SOM (0.66%) (Table 1). The lower SOM of the treated soil represents a potential for decreased microbial habitat (Kladivko and Clapperton, 2011; Doran and Smith, 1987) soil structure (Grandy et al., 2002; Franzluebbers et al., 2000), macropore formation, micronutrient adsorption (Ingram et al., 2005), and available nutrients (Ingram et al., 2005) meaning that SOM levels can impact microbial activity (Franzluebbers et al., 2000). The quality and quantity of the substrate source for soil microbes is impacted by the level of SOM (Kladivko and Clapperton, 2011). Additionally, lower SOM on the treated soil indicates less substrate to be impacted by a priming effect that can occur with the application of soil amendments (Hopkins et al., 2014; Talbot and Treseder, 2012; Nottingham et al., 2009; Fontaine et al., 2004). Additionally, the subsoil SOM of non-treated soils may have been more labile than the SOM of

treated soils. Research has found that subsoils contain high relative levels of polysaccharides and other labile plant derived compounds including cellulose derived compounds, and subsoils contain low relative levels of lignins and aromatics (Vancampenhouta et al., 2012). On the other hand, preferential denaturation of labile substrate and decreased solubility of SOM occurs on heated soils (Certinie, 2005). Thermal desorption may have also negatively impacted the microbial community resulting in a smaller living microbial population. Research has found that heating of soils reduces microbial populations of the soil (Jimenez et al., 2008; Choromanska et al., 2002) and microbial activity (Cebron et al., 2011; Choromanska et al., 2002). Therefore, the application of amendments to treated soils did not improve or sustain microbial activity to the level of non-treated soils proceeding amendment application which is reflected by the lower baseflux and higher rate of change on treated soils compared to non-treated soils.

Interestingly, treated soils had significantly higher POXC than non-treated soils which differs from the microbial activity indicated by the baseflux. Additional research would be required to determine why POXC concentrations were higher on treated soils, but it may indicate that the thermal desorption process converted SOM to the labile portion of SOM measured by POXC. This indicates that the thermal desorption process likely did not have long term negative impacts on microbial activity and supports that the largest concern proceeding oil remediation is the low SOM of soils.

In support of the POXC results, N mineralized was higher on treated soils than non-treated soils. Given that microbial activity is reflected by N mineralization (Franzluebbbers et al., 2018; Haney et al., 2001) and POXC (Hurisso et al., 2016; Wang et al., 2003b), the thermal desorption likely does not have long-term negative impacts on the soil microbial community. It also indicates that the SOM in treated soils might not have been more resistant to microbial

attack as previously hypothesized. An additional explanation for these results could be a larger microbial population reestablished in treated soils. Thus, treated soils had higher concentrations of mineralized N which may have been due to the P concentration of the soil, SOM characteristics, or microbial characteristics.

Impact of Amendments

Amendment additions increased baseflux and POXC due to increased substrate on treated soils (Table 7) indicating that the addition of soil amendments initially improved substrate quantity and quality resulting in improved microbial activity despite the thermal desorption treatment of the soil. Additionally, this indicates that all three amendments have the potential to increase soil quality of soils treated with thermal desorption (Culman et al., 2012; Haney et al., 2004; Franzluebbers et al., 2000). One of the concerns proceeding thermal desorption treatment is low SOM (O'Brien et al., 2016; Vidonish et al., 2016) due to mineralization of SOM during the thermal desorption process (Sierra et al., 2015) leading to low microbial activity (Cebren et al., 2011) resulting in a potential for nutrient stress (O'Brien et al., 2017). The increased microbial activity on the thermal desorption treated soil implies that amendments may have increased nutrient cycling (Laird et al., 2010), which has the potential to decrease nutrient stress on treated soils. Thus, all three amendments significantly improved microbial activity relative to the control as indicated by the baseflux and POXC concentrations suggesting that amendments may be a beneficial step in the reclamation of soils treated with thermal desorption.

Microbial activity showed the greatest response to Proganics additions on treated soils which was reflected by the higher baseflux, rate of change of CO₂ flux, and POXC concentration than all other treatments. Given the elevated baseflux, the rate of change reflects a higher C utilization rate by soil microbes. The POXC results indicate that Proganics increased the

processed labile C pool (Culman et al., 2012) that is easily degradable by soil microbes (Calderon et al., 2017). The higher microbial activity and C utilization rate may be due to a high labile substrate, improved microbial habitat, and additions of soil microbes with Proganics. Proganics has a higher total C content than spent lime and compost (Table 3) which can result in a rapid increase in microbial metabolic activity like seen with additions of readily available C (Kolb, et al., 2009). Based on the low C:N ratio of Proganics (Table 3), Proganics would be expected to be susceptible to microbial decomposition (Johnson et al., 2007) without microbial N limitations (Parr and Papendick, 1978). Additionally, N is particularly important for amendments being applied to soils treated with thermal desorption due to a potential for N limitations (O'Brien et al., 2017). N limitations may be a result of the low SOM that has been observed on soils treated with thermal desorption (O'Brien et al., 2016; Vidonish et al., 2016; Sierra et al., 2015) or to volatilization of N during soil heating (Certinie, 2005). The treated soils contain low total N (0.05 g kg^{-1}) (Table 1), reflecting the N limitations proceeding thermal desorption treatment. Other research has seen higher microbial activity with increasing C levels in amendments (Quideau et al., 2013) provided N is not a limitation for microbial activity (Sparrow et al., 2011; Kolb et al., 2009). Based on the composition of Proganics as listed by the producing company, it is hypothesized that Proganics contains polysaccharide polymers and fatty acids which have the potential to be labile substrate (Bhattacharyya et al., 2016; (Annabi et al., 2007). The biochar applied to the soil with Proganics also has the potential to improve microbial habitat by increasing soil surface area (Rogovska et al., 2011). Increased soil surface area may be particularly beneficial to soil microbes on soils treated with thermal desorption due to the low SOM of these soils. it is hypothesized that Proganics may directly increase microbial biomass due to microbes in Proganics which has been proposed as an explanation for increases in CO_2

flux with the application of various organic amendments (Tejada et al., 2010; Flavel and Murphy, 2006; Rochette et al., 2000). Thus, proganics had a significantly higher baseflux, rate of change, and POXC concentration than all other treatments which may be due to labile components of the amendment, improved physical soil condition, and addition of soil microbes.

Baseflux and POXC concentrations increased with spent lime and compost additions relative to the control on treated soils, but not to the level observed with Proganics which probably resulted from a smaller labile substrate contribution from spent lime and compost compared to Proganics. This can be explained by the lower total C of spent lime and compost compared to Proganics (Table 3, the large inorganic C component of spent lime (Sims et al., 2018), and the recalcitrance of C in compost (Wilson et al., 2018; Tirol-Padre et al., 2007; Zvomuya et al., 2007; Flavel and Murphy, 2006). Despite the recalcitrance of compost, other research has found that compost additions increase POXC concentrations (White et al., 2020). In addition, N may have been a limitation for microbial activity with both the spent lime and the compost substrate due to a higher C:N ratio in spent lime (Table 3) and a majority of N being stable in compost (Dere and Stehouwer, 2011; Zvomuya et al., 2007). A potential for available N limitations on treated soils proceeding heating (O'Brien et al., 2017; Certinie, 2005) make amendments with less microbial available N less favorable for soils treated with thermal desorption. Thus, spent lime and compost showed a lower baseflux and POXC concentration response relative to Proganics which may be due to smaller labile substrate contributions by spent lime and compost compared to Proganics and N limitations for microbial activity.

The insignificant difference between the baseflux and POXC concentration of spent lime and compost amended soils despite spent lime having lower total C than compost (Table 3) as well as the lower rate of change of CO₂ flux of soils amended with compost supports that spent

lime has more initial labile substrate compared to compost, but compost has a greater potential to result in soil accumulation of C and improved microbial habitat. The greater labile substrate of spent lime may be due to a potential for sucrose from sugar beets as an impurity in spent lime which is a labile substrate (Kelliher et al., 2005) compared to recalcitrant substrate in compost (Wilson et al., 2018). Decreased rate of change with elevated or similar microbial activity indicates that compost extended microbial activity into drier soil conditions relative to spent lime and the control soil. The difference between spent lime and compost is likely due to spent lime containing a large inorganic C pool (Sims et al., 2018) and compost containing an organic C pool. The lower rate of change with compost additions indicates a potential for an accumulation of SOM (which has been observed in other research with compost additions (Sadeghpour et al., 2016; Dere and Stehouwer, 2011; Tirol-Padre et al., 2007; Zvomuya et al., 2007)). The low SOM of the treated soil indicates that soil microbes were likely largely dependent on amendment derived SOM, so the recalcitrance of the substrate contained in compost may have resulted in a lower C utilization rate by microbes. Thus, the baseflux, POXC concentration, and rate of change of CO₂ flux indicates that spent lime may initially contain more labile substrate relative to compost, but compost has a greater potential to result in the accumulation of C which can improve microbial habitat.

Compost and spent lime additions to the soil both resulted in a higher mineralized N concentration relative to the control and Proganics, and Proganics did not show significantly higher N mineralized relative to the control soil. Based on the C:N ratio of 9.5:1 (Table 3), N mineralization would be expected (Sullivan et al., 2002). However, some research has found that the majority of N in compost is stable (Dere and Stehouwer, 2011; Sullivan et al., 2002), but characteristics of compost may vary with the source of the compost (Huang et al., 2017), and

maturity of compost (Wilson et al., 2018). the mineralized N concentration at the conclusion of the incubation indicates that a majority of N was not stable in the compost used in this study. Interestingly, spent lime has a C:N ratio of 40.6:1 which should indicate immobilization of N in the soil (Sullivan et al., 2002). However, the inorganic C contained in calcium carbonate is not available for microbial mineralization (Wang et al., 2016). This may have led to a lower effective C:N ratio leading to mineralization of N. Additionally, the sucrose that was hypothesized to exist in spent lime as an impurity has the potential to lead to a priming effect resulting in utilization of SOM derived organic matter (Hopkins et al., 2014; Nottingham et al., 2009). This may have acted as an additional N source for microbial activity and mineralization. Proganics did not significantly impact N mineralization relative to the control despite showing higher microbial activity based on the baseflux and POXC results. the C:N ratio found for Proganics in this study was 10.5:1 (Table 3) which indicates that N mineralization would be expected to occur (Sullivan et al., 2002), but the producing company of Proganics reports the C:N ratio of Proganics as between 40:1 and 60:1 which would indicate that immobilization of N would occur (Sullivan et al., 2002). Proganics contains biochar which has the potential to absorb nutrients including inorganic N (Mukherjee et al., 2014; Zheng et al., 2013) and enhance N immobilization (Zheng et al., 2013) which is a potential explanation for the lack of response of mineralized N to Proganics additions. Thus, compost and spent lime both increased the N mineralized over the incubation period, but Proganics had no impact on N mineralized which may be a result of the biochar component of Proganics.

The baseflux results when both treated and non-treated soils were considered together differed from treated soils with significantly higher microbial activity in response to spent lime relative to compost which may have been due to the difference in SOM levels between treated

and non-treated soils or the microbial community contained within amendments. The higher SOM content of the non-treated soil compared to the treated soil (Table 1) may have sustained a larger priming effect proceeding spent lime addition. Additionally, the SOM in the treated soil may be more resistant to microbial attack proceeding heating (Certin, 2005) compared to subsoil SOM with more labile components (Vancampenhouta et al., 2012) which also contributes to a greater potential for a priming effect on the non-treated soil. The higher microbial activity seen with spent lime on the non-treated soil may also be due to the lack of a microbial community in spent lime. Therefore, the baseflux response to spent lime additions is largely based on the soil microbial community which may have been negatively impacted by heating (Cebon et al., 2011; Jimenez et al., 2008; Choromanska et al., 2002). This differs from compost because compost is expected to have a microbial community (Tejada et al., 2010; Flavel and Murphy, 2006) which may offset the negative impact to the native soil microbial community. Thus, spent lime resulted in significantly higher baseflux when compared to compost when both soils were considered together which differed from the treated soil and may have been due to the SOM content of the soil or the lack of microbial community in spent lime.

The POXC results when both non-treated and treated soils were considered together differed from the treated soil POXC results in that soils amended with compost had significantly higher POXC than soils amended with spent lime which is due to the lack of response of POXC on non-treated soils to spent lime relative to the control. This also differs from the baseflux results when both non-treated and treated soils were considered together. This is supported by the soil \times amendment interaction where spent lime on non-treated soils did not have higher POXC than the non-treated and treated control soils where as POXC concentrations were higher than the control on treated soils amended with spent lime. Additional research would be required

to determine why POXC concentrations responded to spent lime on treated soils and not non-treated soils, but it may be related to impacts on SOM from the thermal desorption process. Thus, soils amended with compost had significantly higher POXC concentrations than soils amended with spent lime when both non-treated and treated soils were considered together due to the lack of response of POXC to spent lime.

The inorganic N concentrations differed when both non-treated and treated soils were considered together and supported the POXC results with compost showing a higher inorganic N concentration at the end of the incubation than spent lime. Based on the soil \times amendment interaction, this difference was due to a higher mineralized N concentration on treated soils proceeding the incubation with compost compared to non-treated soils. This may be due to a larger microbial population in treated soils compared to non-treated subsoils. A larger microbial population would result in a greater microbial response to the large organic pool of compost. It may also be due to greater P limitations on non-treated soils (Table 1) that limited microbial population growth in response to compost substrate quicker than on treated soils resulting in lower N mineralization. The lack of differences in N mineralized for the soil \times amendment interaction for all treatments other than compost indicates that the thermal desorption process has minimal negative impacts on microbial activity over the long-term. This means that low SOM would be the biggest concern on both non-treated and treated soils which would require reclamation with organic amendments proceeding oil remediation. Thus, compost resulted in higher N mineralized on treated soils than non-treated soils which may be due to a larger microbial population on treated soils relative to non-treated subsoils or P acting as a limiting factor for microbial activity on non-treated soils than treated soils.

The soil \times amendment interaction results for baseflux, rate of change, and POXC concentration indicate that the thermal desorption process might not significantly harm soil microbial communities over the long-term and that amendments can be used to improve microbial activity on soils treated with thermal desorption. There was no significant difference in microbial activity measured through baseflux, rate of change, and POXC concentrations between the non-treated control and treated control indicating that thermal desorption did not significantly hinder microbial activity. Despite this, the low SOM of both non-treated and treated soils indicates that reclamation with organic amendments would still be required to improve microbial activity (Zhang et al., 2014; Rogovska et al., 2011; Flavel and Murphy, 2006) and nutrient cycling (Acosta-Martinez et al., 2010; Sardans and Penuelas, 2010; Schjonning et al., 2003; Dick et al., 2001). Proganics showed the greatest potential to improve microbial activity on both non-treated and treated soils as indicated by the baseflux and POXC concentrations which reflects the labile substrate in Proganics. The lack of significant difference in baseflux and rate of change in response to spent lime additions and lack of significant difference in baseflux, rate of change and POXC concentration in response to compost additions between non-treated and treated soils also supports that thermal desorption treatment did not significantly negatively impact microbial communities over the long-term. However, Proganics additions resulted in higher baseflux on non-treated soils relative to treated soils. Additionally, the rate of change was higher on treated soils than non-treated soils in response to Proganics additions indicating that microbial activity may have declined more sharply on treated soils. This reflects a potential that thermal desorption negatively impacted microbial communities which may be due to a smaller soil microbial population since the negative impact of thermal desorption on microbial activity was only observed with the addition of an amendment containing high labile substrate. Thus, the soil \times

amendment interaction indicates that microbial activity is not significantly harmed by thermal desorption and soil amendments can be used to improve microbial activity on soils treated with thermal desorption.

Impact of DW Cycle

The decrease in microbial activity as measured by baseflux and C utilization as measured by rate of change from DW cycle 1 to DW cycle 2 reflects a decrease in labile substrate due to microbial mediated decomposition in the first DW cycle (Birch, 1958) but the lack of difference in baseflux and rate of change between DW cycle 2 and DW cycle 3 indicates that labile substrate was depleted proceeding DW cycle 1 when all treatments were considered together. The increase in microbial activity seen in cycle 1 may have also been due in part to the reestablishment of the microbial population proceeding microbial death from the drying process (Haney et al., 2004). The depletion of labile substrate over DW cycle 1 may be due to the low SOM of this soil of 0.66% (Table 1) because DW cycles can result in the release of SOM that was previously physically protected making this SOM available to microbial attack (Fierer and Schimel, 2003; Degens, 1995). In addition to low levels of SOM that can be released upon rewetting, the SOM released may be stable and therefore resistant to decomposition (Degens, 1995). Soils treated with thermal desorption are particularly likely to contain stable Som due to utilization of labile SOM during soil disturbance or oxidation of labile SOM during the thermal desorption process. Heating of soils can result in aromatization and preferential denaturation of labile sources of C such as carbohydrates, sugars, and lipids (Certinie, 2005). Additionally, oxygen groups can be lost from SOM during heating resulting in a decrease in solubility of SOM. Proceeding heating of soil, remaining SOC may be in the form of black C (Certinie, 2005) which shows limited mineralization from microbial activity (Major et al., 2010). However, the

low SOM of this soil would make the release of physically protected SOM limited making microbial decomposition largely dependent on substrate from amendments. Since all treatments were considered together, the lack of significant decrease in baseflux and rate of change between DW cycle 2 and DW cycle 3 may be due to a combination of low SOM in the soil and low labile substrate contributed by spent lime and compost as previously discussed. The elevated microbial activity in the first DW cycle may be due to reestablishment of the microbial community proceeding air drying prior to the incubation (Haney et al., 2004). Additionally, Proganics was hypothesized to contain living microbes and compost contains living microbes (Tejada et al., 2010; Flavel and Murphy, 2006), but amendment derived microbes may not be adapted to DW cycles as well as native microbes. Thus, the significant difference in baseflux and rate of change between DW cycle 1 and DW cycle 2, but lack of a significant difference between DW cycle 2 and DW cycle 3 may be due to depletion of labile substrate, lack of release of physically protected SOM with DW cycles, or a microbial community that is adjusted to DW cycles.

The impact of DW cycle on baseflux when both non-treated and treated soils were considered together differed from treated soils with a decrease in baseflux and rate of change from DW cycle 2 to DW cycle 3. This likely reflects the lower SOM level (Table 1) and potential loss of labile SOM with heating (Certinie, 2005) of treated soils relative to non-treated subsoils which can contain relatively high labile substrate (Vancampenhouta et al., 2012). These factors may have led to a priming effect sustained into the second DW cycle on the non-treated soils resulting in the significant difference between DW cycle 2 and DW cycle 3. This is further supported by the significantly higher microbial activity in the soil \times cycle interaction for non-treated DW cycle 1 and DW cycle 2 than treated DW cycle 1 and DW cycle 2 respectively. Additionally, given that there was no significant difference between DW cycle 3 of non-treated

and treated soils, the differences in DW cycle 1 and DW cycle 2 are likely due to differences in SOM content and resistance of SOM to microbial attack. Therefore, the non-treated soils may have shown a significant decrease in microbial activity between DW cycle 2 and DW cycle 3 due to a higher labile component of the SOM.

Impact of Amendment × Cycle Interaction

Microbial activity in the control from treated soils did not show a significant difference in baseflux between DW cycles or with the three DW cycles with compost and latter two DW cycles with spent lime. None of the three DW cycles with the control was significantly higher than any other amendment × cycle interaction. This is likely due to the low SOM of this soil (Table 3) proceeding thermal desorption treatment and the recalcitrance of SOM following heating of soils (Certinie, 2005) leading to low microbial activity on these soils (Cebon et al., 2011). The lack of significant difference in microbial activity indicates that physically protected labile SOM was not released from this soil upon rewetting or the SOM that was released was resistant to microbial attack due to the heating process (Certinie, 2005). Thus, the control soils treated by thermal desorption did not have higher microbial activity than any other amendment × cycle interaction and no significant difference was seen between DW cycles which was likely due to limited substrate and resistance of the remaining substrate to microbial decomposition.

Proganics cycle 1 resulted in higher baseflux and rate of change than all other amendment × cycle interactions reflecting higher microbial activity and a higher C utilization rate. A higher C utilization rate could result in depletion of labile substrate, but the continued elevated microbial activity sustained by the high total C (Table 1) on Proganics amended soils indicates that labile substrate was not depleted. Proganics cycle 2 showed a significantly lower baseflux than Proganics cycle 1, but significantly higher baseflux than all other amendment × cycle

interactions with the exception of Proganics cycle 3. this indicates that Proganics maintained higher microbial activity in the first two DW cycles than any other treatment which has the potential to lead to higher nutrient cycling (Laird et al., 2010) which is an important step in reclamation of soils treated with thermal desorption. Proganics Cycle 3 did not have significantly different baseflux results than Proganics Cycle 2, spent lime cycle 1, and compost cycle 1, but it was significantly higher than all other treatment \times cycle interactions with the exception of Proganics Cycle 1. This indicates that Proganics maintained similar microbial activity after the initial decline meaning that Proganics would have the potential to maintain elevated microbial activity through additional DW cycles. Based on microbial response, the labile substrate pool in Proganics cycle 3 was still comparable to spent lime and Compost at their initial application. Therefore, Proganics increased microbial activity relative to the control through all three DW cycles indicating that Proganics can improve microbial activity on soils treated with thermal desorption and the elevation in microbial activity can be sustained through DW cycles.

Spent lime showed the ability to increase microbial activity over the short-term with significantly higher baseflux relative to the control only observed in DW cycle 1. However, microbial activity declined proceeding the first DW cycle with spent lime and baseflux was not significantly different than the three DW cycles with compost and the control soil. This indicates that the labile substrate contributed to the soil from spent lime application was depleted after one DW cycle which supports the hypothesis that sucrose from sugar beets was one of the impurities in spent lime and was rapidly utilized (Wang et al., 2015) leaving minimal labile substrate for DW cycle 2 and DW cycle 3. The low SOM seen on this soil would limit a priming effect like has been observed with sucrose additions to the soil (Hopkins et al., 2014; Nottingham et al., 2009), so microbial activity is largely dependent on the amendment substrate. The lack of

significant difference in rate of change between the three DW cycles of spent lime and the control soil was likely due to the large inorganic C component of spent lime (Sims et al., 2018) that is not mineralized by soil microbes (Wang et al., 2016). The large inorganic c component also indicates that spent lime would likely have limited benefits for increasing SOM which is needed on soils treated with thermal desorption based on the low SOM content (Table 1). Therefore, spent lime would not be an ideal amendment for reclamation of soils treated with thermal desorption due to spent lime only increasing microbial activity over the short term and a large inorganic C component that does not increase SOM.

The application of compost to soils treated with thermal desorption showed no potential for improving microbial activity over DW cycles as indicated by the baseflux results. Compost did not show a significant difference in baseflux between DW cycles or the three DW cycles with the control soil. This reflects the recalcitrant substrate of compost (Wilson et al., 2018). Compost cycle 3 had a significantly lower rate of change than the third DW cycle for the control soil despite there being no significant difference in microbial activity indicating that compost extended microbial activity into drier soil conditions. The baseflux and rate of change indicate that there would be an accumulation of organic matter in the soil which has been seen in other research with the application of composted manure (Sadeghpour et al., 2016; Dere and Stehouwer, 2011; Tirol-Padre et al., 2007; Zvomuya et al., 2007). This indicates that compost may have improved microbial habitat which can occur with the application of organic amendments (Kladivko and Clapperton, 2011; Doran and Smith, 1987). Low SOM (O'Brien et al., 2016; Vidonish et al., 2016; Sierra et al., 2015) is a concern on soils treated with thermal desorption, and compost has the potential to increase SOM. However, nutrient stress (O'Brien et al., 2017) is also a concern on soils treated with thermal desorption which would require a more

labile amendment than compost to increase microbial mediated nutrient cycling as indicated by the baseflux and rate of change. Thus, compost did not improve microbial activity on soils treated with thermal desorption as indicated by the baseflux and rate of change, but this indicates that compost would result in an accumulation of SOM.

Microbial activity in the control soil for both non-treated and treated soils considered together did not show a significant difference in baseflux between DW cycles, and none of the three DW cycles with the control was significantly higher than any other amendment \times cycle interaction. This reflects that soils may require reclamation due to the low SOM of subsoils proceeding oil spill remediation rather than specific impacts from thermal desorption on microbial communities. Proganics showed an ability to sustain elevated microbial activity on low SOM soils regardless of whether the soil was treated by thermal desorption. The spent lime results when considered for both non-treated and treated soils differed from the results of treated soils in that baseflux in spent lime cycle 3 was still significantly higher than baseflux in the three DW cycles with the control soil. This likely reflects the difference in SOM between non-treated and treated soils with higher SOM in non-treated soils (Table 1 and the difference in SOM seen in subsoil (Vancampenhouta et al., 2012) relative to heated soil (Certinie, 2005). Microbial response to compost also differed when considered for both non-treated soils and treated soils compared to treated soils alone with the first DW cycle showing a significantly higher baseflux than the three DW cycles for the control which may reflect negative impacts from soil heating on the microbial community proceeding thermal desorption treatment (Cebren et al., 2011; Jimenez et al., 2008; Choromanska et al., 2002). Proganics cycle 2 showed a significantly higher rate of change than all three DW cycles with the control soil. This indicates that the non-treated soil sustained a higher C utilization rate for a longer time with Proganics additions which is probably

due to negative impacts from soil heating on the microbial community in thermal desorption treated soils. Compost cycle 2 also significantly decreased the rate of change of CO₂ flux relative to the second DW cycle with the control soil. This indicates that compost may have improved microbial habitat as discussed above, but it also suggests that SOM was more resistant to microbial attack in the treated soil. This indicates that microbial activity continued into drier soil conditions on the non-treated soil in DW cycle 2 compared to the second DW cycle with compost on the treated soil. This is likely a result of the negative impact of soil heating on microbial communities. Therefore, the baseflux and rate of change results for both non-treated and treated soils considered together indicate that the low SOM is likely the largest factor hindering microbial activity, but microbial populations may have been negatively impacted by the thermal desorption process.

The baseflux and rate of change results for the soil × amendment × cycle interaction indicate that the low SOM of both non-treated and treated soils is likely the largest concern proceeding remediation rather than specific effects of thermal desorption. However, higher microbial activity with non-treated Proganics to treated Proganics for DW cycle 2 may be a result of the impact of DW cycles on different microbial communities proceeding thermal desorption treatment. Heating of soils can negatively impact soil microbial communities (Jimenez et al., 2008; Choromanska et al., 2002). The dry subsoil may have selected for a native microbial population that is capable of adjusting to DW cycles with rapid increases in soil moisture potential which has been suggested as an explanation to microbial response to rapid rewetting of soil by Fierer and Schimel (2003). Microbes in Proganics have the potential to not be adjusted to rapid changes in soil moisture which would negatively impact the response of amendment derived microbes to DW cycles. The native microbial population may have

recovered over the second DW cycle leading to no significant difference between non-treated and treated soils in the third DW cycle with Proganics. Additionally, the higher rate of change with lower microbial activity in treated Proganics cycle 1 does represent a negative impact on microbial communities from thermal desorption because it indicates a steeper decline in microbial activity proceeding amendment application while labile substrate is still available. Therefore, thermal desorption likely negatively impacted the microbial communities of soils treated with thermal desorption, but the low SOM of soils proceeding oil remediation is likely a larger concern than impacts of thermal desorption on microbial communities.

Study Limitations

This study was a laboratory study which is a limitation of the study design. Prior to incubations, soils were sampled, dried, sieved, and placed into biochambers and all of these disturbances have the potential to impact microbial activity and nutrient availability. Additionally, CO₂ flux measures from the soil are highly variable. Taking three readings per day and measuring the change in CO₂ flux over a five-minute reading were done in an attempt to minimize the impact of the variability of CO₂ measurements. An additional limitation of this study was the lack of ability to take POXC measurements throughout the incubation given that POXC concentrations can change rapidly. Future studies could include additional replicated biochambers where subsamples can be taken to measure POXC concentrations throughout the incubation.

CONCLUSION

This research supports that microbial activity of saline and thermal desorption treated problem soils can be improved with the use of amendments. However, the response of problem soils to amendments varied with the specific problem soil and the measures of microbial activity indicating that site specific characteristics are useful when selecting an amendment for recovering microbial activity of problem soils. When considering baseflux as a measure of microbial activity, Proganics has the greatest ability to improve microbial activity on both saline soils and soils treated with thermal desorption. This is followed by either spent lime or compost on both soils. The rate of change of CO₂ flux indicates that compost decreases the C utilization rate of soil microbes on both saline soils and soils treated with thermal desorption. However, Proganics increases the C utilization rate of microbes on soils treated with thermal desorption, but not saline soils. When considering POXC as a measure of microbial activity, Proganics applications has the greatest ability to improve microbial activity on both saline soils and soils treated with thermal desorption. Spent lime and compost increase microbial activity on soils treated with thermal desorption but decrease microbial activity on saline soils. When considering mineralized N as a measure of microbial activity, compost and spent lime applications have the greatest ability to increase microbial activity on both saline soils and soils treated with thermal desorption. Proganics decreases microbial activity on saline soils and does not impact microbial activity on soils treated with thermal desorption.

DW cycles influence the response of baseflux to soil amendments. Proganics sustained elevated microbial activity through all three DW cycles on both saline soils and soils treated with thermal desorption. Spent lime and compost did not elevate microbial activity through all three

DW cycles but spent lime applications had a higher ability to sustain microbial activity through DW cycles than compost.

Given that the goal of increasing microbial activity on these problem soils is to increase microbial mediated nutrient cycling, compost and spent lime are recommended for improvement of saline soils and compost is recommended for reclamation of thermal desorption treated soils due to their ability to increase microbial activity as measured by baseflux and mineralized N. The saline soils examined for this study did not require increased SOM as part of the efforts to improve soil quality , so either compost or spent lime are effective amendments for reclamation. The soils treated with thermal desorption examined in this study contain low SOM, so compost is recommended for reclamation over spent lime. Despite showing increased microbial activity, Proganics is not recommended for reclamation of either saline soils or soils treated with thermal desorption because it does not increase nutrient cycling on these problem soils based on the mineralized N concentrations at the conclusion of the incubation.

This research indicates several focuses for future research regarding the recovery of microbial activity on problem soils. Research is required to determine ideal application rates of compost and spent lime to problem soils to determine an application rate that improves microbial activity and nutrient cycling but does not result in environmental harm from excess nutrient cycling. Given that this was a laboratory study, field work is required to verify incubation results with field conditions. Additional research is required regarding Proganics to analyze the lack of increase in mineralized N despite higher microbial activity. Research is also required on SOM characteristics proceeding thermal desorption treatment particularly with regards to POXC concentrations.

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