CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF THERMALLY AND CHEMICALLY DISTURBED SOIL IN NORTHWESTERN NORTH DAKOTA

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Chemical and biological characteristics of thermally and chemically disturbed soil in Northwestern North Dakota

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ABSTRACT

Thermal desorption (TD) remediates hydrocarbon-contaminated soil by heating the soil (200 to 500 °C) to volatilize the hydrocarbons, effectively removing the contaminant from the soil. If the soil is then used for agricultural production, reclamation success can be determined by quantifying aspects of soil health. Cation exchange capacity (CEC), cation selectivity and Gibbs free energy (ΔG_{ex}) of TD-treated and untreated soil were compared. Although CEC and ΔG_{ex} differed, cation selectivities were not altered suggesting that alternative fertility management to retain previous soil productivity may not be needed. From field plots, N-transforming genes were lowered in contaminated and TD-treated soils as compared to non-contaminated soil, but the addition of surface soil (1:1 blends) increased N-cycling genes to levels reported in the literature. Thermal desorption may not alter soil chemical as much as biological metrics, but blending treated or contaminated soils with native surface soils can enhance soil function and, ultimately, productivity.

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DEDICATION

I would like to dedicate this to my parents and sister, Joseph and Theresa Ritter and Christine, for their love and support throughout this process. Being raised with an agricultural background has provided significant knowledge that I have grown upon.

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LIST OF ABBREVIATIONS

AOA.....Ammonia-oxidizing archaea

AOB.....Ammonia-oxidizing bacteria

CEC.....Cation exchange capacity

DOC.....Dissolved organic carbon

PMN.....Potentially mineralizable nitrogen

SOC.....Soil organic carbon

SSA.....Specific surface area

TD.....Thermal desorption

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GENERAL INTRODUCTION

As anthropogenic activities like crude-oil production continue, the need for soil remediation and reclamation after disturbance from exploration, recovery, and accidental contamination is necessary. By considering soil health, or the capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health (Karlen et al., 1997), soil remediation and subsequent reclamation can better promote the productivity and functioning of the soil. Reclaimed soils may never fully resemble native soils in terms of quality and health criteria because of the diverse nature, origin and environmental impact of contaminants and treatments. Therefore, it is valuable to assess the ecology and functioning of these soils using a variety of chemical, biological, and physical parameters.

Thermal desorption (TD) can be used as a crude-oil remediation process that uses direct heat energy where the contaminated soil is heated between 200 and 500 °C in a desorber chamber causing volatilization of the associated hydrocarbons. The vaporized contaminants then enter a secondary chamber where the hydrocarbons are converted to CO_2 and water vapor at temperatures commonly greater than 600 °C. The treated soil exits the desorber chamber where it is re-hydrated and cooled. The remediated soil material can then be returned to the original excavation site at the individuals' discretion (Hamby, 1996).

Very little has been reported on the TD process affecting the cation exchange capacity (CEC) and cation preference of the soil that is the focus of the first study (Chapter 2). The study compares CEC, cation preference, and Gibbs free energy (ΔG_{ex}) between native topsoil and subsoil to TD-treated topsoil and subsoil. Determining the soil's chemical properties is important as the oil industry continues to grow, along with the concurrent risk of spills and the

long-term impacts of hydrocarbon contamination and remediation on soil fertility and agricultural productivity.

The second study (Chapter 3) focuses on the soil microbial community and function as related to important nutrient cycles and transformations. Contaminated systems tend to be dominated by organisms capable of utilizing and/or surviving the toxic contamination (Macnaughton et al., 1999), potentially displacing organisms previously dominant in the undisturbed system responsible for transforming important nutrients (N, S, P, C). This study compares N-cycling microorganism quantities and enzyme activities in oil-contaminated, TD-treated soil, native-uncontaminated soil, and 1:1 blends with and without compost over time. This study provides important information on the suitability of TD as a remediation technique for agricultural soils contaminated with hydrocarbons, as little information is available on how the TD process affects microbial communities or their recovery. Since enzymes are critical metabolic catalysts in soil, the effect of reclamation strategies will highlight potential nutrient implications.

CHAPTER 1: LITERATURE REVIEW

1.1. Introduction

A healthy soil is considered a stable soil, which has a high resilience to stress, high quantities of biological diversity, and high levels of internal nutrient cycling (Warkentin, 1995; van Bruggen and Semenov, 2000). Soil provides the foundation for the establishment of stable terrestrial nutrient cycles encompassing plant growth and microbial processes; therefore, a soils' composition and density directly affect the future stability of the ecosystem (Sheoran et al., 2010). Degradation of soil from erosion, compaction, or contamination has led to developing measures to determine the health of the soil (Kibblewhite et al., 2008). Soil health is not a new concept and was often used interchangeably with soil quality (Pankhurst et al., 1995; Karlen et al., 1997). Used synonymously, soil quality-health was defined as: "The capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health" (Pankhurst et al., 1995).

However, attempts have been made to differentiate between soil quality and soil health; where soil quality assesses soil functionality and soil health considers conditions required for sustainability and promoting plant, animal, and human health (Doran et al., 2002; Gil-Sotres et al., 2005; Cebron et al., 2011). Therefore, reclamation, where degraded lands are returned to productivity and biologic function is reestablished (Sheoran et al., 2010), and restoration of a disturbed soil should consider soil health by using a holistic approach to restoring soil function; taking into account soil physical, chemical, and biological properties, their roles, and interactions, thus contributing to enhanced yields or plant productivity (Figure 1).

Estimates indicate that the land area prone to soil degradation is about two billion ha, of which 562 million ha is agricultural land, 685 million ha is permanent pastures, and 719 million

ha is forest and woodland (Lal, 2001). In addition to negatively impacting agronomic production, soil degradation is increasingly linked to food security, water security, energy security, biodiversity, and many ecosystem services (Brevik et al., 2015). Human activities influenced by socio-economic factors such as deforestation, land use conversion, or over-exploitation can exacerbate soil degradation. Similarly, during the production, transportation, and storage of crude oil, the risk of accidental spills can introduce toxic hydrocarbons into the environment that damage the natural functioning of the soil (Chaîneau et al., 2003; Rahman et al., 2003).

Crude oil and natural gas production have increased considerably in the past decade in the Bakken Formation (approximately 34 million barrels of oil per month in 2015; North Dakota Department of Mineral Resources, 2016), which lies in the Williston Basin extending over parts of North Dakota, Montana, and Saskatchewan, Canada. The mobility and fate of hydrocarbons present in crude oil is dependent on the amount and chemistry of oil spilled, the physical and chemical properties of the soil, and the length of exposure (Chaîneau et al., 2000; Ijah and Antai, 2003). Once crude oil is introduced to the soil environment, the hydrocarbons can adsorb to the organic or mineral matter of soil, undergo biodegradation, volatilization, or leaching (Chaîneau et al., 2003), thereby having the risk of threatening ecosystem and human health. Hydrocarbons can influence the physical, chemical and biological properties of soil, such as microbial counts and diversity, enzyme activity, and soil respiration (Ijah and Antai, 2003; Baran et al., 2004). One of the main mechanisms of soil biological degradation caused by oil spills is an imbalance in the C:N ratio. The excess C from the oil can cause N immobilization resulting in N deficiency in oil-saturated soil, conditions which hinder the growth of bacteria and utilization of the C source (Ayotamuno et al., 2006). Although there are naturally occurring soil bacteria capable of

utilizing and degrading complex hydrocarbons, contaminated ecosystems tend to be less diverse in microbial communities and dominated by the organisms capable of utilizing and/or surviving the conditions at the potential expense of other important enzymes that transform other vital nutrients (N, S, P) (Macnaughton et al., 1999). Through reclamation, efforts would be made to restore the diversity of the microbial population to their uncontaminated precursor.

One method to remediate crude-oil spills that is relatively fast and effective at removing hydrocarbons is thermal desorption (TD). Thermal desorption remediates hydrocarbon-contaminated soils in a manner that preserves the remediated soil for re-use and eliminates the need for a landfill (Hamby, 1996). Thermal desorption is a process that uses direct or indirect heat energy where the contaminated soil is heated between 200 and 500 °C in a desorber chamber causing near total volatilization of the associated hydrocarbons. The vaporized contaminants then enter a secondary chamber where the hydrocarbons are converted to CO_2 and water vapor at temperatures greater than 600 °C. The soil exits the desorber chamber reduced or eliminated from hydrocarbons (TD-treated soil) where it is then re-hydrated and allowed to cool. The remediated soil can then be returned to the original excavation site at the individuals' discretion (Hamby, 1996).

Studies have previously reviewed many physical effects of heating soil at high temperatures such as particle size distribution, mineralogical changes, and combustion of soil organic carbon (SOC) (Biache et al., 2008; Dazy et al., 2009; Bonnard et al., 2010; O'Brien et al., 2016). Around 20-30% reduction in SOC was observed after heating (Bonnard et al, 2010' O'Brien et al., 2016); whereas, dissolved organic carbon (DOC) increased after TD treatment (O'Brien et al., 2016). The temperature threshold for clay deterioration is normally above 500 °C, which can result in dramatic textural shifts (Pape et al., 2015). For instance, kaolinite

structure begins to degrade at 530 °C; while, bentonite, often composed of smectite minerals, does not deteriorate due to heating until temperatures reach over 700 °C (Tan et al., 1986). Slight decreases in clay-sized particles resulted in a substantial reduction in specific surface area (SSA) as clay-sized particles generally dictate SSA (O'Brien et al., 2016). The effects of TD on soil physical properties has been well researched; while, the effects of TD on a soil's ion selectivity and the reestablishment of the microorganisms involved in N processing are largely unknown.

1.2. Thermal Desorption: Measuring impacts on soil chemical properties

The heating of clays to temperatures seen in desorber chambers and subsequent destruction of soil organic matter may significantly alter the cation exchange capacity (CEC) of soil and ability to retain important plant nutrients. If remediated soil is to be used for agricultural purposes, information about nutrient retention is vital for developing a nutrient management plan post-remediation. However, there is variability on TD temperature effects on soil CEC. For example, when indirectly heated at 350 °C for 10 min CEC did not significantly decrease for a kaolinite, illite dominated Typic Hapludalf, and only slight changes occurred for exchangeable Ca, Mg, or K. However, when the soil was heated to 600 °C for 10 min a nearly 50% decrease in CEC and 35% decrease in exchangeable Ca occurred (Roh et al., 2000). Additionally, no significant changes in clay content, which is important for soil CEC, were observed when a Hg-contaminated soil in Guizhou Province, China was treated at 400 °C for 20 min (Ma et al., 2014). Similarly, thermal treatment at 700 °C for 20 min decreased CEC by 52% which was contributed to the oxidation of organic matter and the destruction of the clay structure (Ma et al., 2014). Cation exchange values also decreased in an acidic loam soil from 21.9 $cmol_c\ kg^{-1}$ to 9.8 $cmol_c\ kg^{-1}$ and 3.1 $cmol_c\ kg^{-1}$ when heated at 250 °C and 500 °C, respectively, for 60 min (Pape et al.,

2015). Comparable results were recorded for a neutral horticultural soil with an initial CEC of 28.2 $cmol_c\ kg^{-1}$ to 14.2 and 6.3 $cmol_c\ kg^{-1}$ when heated at 250 °C and 500 °C, respectively, for 60 min (Pape et al., 2015). The results of TD on CEC vary depending on heating temperature and time and may alter the ability of the soil to retain cations required for crop nutrients. However, little information is known as to whether TD may influence the cation selectivities, the ability of the soil to adsorb specific cations. Therefore, additional research to determine cation selectivities is necessary.

1.3. Thermal Desorption: Measuring impacts on soil biological properties

Thermal desorption has been shown to remove hydrocarbons from the soil (McAlexander et al., 2015), but has altered some soil chemical properties such as decreases in CEC from the oxidation of organic matter and the destruction of the clay structure due to high temperatures used to remove contamination (Bonnard et al., 2010; Pape et al., 2015). However, little information is known for soil biological properties after TD treatment; therefore, selecting indicators of soil biological function is important to measure remediation success of TD soil. Taking biological functions into consideration requires useful, reproducible indicators that respond quickly to changes in management (Doran and Zeiss, 2000). Soil organisms are largely responsible for nutrient transformations that provide the foundation for many important soil properties like fertility and structure (Pankhurst et al., 1995). However, most agricultural soils are limited in their ability to supply adequate plant-available forms of N due to the susceptibility of loss through leaching, denitrification, or immobilization (Gul and Whalen, 2016). The N cycle consists mainly of microbial-driven processes including ammonification, anaerobic NH_3 oxidation, nitrification, denitrification, and N fixation (You et al., 2009). These transformations consist of a series of dependent processes where the products of one step become substrates in

the following phase and any downstream products may be immobilized by soil organic matter, taken up by plants or lost through leaching or as reactive gases (Phillips et al., 2015).

Soil enzymes, which are the catalysts for biochemical reactions, are a measure of the soil microbial activity. Some enzyme activities respond rapidly to changes caused by both natural and anthropogenic factors such as management (Bandick and Dick, 1999) and pollutants (Andreoni et al., 2004), and are directly related to nutrient cycles and transformations (Gianfreda et al., 2005). Enzyme activities are also sensitive to contamination and have the advantage of being measured without expensive, sophisticated instruments suggesting their use as suitable indicators of soil quality (Gianfreda et al., 2005; Shen et al., 2005). Through enzymes, inorganic N fertilizers, like urea, are converted into NH_3 or NH_4^+. Ammonia-oxidizing prokaryotes perform the first step in nitrification, oxidizing NH_3 into nitrite (NO_2^-) (Hallin et al., 2009) followed by the oxidation to nitrate (NO_3^-) by NO_2^- oxidizers. Nitrate can then be reduced into NO_2^- by nitrate reductase or to N_2 in the final step of denitrification by nitrous oxide reductase (Hallin et al., 2009). Since the N cycle is a series of dependent processes, measuring steps within the N cycle (Figure 2) can determine if the cycle is functioning as a system of soil health.

1.3.1. Urease

Soil urease is important in N transformations and cycling as it enzymatically catalyzes the hydrolysis of urea to NH_3 and CO_2 ($NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2$) resulting in an accumulation of NH_4^+ from NH_3 protonation and a rise in soil pH (Tabatabai and Bremner, 1972; Kim et al., 2008). This is especially critical to current agricultural practices in which the use of urea as a N fertilizer source has increased significantly in recent decades (Cozzi et al., 2014). Urease has been measured as a soil health indicator in other reclamation and management studies (Sahrawat, 1984; Bergstrom et al., 1998; Marschner et al., 2003), and its activity has been

measured in different soil types with ranges from 23 to 390 μg NH_4-N g^{-1} soil 2 h^{-1} (Tabatabai, 1977); the majority of studies reported values on the lower end. From the studies reporting urease activity, the higher values were predominately from soils that were vegetated and not disturbed, and the lower values were from vegetation-free or disturbed soils.

Higher urease values under vegetation have been attributed to higher microbial proliferation and microbial activity at the rhizosphere (Fenn et al., 1992; Bergstrom et al., 1998). Comparably, a reclaimed calcareous Regosol (Entisol) had urease activities that ranged from 25 μg NH_4-N g^{-1} soil 2 h^{-1} with NPK mineral fertilizer and plant residue removed to 30 μg NH_4-N g^{-1} 2 h^{-1} with a straw treatment added at 4.0 Mg ha^{-1} y^{-1} with 10 kg N as $CaCN_2$ Mg $straw^{-1}$ (Marschner et al., 2003). Slightly higher values (45 to 64 μg NH_4-N g^{-1} 2 h^{-1}) were recorded in an Ustollic Camborthids at ambient CO_2 conditions in shortgrass steppe in northeastern Colorado at the end of the growing season (Kandeler et al., 2006). The following activities followed different methods resulting in separate units, but still provide valuable comparisons. Where tillage has been used, urease activity has been higher in conventional tillage (67 μg N g^{-1} h^{-1}) compared to no-tillage (53 μg N g^{-1} h^{-1}) in a fine textured Orthic Gray Brown Luvisol (Hapludalf, Glossudalf) (Bergstrom et al., 1998). Urease concentrations are also found to be reduced at depths below 8 cm with values of 39 and 35 μg N g^{-1} h^{-1} at 0 to 8 cm to 27 and 28 μg N g^{-1} h^{-1} below 8 cm for no-tillage and conventional tillage, respectively in an Orthic Humic Gleysol (Aquoll, Humaquept) (Bergstrom et al., 1998).

Although urease activity can vary due to land management, vegetation, and tillage, there has been a documented negative relationship between urease activities and polycyclic aromatic hydrocarbon concentrations, which suggests the enzyme could be used as a sensitive indicator in soils contaminated with these compounds (Gianfreda et al., 2005). An increase in urease activity

at 70 °C has been shown in a clayey-skeletal Udic Rhodustalfs (Sahrawat, 1984) but heating at 105 °C for 24 hr or autoclaved at 120 °C for 2 hr resulted in zero urease activity in Iowa soils (Zantua and Bremner, 1977). Greater temperatures are utilized by TD and may cause declines in urease activity, which can have implications for the N supplying power of soils amended with urea fertilizer. Recovery of urease levels in the soil following reclamation may be used as a metric for determining when reclamation of soil has been successful and soil health has improved.

1.3.2. Nitrification

Depending on the environmental conditions, nitrification can either benefit or harm N retention since NH_3 is highly volatile and can be readily lost (Kowalchuk and Stephen, 2001). The use of N-rich fertilizers, generated either chemically or through the recycling of organic wastes, is a necessity for the high productivity of modern agricultural practices (Kowalchuk and Stephen, 2001). Nitrification can lead to N loss by increasing mobile N through the conversion of NH_3 to NO_3^- or linking nitrification with denitrification activities (Kowalchuk and Stephen, 2001). Nitrification is a two-step process in which NH_3 is oxidized to NO_2^- by NH_3-oxidizing organisms followed by NO_2^- oxidation which converts NO_2^- to NO_3^- by NO_2^--oxidizing organisms. Ammonia oxidation converts NH_3 to hydroxylamine (H_3NO) by the NH_3 monooxygenase enzyme, the rate-limiting step for nitrification in a wide variety of environments (Kowalchuk and Stephen, 2001; You et al., 2009). Both NH_3-oxidizing bacteria (AOB) and NH_3-oxidizing archaea (AOA) contain the NH_3-oxidizing gene (*amoA*) critical for nitrification (Könneke et al., 2005; You et al., 2009). In contrast to denitrification, which can be accomplished by many different bacteria, archaea, or eukaryotes; the oxidation of NH_3 is performed only by a few phylogenetically constrained NH_3-oxidizing bacteria and archaea

(Hallin et al., 2009). Ammonia-oxidizing bacteria have generally been accepted as utilizing reduced N such as NH_3 and not NH_4^+ as an energy source substrate, CO_2 as a C source, and molecular oxygen as an electron acceptor; therefore, the NH_3/NH_4^+ ratio may affect AOB and AOA population growth (Kowalchuk and Stephen, 2001; You et al., 2009).

Ammonia-oxidizing archaea and bacteria can be found in the same environments with both groups competing directly but typically occupying separate ecological niches (Nicol et al., 2008; Verhamme et al., 2011). Ammonia-oxidizing bacteria are generally found in aerobic environments where NH_3 is available through the mineralization of organic matter or anthropogenic N sources, such as fertilizers and organic wastes (Kowalchuk and Stephen, 2001). Conversely, AOA dominate in systems were the NH_3 concentration is relatively low and nutrients are limited (Adair and Schwartz, 2008; Chen et al., 2015). Although AOB have been shown to proliferate in nutrient-rich environments, other studies suggest AOA still tend to dominate over AOB (Leininger et al., 2006; Di et al., 2010). The range of *amoA* gene copies in soil for AOA are typically 5.0×10^4 to 1×10^8 copies g^{-1} soil (Leininger et al., 2006; Boyle-Yarwood et al., 2008; Di et al., 2010) and depending on fertilizer treatments AOB range from 7.7×10^3 to 7.8×10^7 copies g^{-1} soil (Okano et al., 2004; Di et al., 2010; Pratscher et al., 2011). Ammonia-oxidizing bacteria have also been shown to decline significantly with depth in unfertilized and inorganically fertilized systems unless treated additionally with manure, while AOA tend to maintain copy numbers with increasing depth (Leininger et al., 2006; Di et al., 2010).

Determining the abundance of AOA and AOB in a soil is beneficial in knowing the diversity of the microorganism community and their NH_4 oxidation potential, but quantifying the monooxygenase enzyme's activity can determine the NO_2^- released through NH_4 oxidation. In a

Haplic Chernozem (Mollisol) NH_4 oxidation was between 0.3 and 0.7 μg NO_2-N g^{-1} 5 h^{-1} (Kandeler et al., 1999) whereas activities for nutrient-poor calcareous grassland soils in northwestern Switzerland were around 500 μg NO_2-N g^{-1} 5 h^{-1} (Niklaus et al., 2001). While both AOA and AOB contain the monooxygenase enzyme and contribute to NH_4 oxidation in the soil, they do so at varying rates (Adair and Schwartz, 2008). Known maximum per-cell NH_4 oxidation rates of AOB are much higher than respective values of AOA (Schauss et al., 2009). The growth of AOB has been linked to increased nitrification activity following high levels of NH_4, either directly from mineral fertilizer or from the rapid hydrolysis of urea to NH_4 (Verhamme et al., 2011). Although AOB is favored at high NH_4 concentrations, which is more typical of agricultural soils receiving high inorganic N input, it has been suggested that AOA may contribute significantly to nitrification of NH_3 released through mineralization (Verhamme et al., 2011). However, since AOA generally have higher abundances, they may be able to compensate for their low per-cell oxidation rates, which may play a major role for the overall ecosystem function if the growth conditions of AOB are disturbed (Schauss et al., 2009).

1.3.3. Denitrification

Denitrification is the major biological process producing N_2 from the microbial reduction of NO_3^- into NO_2^- by nitrate reductase, into NO by nitrite reductase, into N_2O by nitric oxide reductase, and finally into N_2 by nitrous oxide reductase (Smith and Tiedje, 1978; Philippot, 2002; Gul and Whalen, 2016). The close relationship between aeration state and denitrification rate indicates the importance of characterizing short-term response to reduced aeration (Smith and Tiedje, 1978). Nitrate reductase values have ranged from 0.92 to 4.38 μg NO_2 –N g^{-1} 24 h^{-1} for soil samples from the Cascade Mountains (Boyle et al., 2006) to as high as 6 to 87 μg NO_2 – N g^{-1} 24 h^{-1} under waterlogged conditions for a Nicollet Aquic Hapludoll and Ames Typic

Haplaquoll, respectively (Abdelmagid and Tabatabai, 1987). Nitrous oxide, a potent greenhouse gas, is produced during incomplete denitrification where it is not reduced to N_2. The activity of N_2O reductase can be quantified by the *nosZ* gene. Henry et al. (2006) observed *nosZ* levels in a Himalayan soil ranging from 2 x 10^5 to 5 x10^5 copies g^{-1} of dry soil and up to 10^7 copies g^{-1} of dry soil from soils in France. Frozen soil from a field previously cropped to spring wheat (*Triticum aestivum* L.) in Fredericton, New Brunswick, Canada recorded similar *nosZ* values of 4.6 x 10^6 nosZ gene copies g^{-1} dry soil (Miller et al., 2008). Since soil can be a major source of N_2O emissions through incomplete denitrification, reclamation efforts to restore the soil microbial community can reduce N lost in undesirable forms.

1.3.4. Active carbon

Carbon is the backbone of functional biological molecules as the microbial community requires C as an energy source (Boopathy, 2000). Higher oxidation states of C correspond to lower energy yields which thus provide less energetic incentive for microorganisms (Boopathy, 2000). The labile fractions of soil C, often termed "active C pool", are readily altered by microbial activities and may provide an early indication of soil improvement or degradation in response to management practices (Weil et al., 2003). Permanganate oxidizable C (POXC) is a relatively new, rapid, and inexpensive method used to quantify labile soil C (Culman et al., 2012) and is intermediately sensitive to management practices compared with particulate organic C and microbial biomass C (Culman et al., 2012). Potentially oxidizable C values for grassland or no-tillage systems have higher oxidizable C values than conventional tillage systems (Culman et al., 2010; Spargo et al., 2011). Permanganate oxidizable C has been measured at ranges from 124 mg C kg^{-1} soil for a fine-silty, mixed, mesic Cumulic Haplustoll (DuPont et al., 2010) to 1468 mg C kg^{-1} soil for a fine, kaolinitic, thermic Typic Kanhapludult (Franzluebbers and

Stuedemann, 2002). Values within this range include 465 mg C kg^{-1} soil for a fine-silty, mixed, superactive, mesic Udic Argiustoll, 467 mg C kg^{-1} soil for corn-soybean rotation compared to 510 mg C kg^{-1} soil for continuous corn in a fine, mixed, mesic, Typic Hapludalf (Mirsky et al., 2008), and 668 mg C kg^{-1} soil for a Mesic Glossic Hapludalf (Culman et al., 2012). Because changes to labile C may disrupt microbial activities, it is valuable to study POXC in TD-treated soils.

1.3.5. Potentially mineralizable nitrogen

Potentially mineralizable nitrogen (PMN) is an indicator of the capacity of the soil microbial community to convert organic N into plant available NH_4^+ (Pankhurst et al., 1995). Potentially mineralizable N can be determined using an anaerobic 7-d incubation from the difference in NH_4 at the beginning of the seven days to the end of the 7 days. Values for PMN determined from this method range from 0.18 to 1.17 mg N kg^{-1} d^{-1} (Accoe et al., 2004; Bowles et al., 2014). Mineralizable N pools also vary by depth with significantly lower PMN at the 30 to 45-cm depths compared to shallower depths for Orthic Humo-Ferric Podzols (Cryorthods, Haplorthods) (Dessureault-Rompré et al., 2013). Measuring PMN can be used to monitor nutrient processing in TD remediation sites and provides useful information to resource managers overseeing reclamation activities.

1.4. Conclusion

The effects of soil remediation on soil productivity for agricultural purposes will be important to determine impacts on nutrient management. Contaminated/polluted and remediated/reclaimed soils may never resemble their original properties in terms of quality and health criteria because of the diverse nature, origin and environmental impact of contaminants/pollutants and treatments. Therefore, it is valuable to assess the ecology and

functioning of these soils under various conditions as information on how the TD process affects cation selectivity or nutrient cycling microbial processes is sparse. Since enzymes are critical metabolic catalysts in soil, the effect of TD on their activity will highlight potential nutrient applications. Knowledge of these factors will indicate if TD has implications for microbial processes, the preference of major cations, long-term nutrient considerations, and overall assessment of soil function. This information will be important as the oil industry continues to grow along with the concurrent risk of spills and the long-term impacts of hydrocarbon contamination and remediation/reclamation on soil fertility and agricultural productivity.

1.5. Figures

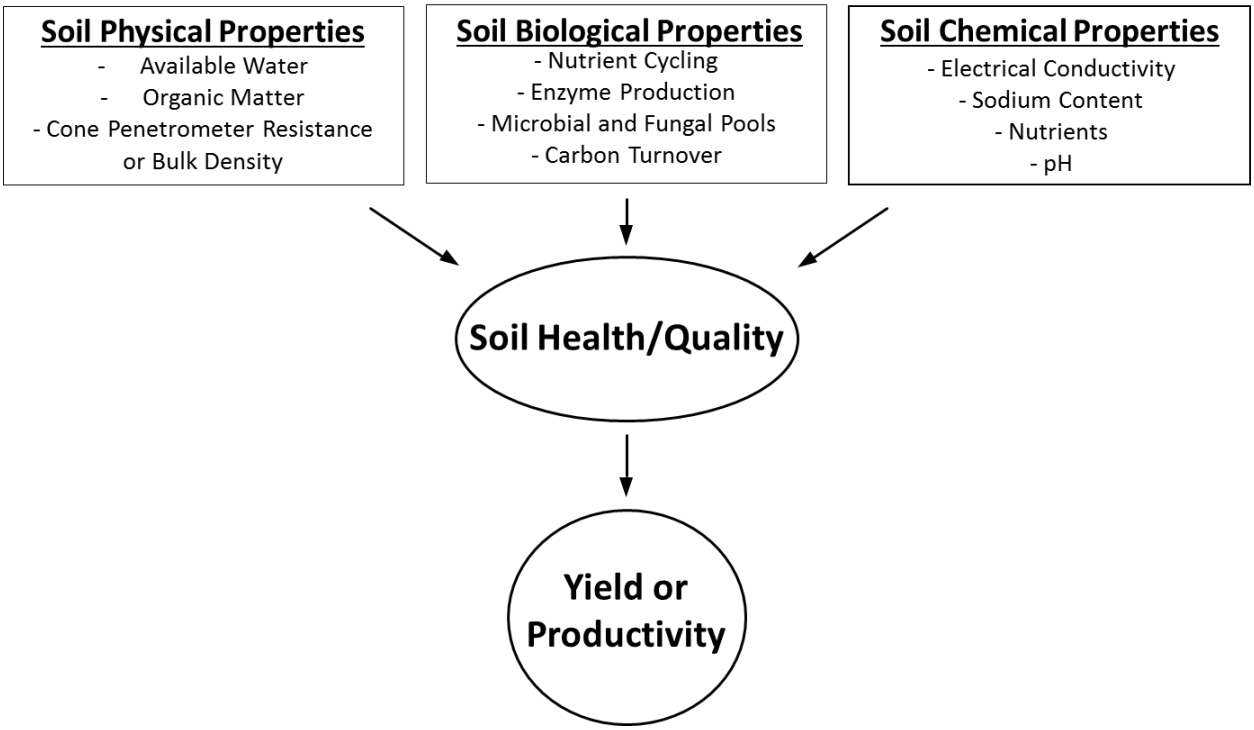

Figure 1. Holistic approach when considering reclamation of soil.

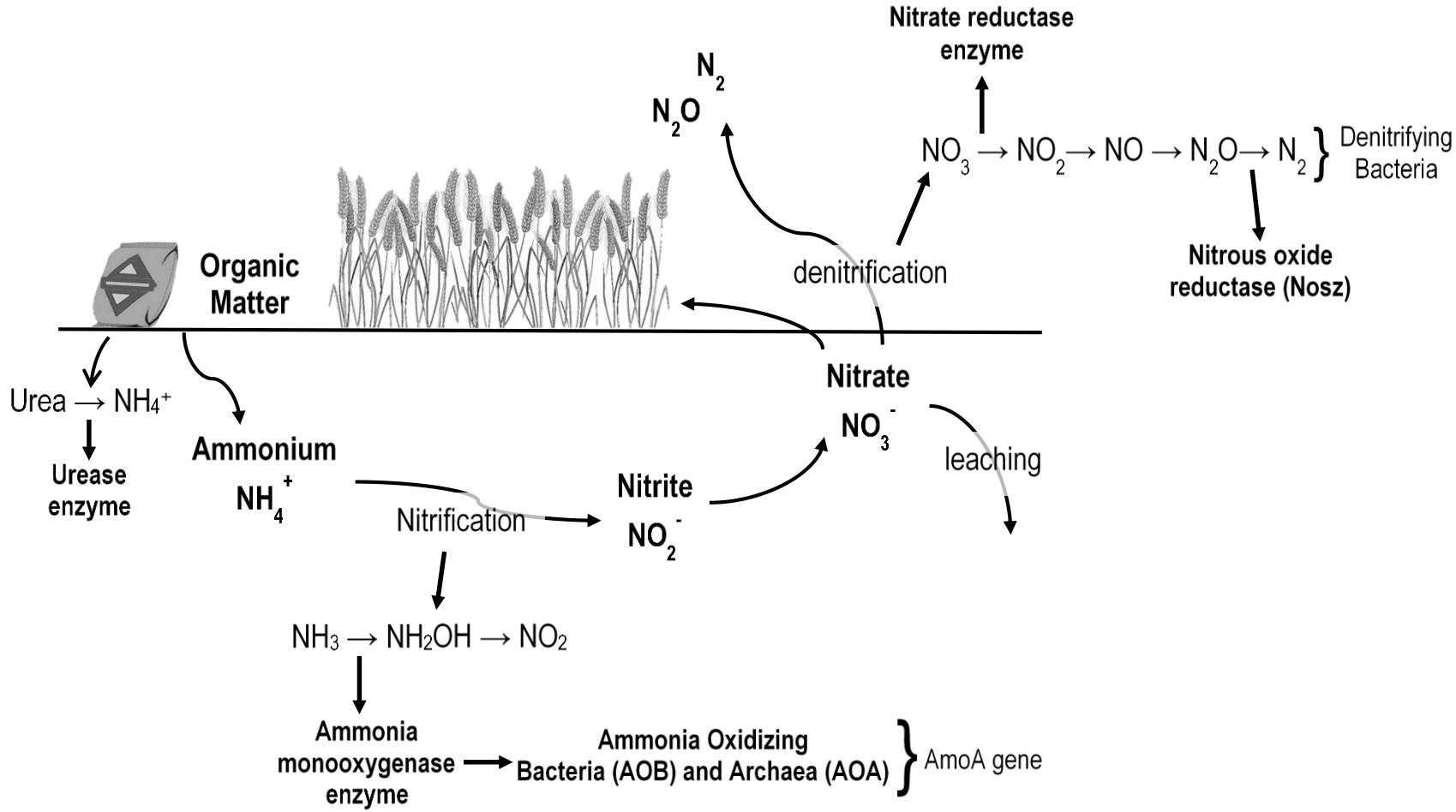

Figure 2. Nitrogen cycle displaying enzymes and genes quantified.

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CHAPTER 2: BINARY EXCHANGES OF CALCIUM, MAGNESIUM, AND POTASSIUM ON THERMALLY DESORBED SOIL[1]

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2.1. Abstract

A lesser-known but effective oil-spill remediation method to remove hydrocarbons from soil is thermal desorption (TD). Thermal desorption involves heating at temperatures between 200 and 500 °C to volatilize the hydrocarbons, effectively removing the contaminants from the soil. Effects of TD on remediated soil for agricultural crop production are limited, but cation exchange capacity (CEC) and selectivity for cations can be good indicators for plant productivity potential. In this study, the CEC and selectivity of cations of TD-treated and untreated topsoil and subsoil were compared using binary exchange measurements of Ca-Mg, Ca-K, and Mg-K. The tested soils were illite and smectite-dominated Mollisols that were collected near an active TD-remediation site in northwest North Dakota (USA). Vanselow selectivity coefficients (K_v) were determined from exchange isotherms and Gibbs free energies (ΔG_{ex}) were computed. For all three exchanges, significant differences were observed in ΔG_{ex} between the untreated and TD-treated topsoil and subsoils. In the Ca-Mg exchange, both the untreated and TD-treated topsoil preferred Ca; whereas, both TD-treated and untreated subsoils preferred Mg. For the Ca-K and Mg-K exchanges, all treatments preferred K. Cation exchange capacity values were significantly greater in the untreated subsoil of the Ca-Mg exchange and the untreated topsoil and subsoil of the Ca-K exchange compared to TD-treated soils. Differences may be due to

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contrasts in soil organic carbon and mineralogies. Although CEC and ΔG_{ex} differed between untreated and TD-treated soils, the cation selectivities were not altered suggesting that the magnitude of the differences may not require alternative fertility management to retain previous soil productivity.

2.2. Introduction

Modern society is dependent on energy, which is most often satisfied by extracting fossil fuels, including oil (Lehmann, 2007). However, this creates a risk of oil spills that can contaminate soils with a broad range of hydrocarbons (Khamehchiyan et al., 2006). The properties of hydrocarbon fluid, and the nature and topography of the terrestrial environment influence the mobility of an oil spill (Osuji et al., 2005). Crude oil has been reported to be increasingly destructive to soil biota and crop growth through its negative effects on soil conditions, microorganisms, and plants (Baker, 1978; Osuji et al., 2005). Attempts to reclaim crude oil-contaminated soils have included additions of poultry manure, sawdust, lime, or Ca and Mg applications (Osuji et al., 2005; Sayed and Zayed, 2006; John-Dewole and Sanni-Awal, 2013). Bioremediation has been used to facilitate recovery efforts by the use of aerobic respiration of microorganisms that transforms petroleum hydrocarbons to CO_2 and H_2O, or other less toxic substances. A simpler method of bioremediation is *in situ* land-farming that, utilizes soil microorganisms and the standard farming procedures of irrigation and aeration to reduce petroleum hydrocarbon concentrations (Onwurah et al., 2007). An alternative method that reliably reduces hydrocarbon concentrations in contaminated soil to desirable levels is thermal desorption (TD).

Thermal desorption remediates hydrocarbon contaminated soils, preserving the remediated soil for re-use and eliminating the liability of a landfill (Hamby, 1996). In the TD

process, contaminated soil is heated between 200 and 500 °C in a chamber in which hydrocarbon desorption is facilitated by volatilization, removing the contaminant from the soil to achieve a desired concentration. The treated soil then exits the chamber, is quenched with water, and cooled. The volatilized hydrocarbon gases are routed from the chamber and through a filtration unit where the dust and particulates are removed. After filtration, the hydrocarbon gases enter a secondary chamber where they are combusted and converted to CO_2 and water vapor. The remediated soil can then be returned to the original excavation site at the discretion of the end-user (Hamby, 1996).

A potential impact from heating the soil during the TD process may be changes to CEC due to alterations in soil mineralogy or loss of soil organic matter (Bonnard et al., 2010; O'Brien et al., 2016). The CEC of a soil is a measure of its ability to hold and exchange nutrients like Ca, K, and Mg, and is a good indicator of soil fertility (Mukherjee et al., 2011; Jeffery et al., 2011). The fate of nutrients in soil is influenced by the ionic radius, valence, and degree of hydration, and selectivity of the exchanger for one cation over another (Teppen and Miller, 2006; Rigon et al., 2015). Thus, any changes to CEC or cation preference may require alternative nutrient management strategies.

The objectives of this study were to determine whether the TD process alters the CEC, cation preference, and magnitude of preference of an agricultural topsoil and subsoil. The null hypothesis was that no differences would be observed in CEC, cation selectivity, or ΔG_{ex} between the untreated and TD treated soils. The results of this study will indicate if TD has implications for the preference of major cations in agricultural soil and thus long-term fertility considerations.

2.3. Materials & Methods

The soil samples were collected in Mountrail County, North Dakota (USA) near an active remediation site that had been contaminated with Bakken crude oil from a pipeline leak (O'Brien et al., 2016). The soils at this site are mapped as Williams-Zahl loams (Williams: fine-loamy, mixed, superactive, frigid Typic Calciustoll) with a productivity index of 76 (NRCS-USDA, 2015). Untreated, non-contaminated topsoil (TS) and subsoil (SS) were collected from uncontaminated stockpiles outside the boundary of the remediation site. Untreated, non-contaminated topsoil and subsoil were then treated separately by an RS40 Thermal Desorption/Oxidation unit (Nelson Environmental Ltd., Edmonton, Alberta) at 350 °C for 15 min to generate TD-treated topsoil (TS-TD) and TD-treated subsoil (SS-TD). This is the same temperature and time used to reduce oil-contaminated soil at the site to less than 500 mg kg^{-1} of total petroleum hydrocarbons.

Previous research (O'Brien et al., 2016) was conducted on the soils to determine particle size distribution, specific surface area, mineralogical analysis and distribution of clay, and soil organic C. Both treated and untreated TS and SS profiles were classified as loams (Table 1; O'Brien et al., 2016). Particle size distribution was conducted by the hydrometer method (Gee and Or, 2002; ASTM Standard D422-63, 2007). Specific surface area (SSA) was calculated using the ethylene glycol monoethyl ether (EGME) retention method (Pennell, 2002). Mineralogical along with clay analysis was performed using X-ray diffraction at a private laboratory (Activation Laboratories Ltd., Ancaster, Ontario, Canada) and total carbon (TC) and soil inorganic carbon (IC) were evaluated using a PrimacsSLC TOC Analyser (Skalar Analytical B.V., Breda, The Netherlands); soil organic carbon (SOC) was determined as the difference between TC and IC.

Methods for cation selectivities between Ca-Mg, Ca-K, and Mg-K followed the batch method described by DeSutter et al. (2006). Initially, soils were equilibrated using 2 *M* $CaCl_2$ for the Ca-Mg and Ca-K exchanges, and 2 *M* $MgCl_2$ for the Mg-K exchange on a horizontal shaker for 20 min at 180 osc min^{-1}. This process was repeated three times to ensure that all exchange sites were saturated with Ca or Mg. After each salt equilibration, soils were rinsed, shaken three times with deionized (DI) water to remove excess salts, then allowed to dry at 25 °C. After drying, the soils were ground to pass through a 1.0 mm sieve. Solution phases were prepared using six predetermined equivalent fractions of cations. Equivalent fractions ranged from 0:1 (Ca/Mg, Ca/K, Mg/K) to 1:0 (Ca/Mg, Ca/K, Mg/K) (Tables 2-7), while maintaining a constant target ionic strength (I) of 0.05 mol L^{-1}. The six solution concentrations were prepared by pipetting appropriate amounts of 2 *M* $CaCl_2$, 2 *M* $MgCl_2$, or 2 *M* KCl solutions into a 1-L volumetric flask and bringing to volume with DI water.

Twenty milliliters of equilibrating solution was then added to 1.0 g of Ca or Mg saturated soil that had been weighed into 50-mL polypropylene-centrifuge tubes. The soil/solution mixtures were shaken for 20 min on a horizontal shaker (180 osc min^{-1}), centrifuged for 20 min at a relative centrifugal force (RCF) of 650 × *g*, and then the supernatant was decanted and discarded. This process was repeated three times. After equilibrations, the soil was washed five times with 20 mL of 95% ethanol by shaking for 10 min on a horizontal shaker (180 osc min^{-1}) followed by centrifugation at a RCF of 650 × *g*. After the washings were completed, soils were allowed to air-dry overnight to remove any excess ethanol. $CaCO_3$ was detected in the soils by placing a drop of 1 *M* HCl on a sample of the dry soil. To lessen the dissolution of $CaCO_3$, the cations (Ca, Mg, and/or K) in each soil were extracted with 20 mL of 1 *M* sodium acetate (adjusted to pH 8.2) by shaking on the same horizontal shaker for 20 min and centrifuging for 20

min at a RCF of 650 × g. The supernatant was removed through pipetting and saved. Ca, Mg, and/or K concentrations were then determined using an atomic absorption spectrophotometer (Buck Scientific 210/211 VGP, Ver 3.94C). For the Ca-Mg and Ca-K exchanges, unintentionally dissolved Ca from $CaCO_3$ in the soil was accounted for by subtracting the recorded Ca concentration from the 0% Ca equilibrating solution extractions from each proceeding Ca concentration.

2.3.1. Analysis

For Ca-Mg exchange, the general binary-exchange reaction is

$$CaX_2 + MgCl_2 \leftrightarrow CaCl_2 + MgX_2 \quad (1)$$

for Ca-K exchange, the general binary-exchange reaction was

$$CaX_2 + 2KCl \leftrightarrow CaCl_2 + 2KX \quad (2)$$

for Mg-K exchange, the general binary-exchange reaction was

$$MgX_2 + 2KCl \leftrightarrow MgCl_2 + 2KX \quad (3)$$

where X represents 1 *M* of surface negative charge on the exchanger phase. The Ca-Mg exchange will be referred to as homoionic exchange and the Ca-K and Mg-K will be referred to as heteroionic exchange owing to the valence charges of the ions. Exchanger mole fractions of Ca (N_{Ca}) and of Mg (N_{Mg}) were defined in homoionic exchange as

$$N_{Ca} = \frac{[CaX_2]}{[CaX_2]+[MgX_2]} \quad (4a)$$

$$N_{Mg} = \frac{[MgX_2]}{[MgX_2]+[CaX_2]} \quad (4b)$$

and exchanger mole fractions of Ca (N_{Ca}), Mg (N_{Mg}), and K (N_K) in heteroionic exchange reactions as

$$N_{Ca,Mg} = \frac{[Ca, MgX_2]}{[Ca, MgX_2] + [KX]} \quad (5a)$$

$$N_K = \frac{[KX]}{[KX] + [Ca, MgX_2]} \quad (5b)$$

where brackets represent the concentrations of adsorbed cations in mol kg^{-1}. For each equilibration, the Vanselow selectivity coefficient (K_v) for homoionic exchange reaction was computed, from Essington (2004), as

$$K_v = \frac{(Ca^{2+})N_{Mg}}{(Mg^{2+})N_{Ca}} \quad (6)$$

and heteroionic exchange reactions computed as

$$K_v = \frac{(Ca^{2+}, Mg^{2+})N_K^2}{(K^+)^2 N_{Ca,Mg}} \quad (7)$$

where parentheses indicate activities. Activities and ionic strength (mol L^{-1}) were determined using Visual MINTEQ, version 3.1 (Gustafsson, 2016) to account for ion-pairing ($CaCl^+$ and $MgCl^+$). The equivalent fraction of Ca^{2+} or Mg^{2+} ($E_{Ca,Mg}$) on the exchanger phase for the homoionic reaction was calculated as

$$E_{Ca} = \frac{N_{Ca}}{N_{Ca} + N_{Mg}} \quad (8)$$

and in the heteroionic as

$$E_{Ca,Mg} = \frac{2N_{Ca,Mg}}{2N_{Ca,Mg} + N_K} \quad (9)$$

Gibbs free energy (ΔG_{ex}) is a measure of the amount of energy available to do work in a system and is used to determine spontaneity of a chemical process. It states that given enough

time, the process will eventually occur. Gibbs free energies were calculated for each binary-exchange reaction from

$$\ln K_{ex} = \int_0^1 \ln K_v dE_{\text{Ca, Mg}} \tag{10}$$

and

$$\Delta G_{ex} = -RT \ln K_{ex} \tag{11}$$

where K_{ex} is the equilibrium exchange constant (Essington, 2004); R is equal to 8.314 J mol^{-1} K^{-1}; and T is the reaction temperature used for this study, 298 K. Each reported ΔG_{ex} is the average of three replications. Selectivity diagrams were constructed by plotting the equivalent fraction of the cation (Mg^{2+} for homoionic and K^+ for heteroionic) in the exchanger phase versus the equivalent fraction of the cation (Mg^{2+} for homoionic and K^+ for heterionic) in the solution phase (Essington, 2004). Non-preference isotherms for homovalent exchanges were 1:1 lines; while, heterovalent equivalent exchange fractions were calculated using

$$E_K = \left\{1 + \frac{1}{\Gamma \mathrm{I}}\left[\frac{3}{\tilde{E}_K^2} - \frac{4}{\tilde{E}_K} + 1\right]\right\}^{-\frac{1}{2}} \tag{12}$$

where E_K is equivalent fraction of K^+ on the exchange complex, Γ is $\frac{\gamma_{K^+}^2}{\gamma_{Ca^{2+} or\ Mg^{2+}}}$ where activities (γ) and concentrations computed by Visual MINTEQ version 3.1 were used to calculate γ, and $\tilde{E}_K$ is the equivalent fraction of K^+ in the solution phase (Essington, 2004) given by

$$\tilde{E}_K = \frac{\{K^+\}}{\{K^+\} + 2\{Ca^{2+}, Mg^{2+}\}} \tag{13}$$

where braces indicate aqueous molar concentrations (mol L^{-1}). If the data lie below the non-preference isotherm, the initial ion or reactant is preferred. For example, the initial ion for the

Ca-Mg and Ca-K exchange is Ca^{2+}. The initial ion for Mg-K is Mg^{2+}. If the experimental data lie above the curvilinear non-preference isotherm, then K_v >1 and the final ion or product is preferred (Sparks, 2003). For example, the final ion for the Ca-Mg exchange is Mg^{2+}. The final ion for Ca-K and Mg-K is K^{+}.

Descriptive statistics were determined for each exchange reaction and a Student's *t* test was used to compare non-contaminated topsoil and subsoil to non-contaminated, thermally desorbed topsoil and subsoil. Significant differences were determined using an alpha = 0.05 (JMP 8; Cary, NC).

2.4. Results and Discussion

Cation exchange capacities ranged between 8.3 and 13.8 $cmol_c\ kg^{-1}$. Similar values were found in Caravaca et al. (1999) with their lowest value at 8.6 $cmol_c\ kg^{-1}$ in cultivated soils with the clay portion dominated by illite and kaolinite and higher values around 13.8 $cmol_c\ kg^{-1}$ for an illite and smectite dominated clay fraction. The average CEC value in the Ca-Mg exchange for TS was significantly greater than TS-TD, while no difference was found between SS and SS-TD (Table 2). In Ca-K exchange, untreated TS and SS both had significantly greater CEC than their TD treated counterparts. Differences in CEC between untreated and TD soils might be attributed to differences in soil organic carbon (SOC), with the untreated TS and SS having significantly higher SOC than their TD-treated counterparts (Table 1; O'Brien et al., 2016). Higher SOC is correlated to higher CEC (Rashidi and Seilsepour, 2008); however, in the Mg-K exchange (Table 6 and 7) there were no differences in CEC between the untreated TS and SS compared to the TD-treated soils.

In the Ca-Mg exchange, the TS and TS-TD preferred Ca as shown by K_v values were dominantly less than one (Table 2 and Eq 1) and lines below 1:1 line of non-preference (Figure

3). Jensen and Babcock (1973) reported Kv values of 0.61 at I = 0.001 and 0.010 in Ca-Mg exchange which were similar to values in this study. Previous studies using soils dominated by montmorillonite and illite clays have observed Ca preference over Mg, which is often attributed to the organic matter's affinity for Ca (Van Bladel and Gheyi, 1980; Curtin et al., 1998). In pure montmorillonite clay, Ca and Mg have equal affinities for exchange sites; however, when OC is present in the exchanger system the preference shifts toward Ca (Sposito et al., 1983). With less OC and a shift toward a smectite dominated clay fraction (Table 1), both the SS and SS-TD soils preferred Mg over Ca as shown by K_v values greater than one (Table 3) and preference lines above the non-preference line (Figure 3).

In the Ca-K and Mg-K exchange, all treatments preferred K as indicated by the greater than one K_v values (Tables 4-7) and preference lines above the non-preference line (Figure 4 and Figure 5). Similar values were observed by Jensen and Babcock (1973) with Kv values of 12.0 and 22.9 in Ca-K and Mg-K exchanges; respectively at I = 0.01 in a Yolo loam. Similar findings of K preference over Ca or Mg have been observed in a variety of soils consisting of mica, smectite, and kaolinite clays (Sinanis et al., 2003; DeSutter and Pierzynski, 2005). Vanselow selectivity coefficients of 9.90 and 23.81 were found in two smectite Ca-K exchanges by Shainberg et al. (1987) using an I = 0.062 mol L^{-1}, similar to values of Kv and I found in this study (Table 4). As stated in Shainberg (1987), illite has a high affinity for K, which may explain the K preference in these soils. Similar to previous findings (Jensen and Babcock, 1973; Sinanis et al., 2003; Agbenin and Yakubu, 2006), the soil's preference for K was higher at lower degrees of K exchanger composition for both Ca-K and Mg-K exchanges, which indicates that the higher selectivity sites for K were filled first followed by low selectivity sites. This selectivity has been attributed to heterogeneity of adsorption sites and to the fact that the cationic

mixture on the exchanger phase does not behave as an ideal solid-solution mixture (Sinanis et al., 2003; Agbenin and Yakubu, 2006). In the Ca-Mg system, K_v was constant and independent of exchanger composition.

Although the cation selectivity preference was not different after TD, ΔG_{ex} significantly differed in values for all exchanges between the untreated and TD treated soils (Table 8). Ca-Mg exchange for the TS and TS-TD were 0.52 and 0.32 kJ mol^{-1}, respectively, similar to the 0.44 kJ mol^{-1} value reported by DeSutter et al. (2006) for a soil composed of mica, smectite, and kaolinite clay. The values are slightly higher than the 0.29 kJ mol^{-1} recorded by Jensen and Babcock (1973) at I = 0.001 and 0.010 in a Yolo loam and 0.26 kJ mol^{-1} observed by Udo (1978) in a kaolinitic clay at 30 °C. Ca-K exchange ΔG_{ex} values ranged from -4.63 to -7.33 kJ mol^{-1}, which were similar to values found by DeSutter and Pierzynski (2005) in two soils dominated by mica, smectite and kaolinite clay fraction that ranged between -4.66 and -5.08 kJ mol^{-1}. Agbenin and Yakubu (2006) observed -3.62 kJ mol^{-1} in the top 0-15 cm and -2.43 kJ mol^{-1} in the 15-30 cm depth of a savanna soil in northern Nigeria. Udo (1978) observed higher ΔG_{ex} values of -1.65 kJ mol^{-1} at 30 °C on a kaolinitic soil clay.

Gibbs free energy values for Mg-K ranged from -4.92 to -5.66 kJ mol^{-1} (Table 8), and these values are within the range of values of -5.70 and -6.70 kJ mol^{-1} reported by Sinanis et al. (2003) for mica and smectite dominated mineralogies, respectively. Agbenin and Yakubu (2006) reported ΔG_{ex} values of -3.79 kJ mol^{-1} in 0-15 cm depth soils and -2.51 kJ mol^{-1} in 15-30 cm depth for Mg-K exchange reaction. Jensen and Babcock (1973) reported ΔG_{ex} values of -1.86 kJ mol^{-1} in Mg-K exchange reaction at I = 0.001. With similar directionality and magnitude to comparable soils for related reactions found in the literature, our results indicate that the TD process did not alter the soils' exchanger phase to any great degree. This suggests that although

there were significant differences in ΔG_{ex} between untreated and TD-treated soils, the magnitude of the differences may not require alternative fertility management practices.

2.5. Conclusion

This study examined the effects of TD on CEC and cation selectivity in non-contaminated agricultural topsoil and subsoil. Slight variations in CEC in the Ca-Mg and Ca-K exchanges existed between the untreated and TD-treated soils with the TD treatments having lower CEC values. No significant differences in K_v preferences were observed in either of the three exchanges between the untreated and TD-treated soils which indicates no implications of the TD process on the soil's natural selection for cations. Vanselow selectivity coefficients indicated that Ca was preferred over Mg when SOC was high; Mg was preferred over Ca when SOC was low, and that K was preferred over Ca and Mg for all treatments. Gibbs free energies were significantly different between the untreated and TD-treated soils for each of the three exchanges, suggesting different degrees of selectivity after TD treatment. However, no trend was noticed between TD-treated soil and greater or lesser selectivity, but magnitudes of ΔG_{ex} were similar between treated and untreated soils across all exchanges. Overall, the selectivities between the topsoil and subsoil were not altered after undergoing TD at 350 °C, and therefore, if used in an agricultural setting similar soils having undergone TD at the same temperatures and retention times should not require alternative fertility management to retain previous productivity.

2.6. Acknowledgements

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2.7. Tables and Figures

Table 1. Previous data (O'Brien et al., 2016) collected on particle size distribution, specific surface area, mineralogical analysis and distribution of clay fraction, and soil organic carbon of untreated and TD-treated soils. Standard errors can be found in O'Brien et al. (2016). Reprinted with permission from Journal of Environmental Quality.

Parameter		Treatment†			
		TS	**TS-TD**	**SS**	**SS-TD**
Particle Size Distribution (g kg^{-1})	Sand	473‡	494	480	490
	Silt	335‡	319	314	322
	Clay	192‡	188	206	188
Textural class		Loam	Loam	Loam	Loam
Specific Surface Area (m^2 g^{-1})		89.6ab	71.2c	93.3a	80.0bc
Mineral (% by weight)	Quartz	48.2	42.3	38.6	40.9
	Plagioclase	17.4	16.8	13.9	13.6
	Microcline	6.7	3.3	5.2	4.8
	Muscovite/Illite	6.2	6.0	5.6	6.9
	Kaolinite	0.6	0.7	0.7	Trace
	Amphibole	Trace	Trace	0.7	Trace
	Dolomite	2.1	2.9	4	2.5
	Calcite	Trace	0.4	1	1.1
	Amorphous	18.9	27.5	30.2	30.1
Clay Fraction (% by weight)	Smectite	42	42	57	51
	Illite	46	47	33	37
	Kaolinite	8	8	7	9
	Chlorite	4	3	3	3
Soil Organic Carbon (g kg^{-1})		28.2a§	19.8b	15.2c	10.9d

† TS, untreated, non-contaminated topsoil; TS-TD, thermal desorbed, non-contaminated topsoil; SS, untreated, non-contaminated subsoil; SS-TD, thermal desorbed, non-contaminated subsoil.

‡ No significant differences within the row ($p < 0.05$).

§ Different letters within rows indicate significance ($p < 0.05$; Tukey's HSD test).

Table 2. Binary exchange of Ca-Mg solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated topsoil profile (TS) and the thermal desorbed, non-contaminated topsoil profile (TS-TD).

Exchanger Test	Solution Composition		TS				TS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Ca	Mg	Mg	Ca			Mg	Ca		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.019	0.000	0.000	0.064	12.8(0.6)†		0.000	0.048	9.7(1.1)	
2	0.016	0.003	0.008	0.055	12.6(0.7)	0.85(0.05)	0.008	0.041	9.9(1.9)	1.11(0.29)
3	0.013	0.007	0.022	0.047	13.8(0.5)	0.90(0.05)	0.018	0.039	11.5(0.3)	0.90(0.01)
4	0.008	0.011	0.034	0.036	14.0(0.4)	0.70(0.04)	0.031	0.030	12.3(0.1)	0.77(0.03)
5	0.006	0.015	0.049	0.024	14.6(0.3)	0.84(0.04)	0.044	0.021	12.9(0.8)	0.87(0.08)
6	0.000	0.020	0.074	0.000	14.9(0.1)		0.064	0.000	12.9(0.3)	
Average					13.8(1.0)a‡				11.5(1.6)b	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 3. Binary exchange of Ca-Mg solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated subsoil profile (SS) and the thermal desorbed, non-contaminated subsoil profile (SS-TD).

Exchanger Test	Solution Composition		SS				SS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Ca	Mg	Mg	Ca			Mg	Ca		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.019	0.000	0.000	0.043	8.5(1.6)†		0.000	0.048	9.6(0.9)	
2	0.016	0.003	0.009	0.040	9.9(1.1)	1.26(0.14)	0.008	0.026	6.9(0.7)	1.75(0.30)
3	0.013	0.007	0.024	0.032	11.2(0.9)	1.40(0.03)	0.021	0.031	10.4(0.6)	1.31(0.07)
4	0.008	0.011	0.039	0.023	12.4(0.75)	1.24(0.03)	0.034	0.022	11.2(0.6)	1.16(0.07)
5	0.006	0.015	0.051	0.015	13.3(0.7)	1.40(0.06)	0.048	0.013	12.3(0.9)	1.50(0.29)
6	0.000	0.020	0.080	0.000	16.0(0.5)		0.068	0.000	13.6(0.6)	
Average					11.9(2.6)a‡				10.7(2.3)a	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 4. Binary exchange of Ca-K solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated topsoil profile (TS) and the thermal desorbed, non-contaminated topsoil profile (TS-TD).

Exchanger Test	Solution Composition		TS				TS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Ca	K	K	Ca			K	Ca		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.017	0.000	0.000	0.047	9.3(0.9)†		0.000	0.044	8.7(0.4)	
2	0.014	0.010	0.023	0.037	9.6(0.2)	22.12(0.41)	0.024	0.034	9.2(0.1)	27.94(1.44)
3	0.010	0.022	0.036	0.031	9.7(0.3)	8.67(0.54)	0.035	0.027	9.0(0.1)	9.84(0.08)
4	0.007	0.030	0.049	0.026	10.1(0.1)	6.51(0.03)	0.048	0.021	9.0(0.1)	7.90(0.11)
5	0.003	0.042	0.066	0.020	10.6(0.1)	2.86(0.06)	0.062	0.015	9.3(0.1)	3.66(0.30)
6	0.000	0.051	0.102	0.000	10.2(0.1)		0.094	0.000	9.4(0.0)	
Average					9.9(0.6)a‡				9.1(0.3)b	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 5. Binary exchange of Ca-K solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated subsoil profile (SS) and the thermal desorbed, non-contaminated subsoil profile (SS-TD).

Exchanger Test	Solution Composition		SS				SS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Ca	K	K	Ca			K	Ca		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.017	0.000	0.000	0.404	8.0(0.1)†		0.000	0.035	6.9(0.1)	
2	0.014	0.010	0.030	0.031	9.3(0.2)	43.56(2.84)	0.024	0.027	7.8(0.1)	36.98(1.86)
3	0.010	0.022	0.043	0.022	8.7(0.3)	17.75(1.06)	0.040	0.019	7.8(0.2)	18.89(0.98)
4	0.007	0.030	0.059	0.022	10.3(0.2)	10.39(0.14)	0.053	0.017	8.7(0.1)	11.86(0.38)
5	0.003	0.042	0.074	0.008	9.0(0.4)	9.77(2.29)	0.068	0.008	8.5(0.3)	8.24(1.39)
6	0.000	0.051	0.108	0.000	10.8(0.1)		0.102	0.000	10.2(0.1)	
Average					9.3(1.0)a‡				8.3(1.0)b	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 6. Binary exchange of Mg-K solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated topsoil profile (TS) and the thermal desorbed, non-contaminated topsoil profile (TS-TD).

Exchanger Test	Solution Composition		TS				TS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Mg	K	K	Mg			K	Mg		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.024	0.000	0.000	0.058	11.7(0.2)†		0.000	0.064	12.7(0.8)	
2	0.020	0.010	0.023	0.044	11.0(0.1)	22.06(0.76)	0.025	0.050	12.6(0.2)	20.91(0.85)
3	0.016	0.019	0.039	0.037	11.4(0.4)	14.67(0.91)	0.037	0.038	11.3(0.9)	13.29(0.87)
4	0.010	0.029	0.052	0.028	10.8(0.9)	9.25(0.33)	0.052	0.029	11.1(0.4)	8.50(0.67)
5	0.005	0.036	0.073	0.023	12.0(0.5)	5.87(0.38)	0.069	0.024	11.7(0.0)	5.17(0.25)
6	0.000	0.043	0.102	0.000	10.2(0.6)		0.094	0.000	9.34(0.1)	
Average					11.2(0.7)a‡				11.5(1.2)a	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 7. Binary exchange of Mg-K solution and exchanger compositions, cation exchange capacity (CEC) of the soils, and Vanselow selectivity coefficients (Kv) for the untreated, non-contaminated subsoil profile (SS) and the thermal desorbed, non-contaminated subsoil profile (SS-TD).

Exchanger Test	Solution Composition		SS				SS-TD			
			Exchanger Composition		CEC	K_v	Exchanger Composition		CEC	K_v
	Mg	K	K	Mg			K	Mg		
	mol L^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}		mol kg^{-1}		$cmol_c$ kg^{-1}	
1	0.024	0.000	0.000	0.065	12.9(1.2)†		0.000	0.068	13.7(0.0)	
2	0.020	0.010	0.023	0.050	12.4(0.9)	17.63(1.54)	0.026	0.054	13.5(0.4)	19.25(0.80)
3	0.016	0.019	0.039	0.041	12.1(0.8)	12.49(0.32)	0.043	0.046	13.5(0.2)	12.20(0.59)
4	0.010	0.029	0.051	0.036	12.3(0.7)	6.40(0.78)	0.056	0.035	12.5(0.4)	7.44(0.20)
5	0.005	0.036	0.063	0.024	11.1(0.6)	4.88(0.71)	0.071	0.024	11.9(0.3)	5.45(0.37)
6	0.000	0.043	0.094	0.000	9.4(0.3)		0.105	0.000	10.5(0.0)	
Average					11.7(1.4)a‡				12.6(1.2)a	

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

Table 8. Gibbs free energies and Kex values for the untreated, non-contaminated topsoil profile (TS), the thermal desorbed, non-contaminated topsoil profile (TS-TD), the untreated, non-contaminated subsoil profile (SS), and the thermal desorbed, non-contaminated subsoil profile (SS-TD) of the Ca-Mg, Ca-K, and Mg-K exchanges.

Exchanger	Treatment	Kex	ΔG (J mol^{-1})
Ca-Mg	TS	0.8(0.01)†A‡	520(44.2)A
	TS-TD	0.9(0.02)B	324(63.1)B
	SS	1.3(0.04)a	-696(65.4)a
	SS-TD	1.4(0.04)b	-855(75.2)b
Ca-K	TS	6.5(0.13)A	-4626(49.3)A
	TS-TD	8.7(0.14)B	-5354(39.4)B
	SS	19.3(1.52)a	-7329(196.5)a
	SS-TD	18.2(0.33)a	-7183(44.4)a
Mg-K	TS	9.8(0.29)A	-5658(73.2)A
	TS-TD	8.6(0.11)B	-5345(31.9)B
	SS	7.3(0.29)a	-4916(99.3)a
	SS-TD	8.2(0.24)b	-5212(73.7)b

† Values in parenthesis are standard deviations

‡ Different letters after values indicate significant difference ($p < 0.05$) between treated and untreated soils

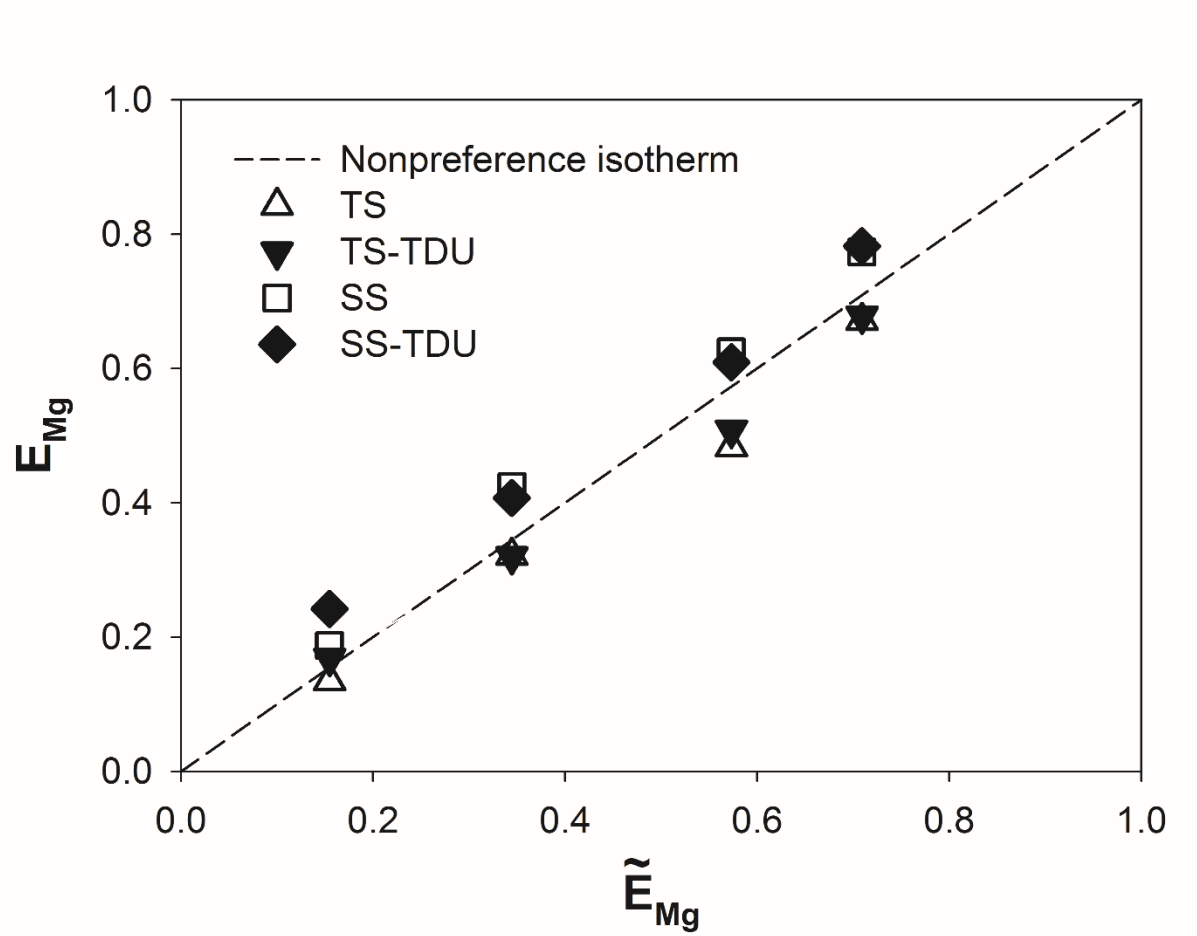

Figure 3. Calcium-Magnesium exchange isotherms showing the equivalent fraction of Mg in the solution ($\tilde{E}_{Mg}$) and exchanger phase (E_{Mg}). Standard deviation values for E_{Mg} <0.04.

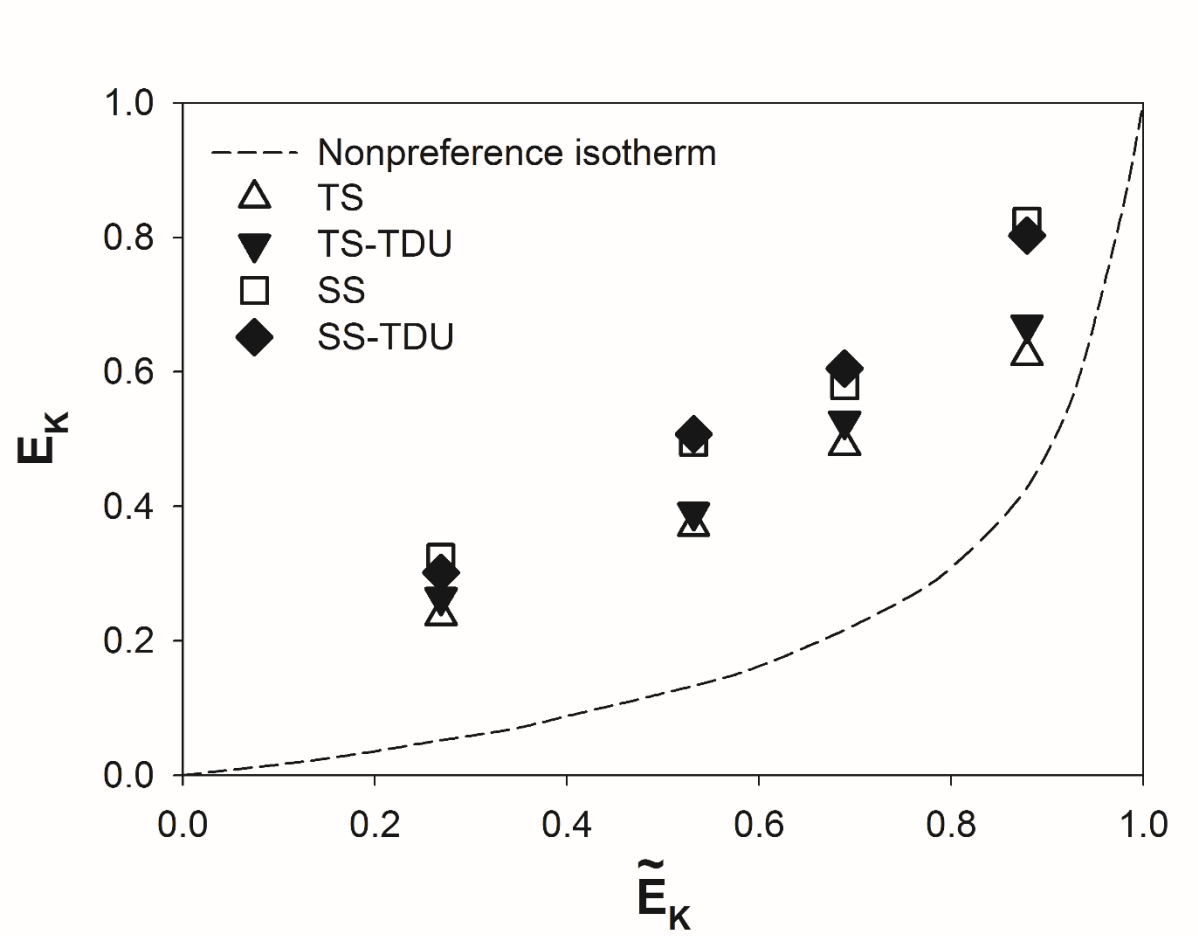

Figure 4. Calcium-Potassium exchange isotherms showing the equivalent fraction of K in the solution ($\tilde{E}_K$) and exchanger phase (E_K). Standard deviation values for E_K <0.03.

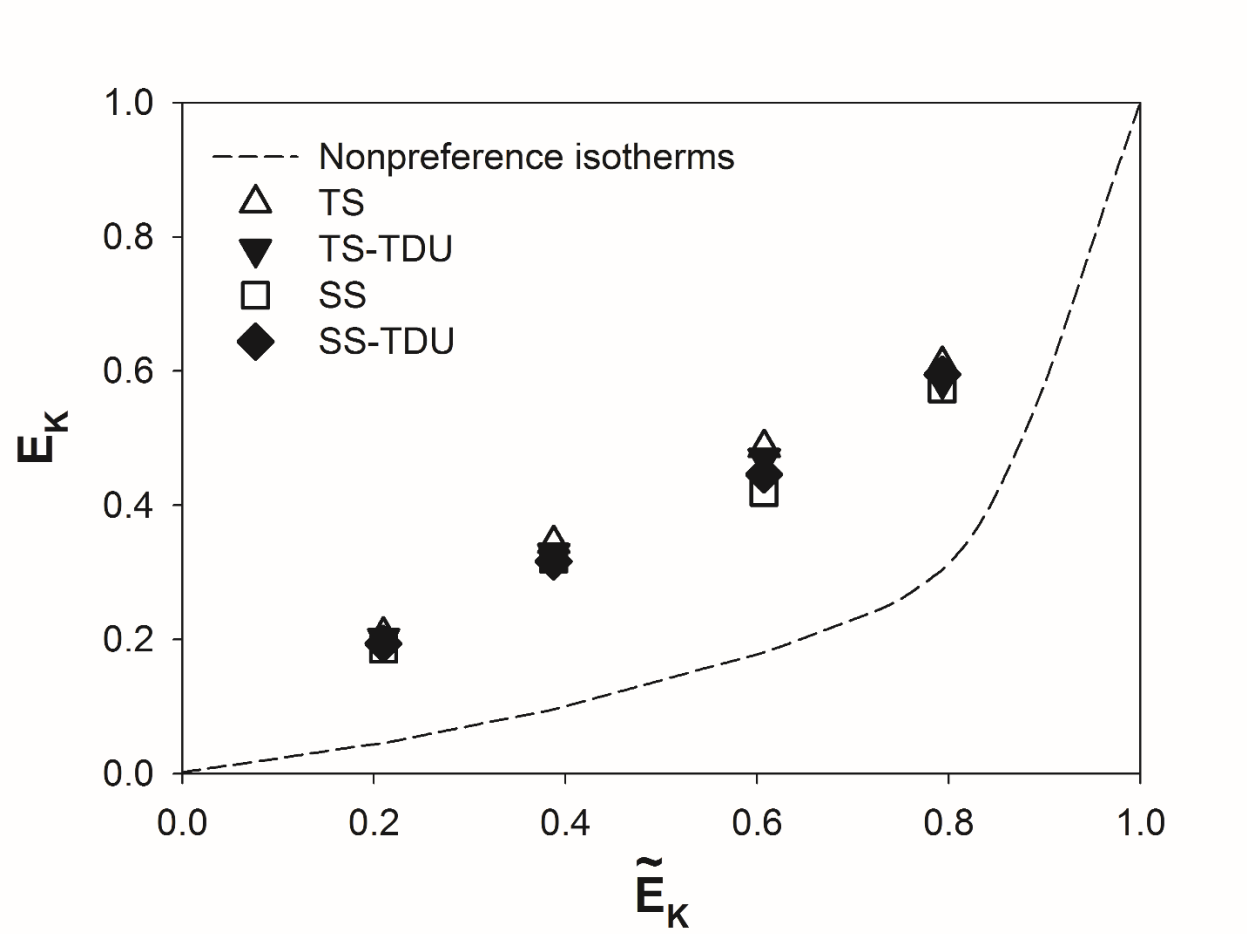

Figure 5. Magnesium-Potassium exchange isotherms showing the equivalent fraction of K in the solution ($\tilde{E}_K$) and exchanger phase (E_K). Standard deviation values for E_K <0.03.

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CHAPTER 3: BIOLOGICAL INDICATORS OF SOIL HEALTH IN THERMALLY AND CHEMICALLY DISTURBED SOILS[1]

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3.1. Abstract

Thermal desorption (TD) remediates hydrocarbon-contaminated soil by heating the soil at high temperatures (200 to 500 °C) to volatilize the hydrocarbons, effectively removing the contaminant from the soil. However, after such drastic treatment soil biological properties are likely to be diminished. A field study utilizing TD-treated soil, contaminated soil, and uncontaminated topsoil was initiated to determine whether the TD treatment alters soil biological properties and whether mixtures of TD-treated soil with non-contaminated soil can reestablish necessary biological functions. The activities of N-cycling enzymes (urease, ammonium oxidation, and nitrate reductase), quantification of N-transforming genes (*amoA* and *nos*Z), active carbon pool, and potentially mineralizable nitrogen were measured in plots having 1) non-contaminated surface soil; 2) crude oil-contaminated subsoil; 3) TD-treated subsoil; and 4) 1:1 blends of each, both with and without compost amendment located in northwest North Dakota, USA. Results indicated that N-transforming microorganisms were lower in contaminated and TD-treated soil as compared to non-contaminated soil. However, the addition of surface soils increased N-cycling organisms needed for successful reclamation to bring soil back to agricultural production.

[1] The material in this chapter was co-authored by Samantha Ritter, Thomas DeSutter, Heather Dose, Peter O'Brien, Francis Casey, Abbey Wick, Caley Gasch, and Eakalak Khan. Samantha Ritter had primary responsibility for sample collection and laboratory analysis. Samantha Ritter was the primary developer of the conclusions that are advanced here. Samantha Ritter also drafted and revised all versions of this chapter.

3.2. Introduction

Microorganisms provide a critical role to nutrient cycling and availability and have been suggested as a measurement of ecological health due to their relatively quick response to changes in the soil environment (Dawson et al., 2007). Nitrogen-transforming organisms are often selected as soil health indicators because of the significance of the N cycle in soil function, plant productivity, and regulating environmental contaminants (Dawson et al., 2007; Das and Chandran, 2011), and because of the specificity of organisms possessing nitrification-transforming enzyme complexes (Das and Chandran, 2011). Through enzymes, inorganic N fertilizers, like urea, are converted into NH_3 or NH_4^+. Ammonia-oxidizing archaea and bacteria containing the *amoA* gene perform the first step in nitrification, oxidizing NH_3 into NO_2^- (Hallin et al., 2009). This step is followed by the oxidation to NO_3^- by NO_2^- oxidizers. Nitrate can then be reduced into NO_2^- by nitrate reductase or to N_2 in the final step of denitrification by nitrous oxide reductase (*nosZ* gene). In contrast to denitrification, which can be accomplished by many different bacteria, archaea, or eukaryotes; the oxidation of NH_3 is performed only by a few phylogenetically constrained ammonia-oxidizing bacteria and archaea (Hallin et al., 2009). Since the N cycle is a series of dependent processes, measuring steps within the N cycle (Figure 2) can determine if the cycle is functioning as a system of soil health.

The activities of certain enzymes in soil have been correlated with plant growth and are thought to be able to serve as indices of soil health since they play an important role in nutrient cycling (Sarkar et al., 1989; Badiane et al., 2001). Similarly, microorganisms require nutrients like C and N for energy and the synthesis of proteins, amino acids, DNA, and RNA. Altered by microbial activities, the labile, active C pool fuels the soil food web and may provide an early indication of soil degradation or improvement in response to management practices (Weil et al.,

2003). Permanganate oxidizable carbon (POXC) has been related to most measures of soil microbial activity and thus can be used to assess active soil C (Weil et al., 2003). Potentially mineralizable nitrogen (PMN) is an indicator of the capacity of the soil microbial community to convert organic N into plant available NH_4^+ (Pankhurst et al., 1995). For biological indicators like these to be effective at determining soil health, they need to have key roles in the functioning of soil ecosystems and have representative and reproducible responses to degradation or improvement of management practices (Dawson et al., 2007).

In contaminated ecosystems, microbial communities tend to be less diverse and dominated by the organisms capable of utilizing and/or surviving the altered conditions at the potential expense of other important microorganisms that transform other vital nutrients (N, S, P) (Macnaughton et al., 1999). In the Bakken Formation, which lies in the Williston Basin extending over parts of North Dakota, Montana, and Saskatchewan, Canada, crude oil and natural gas production persist with the risk of accidental crude-oil spills introducing toxic hydrocarbons into the environment (Chaîneau et al., 2003; Rahman et al., 2003). There are numerous strategies to reduce total petroleum hydrocarbon concentration in soils, including, but not limited to, chemical oxidation, bioremediation, phytoremediation, and thermal desorption (TD). Thermal desorption, is a relatively efficient method to remediate hydrocarbon contaminated soil by heating it between 200 and 500 °C to volatilize the hydrocarbons from the contaminated soil, which effectively removes these hydrocarbons as a gas (Hamby, 1996). The vaporized contaminants then enter a secondary chamber where the hydrocarbons are converted to CO_2 and water vapor at a higher temperature (commonly 600 to 800 °C). The soil then exits the chamber reduced or eliminated from hydrocarbons (TD-treated soil), is re-hydrated, cooled, and can then be returned to the original excavation site (Hamby, 1996). However, after such drastic

treatment, soil biological diversity and functions are likely to be diminished (Cebron et al., 2011). Therefore, it is essential to understand whether these soil functions and microbial populations can recover and be sustainable for agricultural production following TD treatment.

The activities of soil organisms are responsible for nutrient transformations; therefore, changes in soil organism activity may be indicative of environmental stress on metabolic activity of the soil (Pankhurst et al., 1995; Margesin et al., 2007). As reclamation methods attempt to return the health of degraded lands to previous productivity and biologic functioning, the impact on nutrient cycling in remediated soil will be important (Sheoran et al., 2010). Many studies have reported the impacts of oil contamination on enzyme activities (Oudot et al., 1989; Macnaughton et al., 1999; Ghazali et al., 2004), but little information has been found on the effects of TD on enzyme activity or microbial communities. In locations where TD sub- or surface soil is to be used for agriculture production, understanding the ability for nutrient cycling can be used to gauge successful reclamation.

In other instances where topsoil has to be relocated to a remediation site, reclamation efforts can provide options for reducing the amount of topsoil required. One option is blending TD-treated soil with topsoil, which could produce a viable system that may reach the productivity values of the native topsoil. Therefore, a field study was initiated in 2015 to determine whether using TD-treated soil would restore/reclaim soil microbial nutrient transformations and biological functioning. We hypothesized that the high temperatures from the TD process would eliminate soil microbial abundance and activity, but that blending the TD soil with non-TD treated soil could be used to inoculate the TD soil and restore soil microbial function. This study determined soil microbial enzyme activities (urease, ammonia monooxygenase, and nitrate reductase), nitrifier and denitrifier populations, PMN and active C

amounts on non-contaminated surface soil, crude oil-contaminated subsoil, TD-treated subsoil, and 1:1 blends, both with and without compost amendment. Successful biological reclamation in this study was determined by values within significant range of the uncontaminated, native A soil.

3.3. Materials & Methods

3.3.1. Study site

The study site was located in northwest North Dakota (48°31'35.4"N, 102°51'25.72"W) near an active remediation site that had been contaminated by a pipeline leak with Bakken crude oil. The pipeline leak was discovered in 2013 leaving an estimated 3 ha contaminated to an approximate depth of 15 meters. The soil at the site was mapped as a Williams-Zahl (Williams: fine-loamy, mixed, superactive, frigid Typic Argiustolls; Zahl: fine-loamy, mixed, superactive, frigid Typic Calciustolls) (NRCS, 2015). Total precipitation and evapotranspiration at the site, from November 2015 to June 2016 were 16.9 and 68.2 cm, respectively, and from July 2016 to October 2016 were 26.7 and 51.8 cm, respectively (NDAWN, 2017). The mean annual air temperature was 6 °C for 2015 and 2016 (NDAWN, 2017).

3.3.2. Remediation of site and plot construction

The crude-oil contaminated subsoil was treated by a thermal desorption/oxidation unit (RS30; Nelson Environmental Ltd., Edmonton, Alberta) at 350 °C for 10 min, followed by re-hydration to 6 to 10% water content to generate TD soil material. Prior to treatment, total petroleum hydrocarbon (TPH) levels were approximately 1415 mg kg^{-1} (± 530 mg kg^{-1}), and the TPH target value following treatment was less than 500 mg kg^{-1}. To determine whether the TD-treated soil could be successfully remediated to agricultural productivity, a randomized complete block design experiment was established in the Fall of 2015, consisting of 3 replications of 15 x

17 m plots measuring 0.9 m deep with 10 treatments adjacent to the active remediation site. The research plots were constructed using three different soils: 1) native topsoil (A), 2) TD-treated subsoil (TDSS) having a TPH concentration of less than 500 mg kg^{-1} after treatment, and 3) crude oil-contaminated subsoil (SP) having a TPH concentration of about 1300 mg kg^{-1}. The A material was excavated several months prior to plot construction during the course of the remediation project. Thus, the A used in the plots was the original topsoil, and it received no additional treatment other than the excavation and replacement. The SP was obtained from a stockpile of contaminated soil. The stockpile is a mixture of subsoils taken across the entire width and depth of the remediation site, and the exact depth of where this subsoil was in the profile was unknown. This stockpile soil was passed through a 10 cm screener (McCloskey R155) to remove rocks and to better ensure a uniform material. The TDSS was obtained from the contaminated stockpile and treated by TD; therefore, the specific depth from which this soil was originally taken is also unknown.

In total, five soil treatments were built, consisting of the A, TDSS, and SP soils as described above, along with two mixtures. The TDSS+A treatment is a 1:1 mixture (by volume) of the TDSS soil and the A soil, and the SP+A treatment is a 1:1 mixture (by volume) of the SP soil and the A soil. To create the mixtures, the two soil types were added into a McCloskey R155 screener in alternating 0.57 m^3 excavator bucket-loads (Caterpillar 336E hydraulic excavator). For example, one bucket of A was placed into the hopper for the screener, followed by one bucket of TDSS (or SP), followed by one bucket of A, and so forth. After passing through the screener, the mixed soil moved via a material stacker (McCloskey ST80) approximately 4.6 m into the air before being deposited into a staging pile of mixed soil materials. After which, the soil treatments were laid and smoothed into the plots using a bucket

loader (Caterpillar 336E hydraulic excavator). The remaining five treatments consisted of a compost manure bedding (CMB) that was added in the Summer of 2016.

3.3.3. Plot management

In late-May 2016 the plots were prepared with fertilizer for sowing of hard-red spring wheat (*Triticum aestivum* L.). Soil P levels were normalized across plots at 105 kg ha^{-1} by adding variable amounts of monoammonium phosphate (MAP; 11-52-0) to each treatment based on Olsen-P test values and levels of P in the compost (Table 1). High levels of P in the compost (CMB), which was livestock (*Bos taurus*) bedding that had been composted for greater than three years, necessitated the high application rate for normalization. Soil N levels were then normalized at 130 kg ha^{-1} by applying urea (46-0-0) at variable amounts based on inorganic N from baseline soil tests (Table 9). Following the hand application of fertilizer on May 24, 2016; CMB was added to designated plots at a rate of 45 Mg ha^{-1} via skid steer (Caterpillar 272C skid steer loader) hauling with 0.38 m^3 bucket and hand spreading.

Fertilizer and CMB were then incorporated to 15 cm depth using a Howard Selectatilth 2.5 m rotary tiller pulled by a John Deere 6420 tractor used for two passes over all plots. Plots were seeded three days later with hard red spring wheat (cultivar: Barlow) at a rate of 90 kg seed ha^{-1} with 30 cm row spacing using Flexi coil 5000 air drill, with Flexi coil 2320 air cart pulled by a Ford 976 versatile tractor.

3.3.4. Soil physical and chemical analyses

Samples were collected from 0 to 15 and 15 to 30 cm depths from the plots on November 15, 2015 to measure initial soil physical and chemical properties (Table 9). These soils were analyzed for pH using the 1:1 soil:water ratio (Peters et al., 2012; Agvise Laboratories, Northwood, ND, USA). Plant available NO_3-N and NH_4-N were determined using KCl

extraction (Mulvaney, 1996; Agvise Laboratories). Nitrate was extracted using 0.2 M KCl and determined using the Cd Reduction Method. Ammonium was extracted with 2 M KCl extraction and determined using a Timberline Ammonium Analyzer. Plant available K was extracted with NH_4 acetate (pH 7) and quantified by inductively couple plasma optical emission spectrometry (Warnck and Brown, 1998). Soil particle size distribution was conducted by the hydrometer method (Gee and Or, 2002; ASTM Standard D422-63, 2007). Inorganic and organic C were evaluated with a Primacs Total Organic Carbon Analyzer (Skalar Analytical B.V. Breda, The Netherlands). Samples were evaluated for TPH concentration within the C10-C36 range using EPA 8015 method modified with silica gel (Pace Analytical 156 Services, Inc. St. Paul, MN, USA).

3.3.5. Soil biological analysis

Soils samples were then collected for enzyme analysis, quantifying N-cycling genes, active carbon, and potentially mineralizable nitrogen. Samples were collected from the plots on November 15, 2015 (Fall 2015); and after CMB and fertilizer application on June 15, 2016 (Summer 2016); and post-harvest on October 3, 2016 (Fall 2016). Samples were collected by hand using stainless-steel probes at depths of 0 to 15 cm and 15 to 30 cm.

3.3.5.1. Soil enzyme analysis

All soil enzyme activity levels were determined on a colorimetric basis using a spectrophotometer (Thermo Spectonic 20D+, Thermo Fisher Scientific, Madison, WI, USA). The urease enzyme is responsible for the conversion of urea into NH_3 and was quantified by the method of Kandeler and Gerber (1988) using 5 g soil incubated for 2 h at 37 °C with urea solution and 0.01 M borate buffer (pH 10), treated with KCl (2 M)-HCl (0.01 M) and shaken for 0.5 h on rotary shaker. The solution was then filtered and NH_4^+ released was quantified at 660

nm. Ammonium oxidation, the first and rate-limiting step in the nitrification process, was quantified by the method of Berg and Rosswall (1985) using 5 g soil incubated for 5 h on a rotatory shaker with 1 mM $(NH_4)_2SO_4$ and 1.5 M $NaClO_3$, extracted with 2 M KCl and the NO_2^- released was quantified at 520 nm. Nitrate reduction, the first step in denitrification was quantified by the method outlined by Abdelmagid and Tabatabai (1987) using 5 g soil treated with 25 mM KNO_3, 0.9 mM 2,4-dinitrophenol solution, and ultrapure H_2O incubated for 24 h at 25 °C, treated with 4 M KCl, filtered, and the NO_2^- released was quantified at 520 nm.

3.3.5.2. Quantifying N-cycling genes using quantitative Polymerase Chain Reaction

The quantities of N-transforming genes were assessed using quantitative Polymerase Chain Reaction (qPCR) using primers specified for a target gene. Functional gene copy numbers represent quantity of the population of organisms capable of producing selected enzymes responsible for nutrient transformations. Soil DNA was extracted from 0.25 g of soil using the Power Soil DNA Extraction Kit (MOBIO Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The samples were then stored at -80 °C until downstream qPCR application. Real time qPCR was performed using PikoReal (Thermo Scientific, Wilmington, DE, USA).

The *amoA* gene, which encodes for ammonia monooxygenase, the first step in nitrification, was quantified using the forward primer, amoA1F 5'-GGGTTTCTACTGGTGGT-3' and the reverse primer amoA2R 5'-CCCCTCKGSAAAGCCTTCTTC-3' (Rotthauwe et al., 1997) to amplify a 491-bp DNA fragment in nitrifying bacteria (AOB). The forward primer Arch-amoA-for 5'-CTGAYTGGGCYTGGACATC'3' and the reverse primer Arch-amoA-rev 5'TTCTTCTTTGTTGCCCAGTA-3' (Wutcher et al., 2006) were used to amplify a 256-bp DNA fragment of the *amoA* gene in nitrifying archaea (AOA). To determine the quantity of final

denitrifiers, the forward primer, nosZ1 5'-WCSYTGTTCMTCGACAGCCAG-3' and the reverse primer nosZ1R 5'-ATGTCGATCARCTGVKCRTTYTC-3' (Henry et al., 2006) were used to amplify a 259-bp DNA fragment that encodes for the n*osZ* nitrous oxide reductase. All primer pairs were synthesized by Invitrogen, (Carlsbad, CA, USA).

A 20 µL reaction volume was used for qPCR that contained 10 µL of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 0.3 µL of 10 µM forward and reverse primer, 1 µL of DNA template, and 8.4 µL of DNA/RNAse free water. The qPCR thermocycler protocol used was 5 min at 95 °C followed by 40 cycles of denaturing at 95 °C for 15 s, annealing at temperatures optimized for each primer for 30 s with a data acquisition step, and extension at 72 °C for 30 s followed by a melt curve analysis. The annealing temperature was 55 °C for the AOA primers, 56 °C for the AOB primers and 64 °C for the *nosZ* primers. Separate standard curves were generated for each functional gene using five serial dilutions of genomic DNA ranging from 0.01 ng μL^{-1} to 4 ng μL^{-1}. Organismal DNA controls used for the AOA primers were isolated from the nitrifying archaea *Nitrosopumilus maritimus* SCM1, while the DNA controls used for the AOB primers were isolated from the nitrifying bacteria *Nitrosomonas europaea* (DSM 28437, DSMZ, Leibniz-Institute, Braunschweig, Germany). The organismal DNA control used for the *nosZ* primer was isolated from the denitrifying bacteria *Pseudomonas aeruginosa* PAO-1 (ATTC 47085, Manassas, VA, USA). Standard curve coefficient of determination (R^2) values were above 0.97. Genomic DNA isolated from standard curve organismal controls and soil samples were run in triplicate. Units for the field plot samples are reported in copies g^{-1} dry soil.

Preliminary data measured the amount of N-transforming genes in the rehydrating water, and similar sources of TDSS and SPSS before the field plots were established. The rehydrating

water is used to cool the TD-treated soil and bring the soil back to 6 to 10% water content. The water accounted for 1.2 x 10^3, 6.6 x 10^3, and 1.0 x 10^6 copies of AOB, AOA, and *nosZ* mL^{-1} water, respectively. Before entering the TDU the SPSS contained 2.17 x 10^2, 1.8 x 10^3, and 3.5 x 10^4 copies g^{-1} soil of AOA, AOB, and *NosZ* genes, respectively. Immediately after being rehydrated the quantities of AOA, AOB, and *NosZ* in the TDSS decreased to 53.8, 1.3, and 1,407 copies g^{-1} soil.

3.3.5.3. Additional soil health measures

The soil collected was also analyzed for active C and PMN. Methods for active C were adapted from Cornell Soil Health Manual (2009). From a larger, thoroughly mixed composite bulk soil, a subsample was collected and allowed to air dry and passed through a 2 mm sieve. A 2.5 g sample was placed into a 50 mL centrifuge tube and 20 mL of a 0.02 M potassium permanganate ($KMnO_4$) solution was added. The samples were placed in a horizontal shaker at 240 oscillations min^{-1} for 2 min. The samples were then centrifuged for 5 min at 730 x *g*. Immediately after centrifugation, 0.2 mL of the supernatant were pipetted into glass tubes with 10 mL of high-purity water. The supernatant absorbance was read at 550 nm using a spectrophotometer (Thermo Spectonic 20D+). Standards of 0.005, 0.01, 0.015, and 0.02 M $KMnO_4$ concentrations were prepared to establish a standard curve. The absorbance value was converted to active C in units of mg C kg^{-1} soil.

Methods for anaerobic PMN were adapted from Cornell Soil Health Manual (2009). It is essential that soil samples remain completely anaerobic during incubation to eliminate possible nitrification-denitrification reactions at the soil-water interface that would lead to low results. Four 8-g sub-samples were taken from a composite field moist bulk soil sample and placed into 50 mL centrifuge tubes. Forty mL of 2.0 M KCl was added to two of the centrifuge tubes,

shaken on a mechanical shaker for 1 hr, and centrifuged at 804 x *g* for 10 min. Then 20 mL of the supernatant was collected and saved for NH_4-N concentration analysis ("time = 0" measurement). Ten mL of DI water was added to the third and fourth tubes, hand shaken and incubated in the dark for 7 d at 30 °C. After the 7 d anaerobic incubation, 30 mL of 2.67 M KCl was added to the third and fourth tubes (creating a 2.0 M solution). The tubes were shaken on a mechanical shaker at 180 oscillations min^{-1} for 1 h and centrifuged at 804 x *g* for 10 min. Twenty mL of supernatant was collected and analyzed for NH_4-N ("time 7 days" measurement) using EPA methods 353.2 (Keith, 1996) with flow injection (FIAlab 2500, FIAlab Instruments, Inc.). The difference between the time 0 and 7 d concentrations was the PMN. Results are reported in units of micrograms N mineralized per g^{-1} $week^{-1}$.

3.3.5.4. Statistical analysis

Results of the biological tests were analyzed using one-way, fixed-effects ANOVA with mean difference significance at the $\alpha = 0.05$ level. Post hoc calculation of Tukey's Honestly Significant Difference (HSD) values were calculated for $\alpha=0.05$ level with R 3.3.1 software (R Core Team, 2016) using the "agricolae" (de Mendiburu, 2016).

3.4. Results and Discussion

3.4.1. Enzyme activities

The subsurface location where the soils were extracted from and the heating of the soil by TD (TDSS, and TDSS+CMB) resulted in NH_4 released by urease activity to be near zero (Table 11 and Table 12). Deeper depths have resulted in lower urease activity in relation to decreased nutrients for microorganisms (Tabatabai, 1977). Similar decreases in urease activity have been found for Iowa soils dried at 105 °C for 24h or autoclaved at 120 °C for 2 h resulting in zero urease activity for all soils studied (Zantua and Bremner, 1977). This may have consequences

for urea application as soil urease is important in N transformations and cycling as it enzymatically catalyzes the hydrolysis of urea to plant-available NH_3 and CO_2 (Kim et al., 2008). Many current agricultural practices rely on this process as their use of urea as a N fertilizer has significantly increased (Cozzi et al., 2014).

The addition of A soil (SP+A, SP+A+CMB, TDSS+A, and TDSS+A+CMB) increased urease activity to approximately 50% (Table 11 and Table 12) of the activity of the A and A+CMB treatments across both soil depths. Similar urease activity levels of 45 to 64 μg NH_4-N g^{-1} 2 h^{-1} were found in ambient CO_2 conditions shortgrass steppe in northeastern Colorado in an Ustollic Camborthids at the end of the growing season (Kandeler et al., 2006). The blended values are within range of slightly lower activities observed in reclaimed loess soils among various amendments with levels between 25 and 30 μg NH_4-N g^{-1} 2 h^{-1} (Marschner et al., 2003). Sampling time did not have a significant effect on urease enzyme activities. Other studies have shown higher urease activity with higher environmental temperatures near 70 °C (Zantua and Bremner, 1977; Sahrawat, 1984) and in soils under vegetation compared to vegetation free or after vegetation removal (Voets et al., 1974; Reddy et al., 1987), but neither trend was apparent in this study across sampling dates.

In Fall 2015 and 2016, NO_2-N released from NH_4 oxidation in A and A+CMB was significantly higher than the other treatments at the 0-15 cm depth (Table 11). During Summer 2016 for the 0-15 cm depth, most NH_4 oxidation values significantly increased from Fall 2015 with few significant differences observed among treatments (Table 11). At 15-30 cm, values for A and A+CMB were significantly greater than for the other treatments for all sampling dates (Table 12). Similar lower values were recorded for a Haplic Chernozem (Mollisol) cropped from sugar beet (*Beta vulgaris* L. *ssp. vulgaris var. altissima Döll)* to spring barley (*Hordeum*

vulgare L.) in Austria with a range between 0.3 and 0.7 μg NO_2-N g^{-1} 5 h^{-1} (Kandeler et al., 1999). Much higher activities, around 500 μg NO_2-N g^{-1} 5 h^{-1}, were described for calcareous grassland soils in northwestern Switzerland (Niklaus et al., 2001). Ammonia-oxidizing bacteria and archaea use the NH_3 monooxygenase enzyme for the oxidation of NH_3 to NO_2^- as an energy-generating step and is necessary for healthy agricultural soils (Bollmann et al., 2014).

Nitrite released by nitrate reductase activity significantly decreased in the 0-15 cm depth for A+CMB, SP+A, SP+A+CMB and TDSS+A+CMB treatments between the Fall 2015 and Summer 2016 samplings (Table 11). No significant differences occurred within treatments from Summer 2016 to Fall 2016. Again, the SP, SP+CMB, TDSS, and TDSS+CMB generally have the lowest values at both depths (Table 11 and Table 12). Similarly, the blended soils responded with levels near the A and A+CMB for both 0-15 and 15-30 cm depths (Table 11 and Table 12). Samples from the Cascade Mountains produced NO_3^- reductase activity levels from 0.92 to 4.38 μg NO_2 –N g^{-1} 24 h^{-1} (Boyle et al., 2006) and similar values from 1.11 to 1.30 μg NO_2-N g^{-1} 24 h^{-1} were measured in a natural soil in Dehli, India (Singh and Kumar, 2008). Under waterlogged conditions, NO_3^- reductase levels were much higher with values between 6 and 87 μg NO_2 –N g^{-1} 24 h^{-1} (Abdelmagid and Tabatabai, 1987). Lower NO_3^- reductase levels signify less fertilizer N loss through denitrification; however, denitrification is an important process contributing N_2O to the atmosphere, thus balancing the global N cycle (Knowles, 1982).

3.4.2. Quantities of N-cycling genes

Assuming 2.5 copies of *amoA* gene per AOB cell (Norton et al., 2002) and one copy per AOA cell (Hallam et al., 2006), AOB *amoA* genes tended to dominate by one to two-fold greater than AOA for all sampling dates and depths studied except for TDSS and TDSS+CMB in the Fall of 2015 (Table 13 and Table 14). During Summer 2016 at 0-15 cm, the TDSS+CMB had

the lowest of the AOA copies (1.61 x 10^4 copies g^{-1} soil; Table 13) while A had the highest AOA copies (4.24 x 10^5 copies g^{-1} soil). The AOB had a large range of values at 0-15 cm from being as low as 2.25 x 10^3 copies g^{-1} soil for TDSS+CMB in Fall of 2015 to as high as 2.30 x 10^7 copies g^{-1} soil in A+CMB in Summer 2016. Similar numbers for AOB were observed under unfertilized conditions with a mean of 5.5 x 10^6 copies g^{-1} soil in field soils (Okano et al., 2004). However, other studies have found AOA gene copy numbers to be greater than those of the AOB due to the nutrients available (Adair and Schwartz, 2008; Shen et al., 2008; Di et al., 2010). For example, in a Typic Udivitrand the gene copy numbers of AOA ranged from 1.2 x 10^5 to 2.0 x 10^7 copies g^{-1} $soil^{-1}$, whereas, gene copy numbers of AOB ranged from 7.7 x 10^3 to 7.7 x 10^4 copies g^{-1} $soil^{-1}$ (Di et al., 2010). Environmental conditions are likely the basis for the increase in AOB gene copy numbers in this study as they are shown to increase with inorganic N application (Zhang et al., 2010).

During the Summer 2016 sampling after CMB and fertilizer application, AOB copies significantly increased between the A and A+CMB; SP+A and SP+A+CMB; and TDSS+A and TDSS+A+CMB (Table 14); whereas, no increase in AOA copies were observed. Similarly observed, the increase in AOB copies could be promoted at high NH_3 concentrations which would occur due to fertilizer application; whereas, AOA are not influenced by NH_3 input suggesting different growth responses to NH_3 concentration, and indicating that AOA and AOB occupy separate ecological niches (Schauss et al., 2009; Verhamme et al., 2011; Chen et al., 2015). Similar to the NH_4 oxidation, for the 0-15 cm, AOB copy numbers increased after compost application in Summer 2016 but then tended to decrease for Fall 2016 (Table 11 and Table 12). The growth of AOB population has been linked to increased nitrification activity following high levels of NH_4 addition, either directly from mineral fertilizer or from the rapid

hydrolysis of urea to NH_4; therefore, is the likely reason for the AOB gene increase (Verhamme et al., 2011).

After NO_3^- has been produced, denitrification can occur if NO_3^- is not used by the plant or leached. Denitrification can lead to a loss of N from soil systems, but incomplete conversion of mineral N to N_2 can result in the formation of nitric oxide (NO), which can contribute to ozone formation, and N_2O, a greenhouse gas; therefore, the final conversion of N_2O to N_2 by the *nosZ* gene is of importance (Boyle et al., 2006). In Fall 2015 at 0-15 cm, *nosZ* genes in the blended soils (SP+A, SP+A+CMB, TDSS+A, and TDSS+CMB) were significantly lower than A and A+CMB, but significantly higher than treatments without the A addition (SP, SP+CMB, TDSS, TDSS+CMB; Table 15). In the Summer of 2016 at 0-15 cm, copies for SP+CMB, SP+A+CMB, and TDSS+A+CMB were not significantly lower than A and A+CMB. By Fall 2016, A+CMB was significantly the highest in *nosZ* copies (Table 15); otherwise, there were no differences among other treatments. The range of *nosZ* copies are within the range for soils in other studies with *nosZ* genes in Himalayan soils ranging from 2×10^5 to 5×10^5 copies g^{-1} of dry soil irrespective of the sampling site, whereas densities up to 10^7 copies g^{-1} of dry soil were observed in soils from France (Henry et al., 2006). Frozen soil from a field previously cropped to spring wheat in Fredericton, New Brunswick, Canada recorded similar *nosZ* values of 4.6×10^6 nosZ gene copies g^{-1} dry soil (Miller et al., 2008).

3.4.3. Active carbon and potentially mineralizable nitrogen (PMN)

Active C was highest for A or A+CMB for both depths and all sampling dates ranging from 330 to 579 mg C kg^{-1} soil, while SP or SP+m consistently had the lowest means ranging from 91.8 to 156 mg C kg^{-1} soil (Table 17 and Table 18). Depending on the season, the blended treatments (SP+A, SP+A+CMB, TDSS+A, and TDSS+A+CMB), were statistically the same as

the A and/or A+CMB treatments. The treatments without the addition of A (SP, SP+CMB, TDSS, TDSS+CMB) tended to have the lowest active C values for all sampling periods and depths. This is of importance as the labile fractions of soil C, termed active C pool, are readily available as a C and energy source for soil microorganisms (Weil et al., 2003). The ranges for A and A+CMB are within range of POXC values of 378 mg C kg^{-1} for a Kansas soil to 814 mg C kg^{-1} for a fine, kaolinitic, thermic Typic Kanhapludult (Culman et al., 2012). Potentially oxidizable C has also been shown to be intermediately sensitive to management practices compared to particulate organic C and microbial biomass C and may prove to be an indicator of soil degradation or improvement in response to disturbance and management practices (Weil et al., 2003; Culman et al., 2012).

Except for treatments without the addition of A (SP, SP+CMB, TDSS, and TDSS+CMB) which had near zero values during all sampling periods, PMN was significantly higher in Fall 2015 (Table 17 and Table 18) compared to Summer 2016 and Fall 2016 within treatments. Small differences occurred within treatments between Summer 2016 and Fall 2016 for both depths. Similar results to A, A+CMB, and the blends were reported for a variety of loam soils in Yolo County, California with averages between 0.18 and 1.43 mg NH_4^+-N kg^{-1} soil (Bowles et al., 2014). Also during a 7-day incubation of a grassland sandy loam in Belgium values of 1.17 mg N kg^{-1} d^{-1} at 0-10 cm depth and 1.00 mg N kg^{-1} d^{-1} at 10 to 20 cm depth were recorded (Accoe et al., 2004). Another study observed a 19% decrease in mineralizable N from flowering to harvest contributed to a net release of mineral N to crop growth or a decrease in substrate quality, such that more N was immobilized, but was not apparent in our study (Franzluebbers et al., 1994). Potentially mineralizable N is the capacity of the soil microbial community to mineralize N tied up in complex organic residues into NH_4 (Accoe et al., 2004). Although most

values appear low, the near zero values for TDSS, TDSS+CMB, SP, and SP+CMB may be indicative from a lack of organic residue or insufficient ability of the microorganisms to create NH_4.

3.4.4. Biological considerations for reclamation

The data contributed to portions of the N cycle that are important for agricultural crop production. Specified biological indicator values to achieve soil health and promote optimum crop yields after disturbance were not identified and can be different depending on ecosystem and soil type. Given that TDSS and SP are subsoil materials the focus was on bringing these soils to acceptable levels of biological functioning after disturbance without the requirement of bringing in relocated topsoil. Since the N cycle consists mainly of microbial-driven processes that consist of a series of dependent stages where the products of one step become substrates in the following step and any downstream products may be immobilized by soil organic matter, the continuous cycle of N is required for a healthy soil (You et al., 2009; Phillips et al., 2015).

The biological parameters measured indicated if the soil is functioning at levels comparable to the native A soil. Quantitative PCR measured specified genes to determine potential output from a soil, but it cannot determine if the genes are functioning and participating in the N cycle. Since the AOA and AOB have varying oxidation rates for NO_2^- production depending on the energy sources available, the actual productivity of the genes quantified may be difficult to determine (Adair and Schwartz, 2008; Schauss et al., 2009). Therefore, qPCR may not be of interest for cropland managers whose concern is the availability of crop nutrients. However, qPCR is invaluable for understanding the microbial community structure and diversity within the environment as it typically requires less than 1 g of soil and targets specific gene sequences (Jacobsen, 1995; Smith and Osborn, 2009). Whereas, measuring enzymes is relatively

inexpensive and less time consuming while providing reliable data to differentiate between treatments and ensure products of the N cycle are being produced at different steps (Gil-Sotres et al., 2005; Dose et al., 2015). Active C and PMN can also be used to determine energy sources available for the microorganisms (Drinkwater et al., 1996; Culman et al., 2012). However, successful reclamation of disturbed soil still depends on the overall soil health, which cannot overlook the chemical and physical properties of the soil.

3.5. Conclusions

The results indicated a trend with A and A+CMB generally having the highest biological indicator levels, while the 1:1 blends were slightly lower and the treatments without the addition of A were generally the lowest. The lower levels for the parameters measured indicate the possibility of consequences to soil functioning. The blended soils reached levels that have been reported in the literature and thus additions of uncontaminated topsoil may be a viable option for reclamation as the A addition may be acting as an inoculant to help restore these biological functions. Since little improvement was observed across time, additional reclamation strategies may need to be included to ensure suitable biological functioning that is equivalent to the native A.

Reclamation of disturbed soils can be accomplished through management practices and amendments. By measuring soil parameters, remediation success of disturbed soil can be quantified, which assists in the determination of when it may be returned back to productivity. The lowest-valued biological parameters measured in this study were generally from the TD-treated and contaminated subsoils suggesting additional reclamation efforts may be necessary to improve soil biological functioning. Biological indicators responded favorably to the blended soils, indicating it is a viable option when remediating highly disturbed soils. Sampling over

time did not assist in evaluating successful remediation, which may indicate that more time is required for improvement. The treatments used in this study provide information on biological functioning during the remediation of crude-oil contaminated agricultural soil, as well as during subsequent reclamation. This information is critical for determination of successful soil reclamation, which must consider all aspects of soil, including biological, physical, and chemical parameters. This study used the native A as a control for ideal target values. Since optimal parameter values are likely to differ depending on environmental conditions, samples from productive soils within the same environment can indicate successful remediation levels. Future research may concentrate on whether time and cropping sequences can elevate biological indicators to levels found in nearby undisturbed soils to indicate successful reclamation.

3.6. Acknowledgements

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3.7. Tables

Table 9. Initial plot data on soil chemical (pH, NO3, NH4, P, K and TPH, organic C and inorganic C) and physical (sand, silt, clay) properties collected from 0-15 and 15-30 cm on November 15, 2015 (Fall 2015). Composted manure-bedding (CMB) was not added prior to these samples being taken.

Treatment†	Depth	Parameter										
		pH	Sand	Silt	Clay	Organic C	Inorganic C	NO_3-N	NH_4-N	P (Olsen)	K	TPH‡
	cm		g kg^{-1}							mg kg^{-1}		
A	0-15	7.5	461	319	220	20	3.0	16.7	10.2	9.0	244	24.9
	15-30	7.4	460	324	216	20	3.0	18.8	8.6	8.8	254	21.5
A+CMB	0-15	7.5	455	328	217	22	3.0	12.4	16.8	9.4	240	17.2
	15-30	7.3	462	327	211	23	2.0	13.7	16.7	8.9	252	23.3
SP	0-15	8.0	385	346	269	5.0	16	0.6	4.9	2.0	149	1340
	15-30	8.1	385	347	268	5.0	15	0.5	5.8	2.3	147	1210
SP+CMB	0-15	8.0	385	353	262	5.0	17	0.5	4.1	2.6	151	1510
	15-30	8.1	393	342	265	4.0	16	0.5	3.9	2.0	151	1810
SP+A	0-15	7.8	418	326	255	12	11	5.8	4.2	5.1	200	683
	15-30	7.7	424	322	254	12	11	5.9	4.4	5.3	197	941
SP+A+CMB	0-15	7.7	433	317	250	12	11	6.6	5.0	5.2	199	589
	15-30	7.7	432	320	247	15	10	6.4	5.0	5.3	198	608
TDSS	0-15	8.1	428	338	235	3.0	17	0.8	6.8	4.7	195	197
	15-30	8.1	424	325	251	3.0	16	0.7	7.5	3.2	198	228
TDSS+CMB	0-15	8.1	430	313	256	4.0	16	0.7	8.3	3.0	188	258
	15-30	8.1	438	325	237	4.0	16	0.7	9.0	3.0	188	202
TDSS+A	0-15	7.8	452	324	224	11	11	8.3	11.6	7.7	233	90.6
	15-30	7.8	451	331	218	11	11	9.7	10.1	7.6	236	78.7
TDSS+A+CMB	0-15	7.8	455	315	230	10	12	8.1	10.0	7.0	220	99.7
	15-30	7.8	439	325	236	12	11	9.6	9.1	7.3	225	129

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Total petroleum hydrocarbons

Table 10. Amounts of fertilizer (monoammonium phosphate (MAP) and urea) added to each plot on May 24, 2016.

Treatment†	MAP (11-52-0)	Urea (46-0-0)
	kg per plot (lbs)	
A	9.5 (21)	3 (6.6)
A+CMB	0.5 (1.1)	5 (11)
SP	10.5 (23)	4.5 (10)
SP+CMB	1.5 (3.3)	6.5 (14.3)
SP+A	10.5 (23)	4.5 (10)
SP+A+CMB	1.5 (3.3)	6.5 (14.3)
TDSS	10.5 (23)	4.5 (10)
TDSS+CMB	1.5 (3.3)	6.5 (14.3)
TDSS+A	9.5 (21)	3 (6.6)
TDSS+A+CMB	0.5 (1.1)	5 (11)

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

Table 11. Urease, ammonium oxidation, and nitrate reductase enzyme activities for the 0-15 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 0-15 cm	Urease				Ammonium oxidation				Nitrate reductase			
	Time				Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD	Fall 2015	Summer 2016	Fall 2016	HSD
	μg NH_4-N g^{-1} soil 2 h^{-1}				μg NO_2-N g^{-1} soil 5 h^{-1}				μg NO_2-N g^{-1} soil 24 h^{-1}			
A	44.8(5.7)‡	53.8(7.5)	33.5(3.9)	14.8	1.8(0.4)	3.0(0.3)	2.5(0.5)	1.03	3.5(1.1)	2.0(0.4)	1.8(0.1)	2.06
A+CMB	67.2(10.2)	59.1(1.6)	34.1(4.0)	16.1	2.1(0.0)	3.2(1.5)	2.3(0.2)	2.16	2.7(0.5)	1.9(0.1)	2.2(0.1)	0.70
SP	5.8(1.2)	3.9(2.7)	3.1(0.2)	4.4	0.0(0.0)	1.4(0.4)	0.3(0.1)	0.66	0.3(0.1)	0.3(0.3)	0.6(0.1)	0.45
SP+CMB	2.7(2.3)	3.8(2.4)	6.2(1.2)	5.1	0.0(0.0)	0.5(0.2)	0.5(0.1)	0.36	0.4(0.1)	0.7(0.2)	0.5(0.1)	0.32
SP+A	20.6(5.5)	33.9(5.2)	18.8(3.3)	11.9	0.5(0.0)	2.2(0.6)	1.1(0.1)	0.94	3.2(0.9)	1.1(0.1)	1.4(0.2)	1.30
SP+A+CMB	24.5(4.6)	29.3(5.3)	21.5(6.3)	13.6	0.5(0.1)	3.2(0.7)	1.4(0.5)	1.25	3.5(1.1)	1.0(0.2)	0.9(0.1)	2.07
TDSS	0.2(0.4)	2.0(1.8)	1.0(0.9)	3.0	0.1(0.1)	2.2(0.5)	0.5(0.2)	0.80	0.2(0.1)	0.1(0.1)	0.2(0.1)	0.26
TDSS+CMB	4.4(0.5)	1.4(0.6)	5.7(0.8)	1.6	0.1(0.1)	2.1(0.1)	0.8(0.2)	0.28	0.2(0.2)	0.1(0.1)	0.2(0.2)	0.41
TDSS+A	14.6(5.1)	24.2(2.1)	15.2(0.6)	8.0	1.0(0.1)	1.4(0.2)	1.4(0.1)	0.37	1.7(0.6)	0.8(0.2)	0.9(0.2)	0.94
TDSS+A+CMB	21.8(5.0)	27.8(0.7)	18.7(0.8)	7.4	0.8(0.2)	2.0(0.6)	1.3(0.3)	1.05	1.3(0.2)	0.5(0.1)	1.6(0.2)	0.38
HSD§	14.4	10.6	8.46		0.45	1.86	0.78		1.78	0.56	0.46	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 12. Urease, ammonium oxidation, and nitrate reductase enzyme activities for the 15-30 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 15-30 cm	Urease				Ammonium oxidation				Nitrate reductase			
	Time				Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD	Fall 2015	Summer 2016	Fall 2016	HSD
	μg NH_4-N g^{-1} soil 2 h^{-1}				μg NO_2-N g^{-1} soil 5 h^{-1}				μg NO_2-N g^{-1} soil 24 h^{-1}			
A	42.9(13.9)‡	53.4(3.0)	40.6(11.5)	26.4	2.0(0.3)	1.7(0.7)	1.6(0.3)	1.13	3.4(0.7)	2.0(0.2)	1.0(0.2)	1.07
A+CMB	67.4(15.7)	51.2(2.2)	34.2(2.2)	23.2	1.8(0.2)	1.7(0.2)	1.8(0.3)	0.56	4.2(1.0)	2.4(0.5)	0.7(0.1)	2.10
SP	1.8(0.8)	2.9(0.9)	4.9(3.6)	5.46	0.0(0.0)	0.1(0.1)	0.0(0.0)	0.10	0.4(0.2)	0.6(0.5)	0.2(0.0)	0.74
SP+CMB	5.5(1.5)	3.1(0.9)	4.2(1.0)	2.86	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.08	0.7(0.2)	0.7(0.2)	0.3(0.1)	0.43
SP+A	37.2(14.1)	23.7(2.8)	20.9(6.0)	22.5	0.5(0.2)	0.6(0.1)	0.6(0.1)	0.38	5.7(0.9)	1.5(0.2)	0.8(0.3)	1.41
SP+A+CMB	24.5(1.1)	14.4(1.3)	19.5(1.8)	3.59	0.5(0.2)	0.4(0.1)	0.2(0.0)	0.47	5.9(1.6)	1.4(0.4)	1.2(0.3)	2.40
TDSS	0.7(0.7)	0.1(0.1)	0.3(0.1)	0.98	0.0(0.0)	0.6(0.3)	0.0(0.0)	0.53	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.04
TDSS+CMB	0.7(0.8)	0.2(0.1)	0.0(0.0)	1.13	0.0(0.0)	0.6(0.4)	0.2(0.1)	0.64	0.0(0.0)	0.1(0.0)	0.1(0.0)	0.04
TDSS+A	19.3(1.2)	24.6(3.2)	19.2(1.6)	5.46	0.9(0.1)	0.6(0.1)	0.5(0.2)	0.17	2.4(0.4)	0.8(0.3)	0.6(0.1)	0.73
TDSS+A+CMB	15.7(2.8)	20.1(1.8)	14.2(.7)	5.33	0.6(0.1)	0.7(0.2)	0.6(0.1)	0.32	2.2(0.2)	0.8(0.2)	0.5(0.2)	0.47
HSD§	23.4	5.68	12.8		0.44	0.85	0.45		2.06	0.87	0.54	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 13. Quantitative polymerase chain reaction values for ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) for the 0-15 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 0-15 cm	**Ammonia-oxidizing archaea (AOA)**				**Ammonia-oxidizing bacteria (AOB)**			
	Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD
	Copies g^{-1} soil				Copies g^{-1} soil			
A	$1.17 \times 10^6(1.05 \times 10^5)$‡	$4.24 \times 10^5(1.62 \times 10^5)$	$4.21 \times 10^5(4.59 \times 10^4)$	2.87×10^5	$2.28 \times 10^6(5.99 \times 10^5)$	$1.54 \times 10^7(1.89 \times 10^6)$	$9.96 \times 10^6(1.30 \times 10^6)$	3.43×10^6
A+CMB	$2.68 \times 10^6(5.58 \times 10^5)$	$4.07 \times 10^5(1.05 \times 10^5)$	$5.06 \times 10^5(6.25 \times 10^4)$	8.26×10^5	$4.37 \times 10^6(1.68 \times 10^6)$	$2.30 \times 10^7(1.12 \times 10^6)$	$9.96 \times 10^6(1.71 \times 10^6)$	3.82×10^6
SP	$1.64 \times 10^5(1.21 \times 10^5)$	$3.58 \times 10^4(1.31 \times 10^4)$	$9.14 \times 10^4(1.59 \times 10^4)$	1.78×10^5	$1.20 \times 10^5(1.28 \times 10^5)$	$1.18 \times 10^6(2.58 \times 10^5)$	$2.92 \times 10^6(3.61 \times 10^6)$	5.24×10^6
SP+CMB	$4.20 \times 10^5(3.06 \times 10^5)$	$1.02 \times 10^5(5.44 \times 10^4)$	$1.33 \times 10^5(7.82 \times 10^4)$	4.63×10^5	$5.96 \times 10^4(3.27 \times 10^4)$	$2.13 \times 10^6(4.69 \times 10^5)$	$3.98 \times 10^6(2.65 \times 10^6)$	3.89×10^6
SP+A	$2.88 \times 10^5(8.93 \times 10^4)$	$9.30 \times 10^4(5.72 \times 10^4)$	$2.76 \times 10^5(8.33 \times 10^4)$	2.20×10^5	$6.48 \times 10^5(8.37 \times 10^4)$	$1.21 \times 10^7(6.07 \times 10^5)$	$8.27 \times 10^6(4.64 \times 10^5)$	1.11×10^6
SP+A+CMB	$7.21 \times 10^5(2.55 \times 10^4)$	$2.19 \times 10^5(6.47 \times 10^4)$	$3.00 \times 10^5(2.97 \times 10^4)$	4.78×10^5	$7.81 \times 10^5(5.67 \times 10^4)$	$1.99 \times 10^7(2.62 \times 10^6)$	$4.20 \times 10^6(7.35 \times 10^5)$	4.86×10^6
TDSS	$1.12 \times 10^5(6.74 \times 10^3)$	$3.10 \times 10^4(2.60 \times 10^4)$	$3.07 \times 10^4(4.14 \times 10^3)$	3.93×10^4	$2.64 \times 10^3(1.09 \times 10^3)$	$6.73 \times 10^5(1.12 \times 10^5)$	$1.25 \times 10^5(1.58 \times 10^5)$	2.80×10^5
TDSS+CMB	$8.80 \times 10^4(5.28 \times 10^4)$	$1.61 \times 10^4(8.48 \times 10^3)$	$1.01 \times 10^5(4.06 \times 10^4)$	9.71×10^4	$2.25 \times 10^3(3.76 \times 10^2)$	$1.60 \times 10^6(5.61 \times 10^5)$	$1.50 \times 10^6(2.18 \times 10^5)$	8.70×10^5
TDSS+A	$3.96 \times 10^5(1.45 \times 10^5)$	$1.01 \times 10^5(3.45 \times 10^4)$	$2.08 \times 10^5(5.11 \times 10^4)$	2.27×10^5	$1.43 \times 10^6(3.89 \times 10^4)$	$7.09 \times 10^6(5.48 \times 10^5)$	$4.78 \times 10^6(2.10 \times 10^6)$	3.14×10^6
TDSS+A+CMB	$6.36 \times 10^5(6.07 \times 10^5)$	$2.04 \times 10^5(8.36 \times 10^4)$	$2.11 \times 10^5(1.33 \times 10^5)$	9.07×10^5	$7.96 \times 10^5(8.80 \times 10^4)$	$1.46 \times 10^7(1.58 \times 10^6)$	$4.74 \times 10^6(3.92 \times 10^6)$	5.80×10^6
HSD§	8.65×10^5	2.18×10^5	2.00×10^5		1.64×10^6	3.60×10^6	6.25×10^6	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 14. Quantitative polymerase chain reaction values for ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) for the 15-30 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 15-30 cm	**Ammonia-oxidizing archaea (AOA)**				**Ammonia-oxidizing bacteria (AOB)**			
	Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD
		Copies g^{-1} soil				Copies g^{-1} soil		
A	$1.83 \times 10^6 (1.03 \times 10^6)$‡	$4.37 \times 10^5 (1.23 \times 10^5)$	$5.84 \times 10^5 (1.32 \times 10^5)$	1.51×10^6	$2.12 \times 10^6 (5.93 \times 10^5)$	$7.93 \times 10^6 (4.93 \times 10^6)$	$3.97 \times 10^6 (7.08 \times 10^5)$	7.26×10^6
A+CMB	$2.65 \times 10^6 (3.36 \times 10^5)$	$3.93 \times 10^5 (8.72 \times 10^4)$	$5.06 \times 10^5 (7.33 \times 10^4)$	5.13×10^5	$4.02 \times 10^6 (1.44 \times 10^6)$	$1.21 \times 10^7 (2.49 \times 10^6)$	$6.55 \times 10^6 (6.59 \times 10^5)$	4.26×10^6
SP	$3.61 \times 10^5 (1.11 \times 10^5)$	$1.36 \times 10^4 (4.18 \times 10^3)$	$1.22 \times 10^5 (4.11 \times 10^4)$	1.61×10^5	$2.94 \times 10^4 (4.64 \times 10^4)$	$4.57 \times 10^3 (2.98 \times 10^2)$	$1.41 \times 10^4 (1.01 \times 10^3)$	6.71×10^4
SP+CMB	$1.12 \times 10^5 (9.59 \times 10^4)$	$3.63 \times 10^4 (7.68 \times 10^3)$	$4.53 \times 10^4 (1.88 \times 10^3)$	1.24×10^5	$2.06 \times 10^4 (9.09 \times 10^3)$	$7.89 \times 10^5 (2.15 \times 10^5)$	$5.38 \times 10^3 (9.46 \times 10^2)$	3.11×10^5
SP+A	$8.11 \times 10^5 (4.75 \times 10^5)$	$1.42 \times 10^5 (3.24 \times 10^4)$	$2.23 \times 10^5 (8.35 \times 10^4)$	6.99×10^5	$8.04 \times 10^5 (5.87 \times 10^5)$	$1.53 \times 10^6 (4.38 \times 10^5)$	$1.42 \times 10^6 (3.72 \times 10^5)$	1.19×10^6
SP+A+CMB	$1.24 \times 10^6 (5.43 \times 10^5)$	$1.42 \times 10^5 (5.09 \times 10^4)$	$1.70 \times 10^5 (2.12 \times 10^4)$	7.89×10^5	$1.30 \times 10^6 (3.11 \times 10^4)$	$1.77 \times 10^6 (9.21 \times 10^5)$	$1.15 \times 10^6 (3.99 \times 10^5)$	1.39×10^6
TDSS	$2.14 \times 10^5 (1.57 \times 10^5)$	$2.01 \times 10^4 (5.16 \times 10^3)$	$2.07 \times 10^4 (5.18 \times 10^3)$	2.27×10^5	$2.12 \times 10^3 (6.88 \times 10^2)$	$2.70 \times 10^5 (5.96 \times 10^4)$	$5.14 \times 10^4 (6.34 \times 10^4)$	1.26×10^5
TDSS+CMB	$1.69 \times 10^5 (1.47 \times 10^5)$	$1.46 \times 10^4 (1.17 \times 10^4)$	$8.57 \times 10^4 (4.74 \times 10^4)$	2.24×10^5	$4.93 \times 10^3 (9.80 \times 10^2)$	$6.21 \times 10^4 (1.55 \times 10^4)$	$3.06 \times 10^5 (2.25 \times 10^5)$	3.26×10^5
TDSS+A	$6.55 \times 10^5 (1.50 \times 10^5)$	$1.54 \times 10^5 (4.24 \times 10^4)$	$3.35 \times 10^5 (1.10 \times 10^5)$	2.76×10^5	$1.57 \times 10^6 (1.74 \times 10^5)$	$3.15 \times 10^6 (3.36 \times 10^5)$	$2.44 \times 10^6 (6.92 \times 10^5)$	1.14×10^6
TDSS+A+CMB	$3.92 \times 10^5 (2.24 \times 10^5)$	$1.28 \times 10^5 (3.41 \times 10^4)$	$1.33 \times 10^5 (7.89 \times 10^4)$	3.47×10^5	$1.25 \times 10^6 (3.52 \times 10^5)$	$1.80 \times 10^6 (1.15 \times 10^6)$	$9.68 \times 10^5 (2.79 \times 10^5)$	1.79×10^6
HSD§	1.38×10^6	1.57×10^5	2.09×10^5		1.57×10^6	5.52×10^6	1.24×10^6	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 15. Quantitative polymerase chain reaction values for nitrous oxide reductase gene (*nos*Z) for the 0-15 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 0-15 cm	**Nitrous oxide reductase (*nos*Z)**			
	Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§
	Copies g^{-1} soil			
A	$8.20 \times 10^6 (1.23 \times 10^5)$‡	$1.03 \times 10^7 (3.84 \times 10^6)$	$4.03 \times 10^6 (1.16 \times 10^6)$	5.81×10^6
A+CMB	$1.03 \times 10^7 (9.24 \times 10^5)$	$1.39 \times 10^7 (5.06 \times 10^6)$	$3.53 \times 10^7 (8.11 \times 10^6)$	1.39×10^7
SP	$4.64 \times 10^5 (1.65 \times 10^5)$	$2.88 \times 10^6 (2.63 \times 10^5)$	$1.96 \times 10^6 (1.37 \times 10^5)$	6.33×10^5
SP+CMB	$1.54 \times 10^6 (7.67 \times 10^4)$	$7.17 \times 10^6 (6.06 \times 10^5)$	$2.54 \times 10^6 (1.58 \times 10^5)$	1.07×10^6
SP+A	$3.63 \times 10^6 (8.99 \times 10^5)$	$2.20 \times 10^6 (4.08 \times 10^5)$	$5.68 \times 10^6 (1.19 \times 10^6)$	2.23×10^6
SP+A+CMB	$4.80 \times 10^6 (3.63 \times 10^5)$	$7.21 \times 10^6 (8.54 \times 10^5)$	$8.46 \times 10^6 (9.38 \times 10^5)$	2.07×10^6
TDSS	$2.92 \times 10^4 (5.95 \times 10^3)$	$5.77 \times 10^5 (3.02 \times 10^4)$	$2.86 \times 10^5 (8.79 \times 10^4)$	1.26×10^5
TDSS+CMB	$3.14 \times 10^4 (9.90 \times 10^3)$	$8.49 \times 10^5 (7.92 \times 10^4)$	$6.52 \times 10^5 (3.44 \times 10^5)$	4.64×10^5
TDSS+A	$3.82 \times 10^6 (7.28 \times 10^5)$	$2.80 \times 10^6 (3.98 \times 10^5)$	$3.61 \times 10^6 (7.14 \times 10^5)$	1.58×10^6
TDSS+A+CMB	$2.26 \times 10^6 (2.67 \times 10^5)$	$4.60 \times 10^6 (3.40 \times 10^5)$	$4.56 \times 10^6 (7.94 \times 10^5)$	1.29×10^6
HSD§	1.43×10^6	6.61×10^6	1.09×10^7	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 16. Quantitative polymerase chain reaction values for nitrous oxide reductase gene (*nos*Z) for the 15-30 cm of each treatment from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 15-30 cm	**Nitrous oxide reductase (*nos*Z)**			
	Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§
	Copies g^{-1} soil			
A	8.54 x 10^6(2.45 x 10^5)‡	8.34 x 10^6(1.83 x 10^6)	2.35 x 10^7(5.73 x 10^6)	4.46 x 10^7
A+CMB	6.77 x 10^6(9.22 x 10^5)	1.19 x 10^7(1.85 x 10^6)	1.45 x 10^7(2.29 x 10^6)	4.46 x 10^6
SP	1.03 x10^6(1.31 x 10^5)	7.47 x 10^5(2.61 x 10^5)	1.71 x 10^6(9.80 x 10^5)	1.48 x 10^6
SP+CMB	1.55 x10^6(1.73 x 10^5)	2.35 x 10^6(1.16 x 10^6)	2.33 x 10^6(1.61 x 10^6)	2.88 x 10^6
SP+A	4.04 x10^6(5.48 x 10^5)	4.64 x 10^6(5.83 x 10^5)	3.20 x 10^6(5.12 x 10^5)	1.57 x 10^6
SP+A+CMB	6.31 x10^6(1.21 x 10^6)	3.10 x 10^6(1.07 x 10^6)	2.43 x 10^6(3.11 x 10^5)	2.38 x 10^6
TDSS	2.11 x 10^4(2.39 x 10^3)	3.21 x 10^5(8.49 x 10^4)	4.75 x 10^5(6.13 x 10^4)	1.73 x 10^5
TDSS+CMB	3.39 x 10^4(1.67 x 10^4)	9.61 x 10^5(4.67 x 10^5)	1.05 x 10^6(0.0)	1.24 x 10^6
TDSS+A	1.67 x10^6(2.75 x 10^5)	3.92 x 10^6(1.35 x 10^6)	2.83 x 10^6(1.10 x 10^6)	2.55 x 10^6
TDSS+A+CMB	1.80 x10^6(7.91 x 10^4)	3.32 x 10^6(2.17 x 10^5)	2.27 x 10^6(1.20 x 10^6)	1.77 x 10^6
HSD§	1.58 x10^6	3.14 x 10^6	7.56 x 10^6	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 17. Active carbon and potentially mineralizable nitrogen for 0-15 cm treatments from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 0-15 cm	Active Carbon				Potentially Mineralizable Nitrogen			
	Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD
	mg C kg^{-1} soil				mg NH_4^+-N kg^{-1} soil 7 d^{-1}			
A	359(10.2)‡	417(36.2)	269(1.7)	54.5	5.8(0.8)	0.8(0.6)	2.8(0.5)	1.6
A+CMB	406(16.7)	554(69.5)	330(23.7)	109	5.4(1.0)	1.8(0.7)	3.4(1.0)	2.3
SP	161(13.2)	156(14.4)	99.8(42.5)	67.7	0.0(0.0)	0.0(0.0)	0.2(0.3)	0.4
SP+CMB	119(13.8)	164(75.8)	91.8(23.7)	143	0.0(0.0)	0.0(0.0)	0.2(0.1)	0.2
SP+A	285(50.1)	321(28.1)	256(8.4)	83.9	4.0(1.0)	0.5(0.1)	0.9(0.5)	1.7
SP+A+CMB	308(13.5)	399(121.5)	253(10.6)	178	4.4(0.7)	0.8(0.5)	1.6(0.5)	1.4
TDSS	197(48.6)	230(33.8)	132(29.8)	112	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0
TDSS+CMB	198(21.2)	310(50.3)	180(15.9)	82.2	0.1(0.1)	0.0(0.0)	0.1(0.1)	0.2
TDSS+A	280(10.5)	395(63.3)	219(20.6)	97.5	1.9(0.1)	0.2(0.0)	0.4(0.1)	0.3
TDSS+A+CMB	278(20.2)	453(108.9)	206(77.0)	195	2.0(0.2)	0.1(0.0)	1.0(0.9)	1.3
HSD§	75.2	209	98.3		1.7	0.9	1.5	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TDSS is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

Table 18. Active carbon and potentially mineralizable nitrogen for 15-30 cm treatments from soil collected in Fall 2015, Summer 2016, and Fall 2016.

Treatment† 15-30 cm	Active Carbon				Potentially Mineralizable Nitrogen			
	Time				Time			
	Fall 2015¶	Summer 2016	Fall 2016	HSD§	Fall 2015	Summer 2016	Fall 2016	HSD
	mg C kg^{-1} soil				mg NH_4^+-N kg^{-1} soil 7 d^{-1}			
A	420(19.8)‡	402(7.2)	250(21.3)	43.3	7.1(0.6)	1.9(0.6)	1.7(0.8)	1.7
A+CMB	396(65.9)	580(153.8)	250(25.0)	245	6.9(1.9)	1.1(0.2)	1.9(0.4)	2.8
SP	148(4.9)	148(18.0)	119(18.5)	38.0	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0
SP+CMB	111(31.0)	130(68.3)	97(2.9)	109	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0
SP+A	292(102.4)	266(83.9)	239(18.6)	193	6.4(1.0)	1.0(1.3)	0.9(0.5)	2.5
SP+A+CMB	250(15.4)	340(83.0)	148(23.1)	127	6.4(1.8)	0.9(0.7)	0.7(0.3)	2.8
TDSS	243(1.0)	212(12.9)	149(54.7)	74.1	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0
TDSS+CMB	196(24.5)	224(33.1)	107(24.2)	69.0	0.1(0.1)	0.0(0.0)	0.0(0.0)	0.1
TDSS+A	257(27.3)	314(27.3)	181(26.6)	67.9	3.3(0.5)	0.1(0.1)	0.1(0.1)	0.7
TDSS+A+CMB	280(93.0)	430(109.4)	170(25.3)	211	3.8(0.5)	0.2(0.1)	0.2(0.1)	0.8
HSD§	149	217	73.3		2.7	1.5	1.0	

†; A is native topsoil, SP is crude oil-contaminated soil from the stockpile at an active remediation site, TD is thermal desorbed-treated subsoil and CMB is composted manure-bedding.

‡; Values in parenthesis indicate standard deviation

§; HSD; Tukey's Honest Significant Difference at α=0.05

¶; Fall 2015 is November 15, 2015; Summer 2016 is June 15, 2015; and Fall 2016 is October 3, 2016

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GENERAL CONCLUSIONS

Remediation success can be quantified by a variety of parameters with different soil properties of more importance depending on land use. However, to promote soil health, physical, biological, and chemical properties should be measured to determine soil functionality and productivity. The impact of TD has been quantified in both chemical and biological aspects. Significant differences were observed in Gibbs free energies between the untreated and thermally desorbed treatments for all three exchanges and cation exchange values for some of the exchanges. In the Ca-Mg exchange, both the untreated and TD topsoil preferred Ca, whereas both subsoils favored Mg. For the Ca-K and Mg-K exchanges, all treatments preferred K. However, the magnitude of difference may not require alternative fertility management to retain previous soil productivity. Furthermore, N-transforming microorganisms, measured by enzyme activities and gene quantification, were lower in contaminated and TD-treated soil as compared to uncontaminated soil, thus the possibility of consequences for soil functioning. The 1:1 blended soils reached levels that have been reported in the literature and thus additions of uncontaminated topsoil may be a viable option for reclamation. Overall, TD treatment significantly affected selected chemical and biological parameters; therefore, these possibilities should be accounted for when using TD-treated soil for agricultural production.

APPENDIX. DEPTH COMPARISONS

Table A1. Tukey's Honest Significant Difference at α=0.05 comparison between 0-15 and 15-30 depths for Fall 2016.

Treatment	Depth	Urease	Ammonium oxidation	Nitrate reductase	Ammonia-oxidizing archaea (AOA)	Ammonia-oxidizing bacteria (AOB)	Nitrous oxide reductase (*nosZ*)	Active C	Potentially mineralizable N
	cm	μg NH_4-N g^{-1} soil	μg NO_2-N g^{-1} soil 5 h^{-1}	μg NO_2-N g^{-1} soil 24 h^{-1}	Copies g^{-1} soil			mg C kg^{-1} soil	mg NH_4^+-N kg^{-1} soil 7 d^{-1}
A	0-15	33.5(3.9)	2.5(0.5)	1.8(0.1)a	$4.21 \times 10^5(4.59 \times 10^4)$	$9.96 \times 10^6(1.30 \times 10^6)$a	$4.03 \times 10^6(1.16 \times 10^6)$b	269(1.7)	2.8(0.5)
	15-30	40.6(11.5)	1.6(0.3)	1.0(0.2)b	$5.84 \times 10^5(1.32 \times 10^5)$	$3.97 \times 10^6(7.08 \times 10^5)$b	$2.35 \times 10^7(5.73 \times 10^6)$a	250(21.3)	1.7(0.8)
A+CMB	0-15	34.1(4.0)	2.3(0.2)	2.2(0.1)a	$5.06 \times 10^5(6.25 \times 10^4)$	$9.96 \times 10^6(1.71 \times 10^6)$a	$3.53 \times 10^7(8.11 \times 10^6)$a	330(23.7)a	3.4(1.0)
	15-30	34.2(2.2)	1.8(0.3)	0.7(0.1)b	$5.06 \times 10^5(7.33 \times 10^4)$	$6.55 \times 10^6(6.59 \times 10^5)$b	$1.45 \times 10^7(2.29 \times 10^6)$b	250(25.0)b	1.9(0.4)
SP	0-15	3.1(0.2)	0.3(0.1)a	0.6(0.1)a	$9.14 \times 10^4(1.59 \times 10^4)$	$2.92 \times 10^6(3.61 \times 10^6)$	$1.96 \times 10^6(1.37 \times 10^5)$	99.8(42.5)	0.2(0.3)
	15-30	4.9(3.6)	0.0(0.0)b	0.2(0.0)b	$1.22 \times 10^5(4.11 \times 10^4)$	$1.41 \times 10^4(1.01 \times 10^3)$	$1.71 \times 10^6(9.80 \times 10^5)$	119(18.5)	0.0(0.0)
SP+CMB	0-15	6.2(1.2)	0.5(0.1)a	0.5(0.1)	$1.33 \times 10^5(7.82 \times 10^4)$	$3.98 \times 10^6(2.65 \times 10^6)$	$2.54 \times 10^6(1.58 \times 10^5)$	91.8(23.7)	0.2(0.1)
	15-30	4.2(1.0)	0.0(0.0)b	0.3(0.1)	$4.53 \times 10^4(1.88 \times 10^3)$	$5.38 \times 10^3(9.46 \times 10^2)$	$2.33 \times 10^6(1.61 \times 10^6)$	97(2.9)	0.0(0.0)
SP+A	0-15	18.8(3.3)	1.1(0.1)a	1.4(0.2)	$2.76 \times 10^5(8.33 \times 10^4)$	$8.27 \times 10^6(4.64 \times 10^5)$a	$5.68 \times 10^6(1.19 \times 10^6)$a	256(8.4)	0.9(0.5)
	15-30	20.9(6.0)	0.6(0.1)b	0.8(0.3)	$2.23 \times 10^5(8.35 \times 10^4)$	$1.42 \times 10^6(3.72 \times 10^5)$b	$3.20 \times 10^6(5.12 \times 10^5)$b	239(18.6)	0.9(0.5)
SP+A+CMB	0-15	21.5(6.3)	1.4(0.5)	0.9(0.1)	$3.00 \times 10^5(2.97 \times 10^4)$a	$4.20 \times 10^6(7.35 \times 10^5)$a	$8.46 \times 10^6(9.38 \times 10^5)$a	253(10.6)a	1.6(0.5)a
	15-30	19.5(1.8)	0.2(0.0)	1.2(0.3)	$1.70 \times 10^5(2.12 \times 10^4)$b	$1.15 \times 10^6(3.99 \times 10^5)$b	$2.43 \times 10^6(3.11 \times 10^5)$b	148(23.1)b	0.7(0.3)b
TDSS	0-15	1.0(0.9)	0.5(0.2)	0.2(0.1)a	$3.07 \times 10^4(4.14 \times 10^3)$	$1.25 \times 10^5(1.58 \times 10^5)$	$2.86 \times 10^5(8.79 \times 10^4)$	132(29.8)	0.0(0.0)
	15-30	0.3(0.1)	0.0(0.0)	0.0(0.0)b	$2.07 \times 10^4(5.18 \times 10^3)$	$5.14 \times 10^4(6.34 \times 10^4)$	$4.75 \times 10^5(6.13 \times 10^4)$	149(54.7)	0.0(0.0)
TDSS+CMB	0-15	5.7(0.8)a†	0.8(0.2)a	0.2(0.2)	$1.01 \times 10^5(4.06 \times 10^4)$	$1.50 \times 10^6(2.18 \times 10^5)$a	$6.52 \times 10^5(3.44 \times 10^5)$	180(15.9)a	0.1(0.1)
	15-30	0.0(0.0)b	0.2(0.1)b	0.1(0.0)	$8.57 \times 10^4(4.74 \times 10^4)$	$3.06 \times 10^5(2.25 \times 10^5)$b	$1.05 \times 10^6(0.0)$	107(24.2)b	0.0(0.0)
TDSS+A	0-15	15.2(0.6)b	1.4(0.1)a	0.9(0.2)	$2.08 \times 10^5(5.11 \times 10^4)$	$4.78 \times 10^6(2.10 \times 10^6)$	$3.61 \times 10^6(7.14 \times 10^5)$	219(20.6)	0.4(0.1)
	15-30	19.2(1.6)a	0.5(0.2)b	0.6(0.1)	$3.35 \times 10^5(1.10 \times 10^5)$	$2.44 \times 10^6(6.92 \times 10^5)$	$2.83 \times 10^6(1.10 \times 10^6)$	181(26.6)	0.1(0.1)
TDSS+A+CMB	0-15	18.7(0.8)a	1.3(0.3)a	1.6(0.2)a	$2.11 \times 10^5(1.33 \times 10^5)$	$4.74 \times 10^6(3.92 \times 10^6)$	$4.56 \times 10^6(7.94 \times 10^5)$	206(77.0)	1.0(0.9)
	15-30	14.2(.7)b	0.6(0.1)b	0.5(0.2)b	$1.33 \times 10^5(7.89 \times 10^4)$	$9.68 \times 10^5(2.79 \times 10^5)$	$2.27 \times 10^6(1.20 \times 10^6)$	170(25.3)	0.2(0.1)

†; Different letters indicate significant difference (α=0.05) between 0-15 and 15-30 cm depths within treatment. Only significant differences are noted.

Table A2. Tukey's Honest Significant Difference at α=0.05 comparison between 0-15 and 15-30 depths for Summer 2016.

Treatment	Depth	Urease	Ammonium oxidation	Nitrate reductase	Ammonia-oxidizing archaea (AOA)	Ammonia-oxidizing bacteria (AOB)	Nitrous oxide reductase (*nos*Z)	Active C	Potentially mineralizable N
	cm	μg NH_4-N g^{-1} soil	μg NO_2-N g^{-1} soil 5 h^{-1}	μg NO_2-N g^{-1} soil 24 h^{-1}	Copies g^{-1} soil			mg C kg^{-1} soil	mg NH_4^+-N kg^{-1} soil 7 d^{-1}
A	0-15	53.8(7.5)	3.0(0.3)a	2.0(0.4)	$4.24 \times 10^5(1.62 \times 10^5)$	$1.54 \times 10^7(1.89 \times 10^6)$	$1.03 \times 10^7(3.84 \times 10^6)$	417(36.2)	0.8(0.6)
	15-30	53.4(3.0)	1.7(0.7)b	2.0(0.2)	$4.37 \times 10^5(1.23 \times 10^5)$	$7.93 \times 10^6(4.93 \times 10^6)$	$8.34 \times 10^6(1.83 \times 10^6)$	402(7.2)	1.9(0.6)
A+CMB	0-15	59.1(1.6)	3.2(1.5)	1.9(0.1)	$4.07 \times 10^5(1.05 \times 10^5)$	$2.30 \times 10^7(1.12 \times 10^6)$a	$1.39 \times 10^7(5.06x\ 10^6)$	554(69.5)	1.8(0.7)
	15-30	51.2(2.2)	1.7(0.2)	2.4(0.5)	$3.93 \times 10^5(8.72 \times 10^4)$	$1.21 \times 10^7(2.49 \times 10^6)$b	$1.19 \times 10^7(1.85 \times 10^6)$	580(153.8)	1.1(0.2)
SP	0-15	3.9(2.7)	1.4(0.4)a	0.3(0.3)	$3.58 \times 10^4(1.31 \times 10^4)$a	$1.18 \times 10^6(2.58 \times 10^5)$a	$2.88 \times 10^6(2.63 \times 10^5)$a	156(14.4)	0.0(0.0)
	15-30	2.9(0.9)	0.1(0.1)b	0.6(0.5)	$1.36 \times 10^4(4.18 \times 10^3)$b	$4.57 \times 10^3(2.98 \times 10^2)$b	$7.47 \times 10^5(2.61 \times 10^5)$b	148(18.0)	0.0(0.0)
SP+CMB	0-15	3.8(2.4)	0.5(0.2)a	0.7(0.2)	$1.02 \times 10^5(5.44 \times 10^4)$	$2.13 \times 10^6(4.69 \times 10^5)$a	$7.17 \times 10^6(6.06 \times 10^5)$a	164(75.8)	0.0(0.0)
	15-30	3.1(0.9)	0.0(0.0)b	0.7(0.2)	$3.63 \times 10^4(7.68 \times 10^3)$	$7.89 \times 10^5(2.15 \times 10^5)$b	$2.35 \times 10^6(1.16 \times 10^6)$b	130(68.3)	0.0(0.0)
SP+A	0-15	33.9(5.2)a	2.2(0.6)a	1.1(0.1)b	$9.30 \times 10^4(5.72 \times 10^4)$	$1.21 \times 10^7(6.07 \times 10^5)$a	$2.20 \times 10^6(4.08 \times 10^5)$b	321(28.1)	0.5(0.1)
	15-30	23.7(2.8)b	0.6(0.1)b	1.5(0.2)a	$1.42 \times 10^5(3.24 \times 10^4)$	$1.53 \times 10^6(4.38 \times 10^5)$b	$4.64 \times 10^6(5.83 \times 10^5)$a	266(83.9)	1.0(1.3)
SP+A+CMB	0-15	29.3(5.3)a	3.2(0.7)a	1.0(0.2)	$2.19 \times 10^5(6.47 \times 10^4)$	$1.99 \times 10^7(2.62 \times 10^6)$a	$7.21 \times 10^6(8.54 \times 10^5)$a	399(121.5)	0.8(0.5)
	15-30	14.4(1.3)b	0.4(0.1)b	1.4(0.4)	$1.42 \times 10^5(5.09 \times 10^4)$	$1.77 \times 10^6(9.21 \times 10^5)$b	$3.10 \times 10^6(1.07 \times 10^6)$b	340(83.0)	0.9(0.7)
TDSS	0-15	2.0(1.8)	2.2(0.5)a	0.1(0.1)	$3.10 \times 10^4(2.60 \times 10^4)$	$6.73 \times 10^5(1.12 \times 10^5)$a	$5.77 \times 10^5(3.02 \times 10^4)$a	230(33.8)	0.0(0.0)
	15-30	0.1(0.1)	0.6(0.3)b	0.0(0.0)	$2.01 \times 10^4(5.16 \times 10^3)$	$2.70 \times 10^5(5.96 \times 10^4)$b	$3.21 \times 10^5(8.49 \times 10^4)$b	212(12.9)	0.0(0.0)
TDSS+CMB	0-15	1.4(0.6)a	2.1(0.1)a	0.1(0.1)	$1.61 \times 10^4(8.48 \times 10^3)$	$1.60 \times 10^6(5.61 \times 10^5)$a	$8.49 \times 10^5(7.92 \times 10^4)$	310(50.3)	0.0(0.0)
	15-30	0.2(0.1)b	0.6(0.4)b	0.1(0.0)	$1.46 \times 10^4(1.17 \times 10^4)$	$6.21 \times 10^4(1.55 \times 10^4)$b	$9.61 \times 10^5(4.67 \times 10^5)$	224(33.1)	0.0(0.0)
TDSS+A	0-15	24.2(2.1)	1.4(0.2)a	0.8(0.2)	$1.01 \times 10^5(3.45 \times 10^4)$	$7.09 \times 10^6(5.48 \times 10^5)$a	$2.80 \times 10^6(3.98 \times 10^5)$	395(63.3)	0.2(0.0)a
	15-30	24.6(3.2)	0.6(0.1)b	0.8(0.3)	$1.54 \times 10^5(4.24 \times 10^4)$	$3.15 \times 10^6(3.36 \times 10^5)$b	$3.92 \times 10^6(1.35 \times 10^6)$	314(27.3)	0.1(0.1)b
TDSS+A+CMB	0-15	27.8(0.7)a	2.0(0.6)a	0.5(0.1)	$2.04 \times 10^5(8.36 \times 10^4)$	$1.46 \times 10^7(1.58 \times 10^6)$a	$4.60 \times 10^6(3.40 \times 10^5)$a	453(108.9)	0.1(0.0)
	15-30	20.1(1.8)b	0.7(0.2)b	0.8(0.2)	$1.28 \times 10^5(3.41 \times 10^4)$	$1.80 \times 10^6(1.15 \times 10^6)$b	$3.32 \times 10^6(2.17 \times 10^5)$b	430(109.4)	0.2(0.1)

†; Different letters indicate significant difference (α=0.05) between 0-15 and 15-30 cm depths within treatment. Only significant differences are noted.

Table A3. Tukey's Honest Significant Difference at α=0.05 comparison between 0-15 and 15-30 depths for Fall 2015.

Treatment	Depth	Urease	Ammonium oxidation	Nitrate reductase	Ammonia-oxidizing archaea (AOA)	Ammonia-oxidizing bacteria (AOB)	Nitrous oxide reductase (*nos*Z)	Active C	Potentially mineralizable N
	cm	μg NH_4-N g^{-1} soil	μg NO_2-N g^{-1} soil 5 h^{-1}	μg NO_2-N g^{-1} soil 24 h^{-1}	Copies g^{-1} soil			mg C kg^{-1} soil	mg NH_4^+-N kg^{-1} soil 7 d^{-1}
A	0-15	44.8(5.7)	1.8(0.4)	3.5(1.1)	$1.17 \times 10^6(1.05 \times 10^5)$	$2.28 \times 10^6(5.99 \times 10^5)$	$8.20 \times 10^6(1.23 \times 10^5)$	359(10.2)b	5.8(0.8)
	15-30	42.9(13.9)	2.0(0.3)	3.4(0.7)	$1.83 \times 10^6(1.03 \times 10^6)$	$2.12 \times 10^6(5.93 \times 10^5)$	$8.54 \times 10^6(2.45 \times 10^5)$	420(19.8)a	7.1(0.6)
A+CMB	0-15	67.2(10.2)	2.1(0.0)	2.7(0.5)	$2.68 \times 10^6(5.58 \times 10^5)$	$4.37 \times 10^6(1.68 \times 10^6)$	$1.03 \times 10^7(9.24 \times 10^5)$a	406(16.7)	5.4(1.0)
	15-30	67.4(15.7)	1.8(0.2)	4.2(1.0)	$2.65 \times 10^6(3.36 \times 10^5)$	$4.02 \times 10^6(1.44 \times 10^6)$	$6.77 \times 10^6(9.22 \times 10^5)$b	396(65.9)	6.9(1.9)
SP	0-15	5.8(1.2)a	0.0(0.0)	0.3(0.1)	$1.64 \times 10^5(1.21 \times 10^5)$	$1.20 \times 10^5(1.28 \times 10^5)$	$4.64 \times 10^5(1.65 \times 10^5)$b	161(13.2)	0.0(0.0)
	15-30	1.8(0.8)b	0.0(0.0)	0.4(0.2)	$3.61 \times 10^5(1.11 \times 10^5)$	$2.94 \times 10^4(4.64 \times 10^4)$	$1.03 \times 10^6(1.31 \times 10^5)$a	148(4.9)	0.0(0.0)
SP+CMB	0-15	2.7(2.3)	0.0(0.0)	0.4(0.1)	$4.20 \times 10^5(3.06 \times 10^5)$	$5.96 \times 10^4(3.27 \times 10^4)$	$1.54 \times 10^6(7.67 \times 10^4)$	119(13.8)	0.0(0.0)
	15-30	5.5(1.5)	0.0(0.0)	0.7(0.2)	$1.12 \times 10^5(9.59 \times 10^4)$	$2.06 \times 10^4(9.09 \times 10^3)$	$1.55 \times 10^6(1.73 \times 10^5)$	111(31.0)	0.0(0.0)
SP+A	0-15	20.6(5.5)	0.5(0.0)	3.2(0.9)b	$2.88 \times 10^5(8.93 \times 10^4)$	$6.48 \times 10^5(8.37 \times 10^4)$	$3.63 \times 10^6(8.99 \times 10^5)$	285(50.1)	4.0(1.0)b
	15-30	37.2(14.1)	0.5(0.2)	5.7(0.9)a	$8.11 \times 10^5(4.75 \times 10^5)$	$8.04 \times 10^5(5.87 \times 10^5)$	$4.04 \times 10^6(5.48 \times 10^5)$	292(102.4)	6.4(1.0)a
SP+A+CMB	0-15	24.5(4.6)	0.5(0.1)	3.5(1.1)	$7.21 \times 10^5(2.55 \times 10^4)$	$7.81 \times 10^5(5.67 \times 10^4)$b	$4.80 \times 10^6(3.63 \times 10^5)$	308(13.5)a	4.4(0.7)
	15-30	24.5(1.1)	0.5(0.2)	5.9(1.6)	$1.24 \times 10^6(5.43 \times 10^5)$	$1.30 \times 10^6(3.11 \times 10^4)$a	$6.31 \times 10^6(1.21 \times 10^6)$	250(15.4)b	6.4(1.8)
TDSS	0-15	0.2(0.4)	0.1(0.1)	0.2(0.1)	$1.12 \times 10^5(6.74 \times 10^3)$	$2.64 \times 10^3(1.09 \times 10^3)$	$2.92 \times 10^4(5.95 \times 10^3)$	197(48.6)	0.0(0.0)
	15-30	0.7(0.7)	0.0(0.0)	0.0(0.0)	$2.14 \times 10^5(1.57 \times 10^5)$	$2.12 \times 10^3(6.88 \times 10^2)$	$2.11 \times 10^4(2.39 \times 10^3)$	243(1.0)	0.0(0.0)
TDSS+CMB	0-15	4.4(0.5)a	0.1(0.1)	0.2(0.2)	$8.80 \times 10^4(5.28 \times 10^4)$	$2.25 \times 10^3(3.76 \times 10^2)$b	$3.14 \times 10^4(9.90 \times 10^3)$	198(21.2)	0.1(0.1)
	15-30	0.7(0.8)b	0.0(0.0)	0.0(0.0)	$1.69 \times 10^5(1.47 \times 10^5)$	$4.93 \times 10^3(9.80 \times 10^2)$a	$3.39 \times 10^4(1.67 \times 10^4)$	196(24.5)	0.0(0.1)
TDSS+A	0-15	14.6(5.1)	1.0(0.1)	1.7(0.6)	$3.96 \times 10^5(1.45 \times 10^5)$	$1.43 \times 10^6(3.89 \times 10^4)$	$3.82 \times 10^6(7.28 \times 10^5)$a	280(10.5)	1.9(0.1)b
	15-30	19.3(1.2)	0.9(0.1)	2.4(0.4)	$6.55 \times 10^5(1.50 \times 10^5)$	$1.57 \times 10^6(1.74 \times 10^5)$	$1.67 \times 10^6(2.75 \times 10^5)$b	257(27.3)	3.3(0.5)a
TDSS+A+CMB	0-15	21.8(5.0)	0.8(0.2)	1.3(0.2)b	$6.36 \times 10^5(6.07 \times 10^5)$	$7.96 \times 10^5(8.80 \times 10^4)$	$2.26 \times 10^6(2.67 \times 10^5)$a	278(20.2)	2.0(0.2)b
	15-30	15.7(2.8)	0.6(0.1)	2.2(0.2)a	$3.92 \times 10^5(2.24 \times 10^5)$	$1.25 \times 10^6(3.52 \times 10^5)$	$1.80 \times 10^6(7.91 \times 10^4)$b	280(93.0)	3.8(0.5)a

†; Different letters indicate significant difference (α=0.05) between 0-15 and 15-30 cm depths within treatment. Only significant differences are noted.