ASSESSING SOIL PHYSICOCHEMICAL PROPERTIES AND SOIL ORGANIC MATTER STABILITY ACROSS A GRASSLAND-CROPLAND EDGE AT AN ECOTONE IN SASKATCHEWAN

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ABSTRACT

Farming production may move northward since climate change is making the south warmer and drier. The expansion of agricultural land impacts several properties and functions of native soils due to changes in land use management and vegetation covers. The current edge between native land and cropland that exists within the prairie-forest ecotone might be the first area to be impacted by changes in both land use management and climatic conditions (i.e., temperature and moisture conditions). Our goal was to find out which land use in this area would be more stable under land use change induced by climatic conditions changes. To achieve our goal, we evaluated physicochemical properties, soil organic matter (SOM) fractionation and biological stability of two main aggregate size fractions (> 2000 μm, and 150–2000 μm) of soils across a grassland-cropland edge in central Saskatchewan, Canada. Additionally, we examined changes in SOM stability of these soils under different temperature and moisture conditions. Our result showed that both land use and soil depth were primary factors controlling soil physiochemical properties. The amount of TOC, TC, TN, and OC:TN ratio gradually declined from grassland to cropland in topsoil (0–12 cm). Since SOM light fraction (LF) is decomposed faster than heavy fraction (HF), HF had significantly higher mass, C and N contents, and C:N ratio compared to LF. Among land uses, cropland had the highest HF mass within two main aggregate size fractions, while all land uses had similar amount of LF mass. Furthermore, the proportion of decomposable C within two main aggregate size fractions of grassland was higher than that of cropland, but the large aggregate size fraction of cropland mineralized more N per g TN than that of grassland. As for SOM stability under different climatic conditions, soils from all land uses mineralized more C and N at higher temperatures regardless of moisture conditions. Besides temperature, land use was another main factor influencing C mineralization, but soil moisture was the other main factor affecting N mineralization. Our results indicated that cropland was more stable than the edge and grassland in terms of C mineralization, but less stable in terms of N mineralization. In addition, the edge was more sensitive to climactic conditions than other land uses in terms of N mineralization. Understanding soil physiochemical properties, SOM stabilization and fractionation, and how these properties of soil might react to land use management change induced by climate change would aid in developing sustainable management which brings benefits to both the producers and the environment.

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List of Abbreviations

C	Carbon
DON	Dissolved Organic Nitrogen
HF	Heavy Fraction
LF	Light Fraction
MAT	Mean annual temperature
N	Nitrogen
OC	Organic Carbon
OFL	Occluded Light Fraction
SD	Standard Deviation
SDNWA	St. Denis National Wildlife Area
SOC	Soil Organic Carbon
SOM	Soil Organic Matter

1. INTRODUCTION

Due to food security problems created by the rapid growth of global populations and climate change, crop production has been increasing, and agriculture land has also expanded (FAO, 2009). The intensification of cultivation has created many environmental issues related to ecosystem functions and services provided by soils including reducing water quality and quantity, creating more greenhouse gases, altering biodiversity, and most importantly reducing the resiliency of the soil itself (Conrstanje, 2015). Soil resilience is the ability of soil to resist or recover from stresses and disturbances from both natural phenomena and human activities (Lal et al., 1997; Seybold et al., 1998). With an appropriate land use and crop management, soil can slowly restore some degraded functions and demonstrate resiliency (Conrstanje, 2015). But with intensive and inappropriate land use management, soil resilience can rapidly decline, and soil can become more degraded (Power, 2010). Hence, it is important to study soil resilience to help development of sustainable soil management to ensure adequate maintenance of soil properties and functions.

Land use change induced by climate change and food security concerns can alter soil resilience by disturbing soil structure and soil microbial communities (Yannikos et al., 2014; Davidson and Jassens, 2016), and cause significant decline in soil properties (Lal et al., 1997). In the prairies of Saskatchewan, increasing cultivation activities on a hummocky landscape has increased both water and wind erosion, leading to the loss of soil organic matter (SOM) and many essential soil nutrients (Ellert and Gregorich, 1996). In addition, mechanical tillage in agriculture can also enhance wind and water erosion by disrupting soil structure, and increasing soil susceptibility to erosion (Brye and Pirani, 2004). The frequent use of heavy farm equipment in cultivation also causes an increase in bulk density of soil (Brye and Pirani, 2004; Rosenzweig et al., 2016; Hebb et al., 2017; Cade-Menun et al., 2017). Since the change in soil properties could reflect the change in soil resilience (Lal et al., 1997), studying soil properties of different land use managements could help predict how soil functions would respond to land use change in the future.

In the Canadian prairie, the hotter and drier conditions created by climate change will drive agriculture to expand northward (Barrow, 2009), leading to a rapid change in dominant vegetation covers and land use managements. Since the prairie-forest ecotone in the Canadian prairie is a particular sensitive area to environmental changes (William et al., 2009), soils in this region will be impacted by land use change induced by climate change. Especially, the anthropogenic edge between cropland and native lands that exist within the prairie-forest ecotone may be the first area to show changes. Clearly, there is a need to evaluate soil resilience in this area, and how this ability of soil reacts to the warming climate. There are many studies that have evaluated the differences in soil properties, especially SOM between land uses (Wang et al., 2000; Baskan et al., 2015), and SOM loss due to cultivation (Ellert and Gregorich, 1996; McGill et al, 1998; and Smith et al., 2015). But there have not been many studies using SOM stabilization within different aggregate sizes, and SOM stabilization under different climatic conditions to predict soil adaptability to land use change associated with climate change.

As an important indicator of soil resilience and soil quality (Girvan et al., 2005), SOM has showed rapid change due to the intensification of agriculture (Yannikos et al., 2014). Soil organic C loss in Canada has been estimated to range from 25 – 35% due to cultivation (Ellert and Gregorich, 1996; McGill et al., 1998; and Smith et al., 2000). Since soil contains more organic carbon (OC) than atmosphere and vegetation combined, the increase of agricultural land and practices could release a large amount of CO_2, contributing toward climate change (Liang et al., 2017). In the US, the conversion to cropland alone has transferred 993 Tg of C to the atmospheric as CO_2 (Rosenzweig et al., 2016). Evaluating SOM stabilization, decomposition, and fractionation in different land uses is essential to help maintain an adequate amount of SOM to sustain soil resilience (Lal et al., 1997), soil quality, and agricultural productivity (Malhi et al, 2003).

There are many factors that can alter SOM decomposition including climate, and land use managements. Temperature, and moisture conditions are the two main climate-dependent factors that control SOM decomposition. Temperature can influence SOM decomposition processes including root respiration and microbial decomposition, as well as the rate of SOM stabilization process, and the adsorption of SOM to mineral surfaces (Paré et al., 2006). Moisture can alter substrate availability for SOM decomposition through controlling water access for the main processes of SOM decomposition, changing water films thickness, and regulating oxygen diffusion (Davidson and Janssens, 2006). Most studies have focused on the effects of temperature on SOM decomposition, a few has looked at water conditions, and not many have evaluated the combined effect of both. Some studies have also observed a difference in SOM mineralization due to the differences in vegetation type (Paré et al., 2006). The composition and activity of soil microbial community can be impacted by the quality and quantity of C input, which depend on the type of vegetation cover (Raich and Tufekcioglu, 2000). Differences in soil structure of different land uses also contribute to altering SOM mineralization (Six et al., 2002). Intense physical disturbance of cultivation has disrupted soil structure, and released SOM protected within soil aggregates to soil microbial communities (Besnard et al., 1996; Hebb et al., 2017). Different aggregate sizes provide different potential for SOM stabilization (Rabbi et al., 2014). Soil organic matter in smaller size aggregates was reported to be more stable, and degrade slower than SOM in larger aggregates (Rabbi et al., 2014). The breakdown of large aggregates size was reported to have a close relationship to SOM loss due to cultivation (Tisdall and Oades, 1982; Akinsete and Nortcliff, 2014). Previous studies on soil aggregation and SOM have focused on the impact of different cultivation practices, studies about SOM stabilization within different aggregate size fractions of different land use managements remain rare.

Our objective is to explore the question: at an edge between land uses, which soil would be more stable under land use change induce by climatic conditions change (i.e., changing temperature and moisture conditions)? We evaluated soil properties, SOM fractionation and biological stability within different aggregate size fractions, and examined changes in the stability of SOM under different climatic conditions along an edge between grassland and cropland at St. Denis National Wildlife Area (East of Saskatoon, Saskatchewan), which is located within the eco-regions of Aspen Parkland and Moisture Mixed Grass.

This thesis is written in manuscript-type format with 6 chapters. Chapter 1 is an overall introduction, followed by a literature review in chapter 2. Chapter 3 and 4 are stand-alone manuscripts started with a preface to link the objectives of two chapters together to the overall goal of the thesis. Chapter 3 focuses on characterizing the differences in soil properties along the edge of grassland and cropland, and differences in SOM fractions and stability within different aggregate size fractions, with the aim of connecting these differences to predict the stability of soil. Chapter 4 builds on chapter 3 and goes further to explore the changes of SOM mineralization toward different climatic conditions using incubations with different temperature and moisture. The goal is to link these changes to the biological stability of soils from different land uses across the edge under warming climate. Finally, chapter 5 summarizes the two research chapters (Chapter 3 and 4), and provides an overall conclusion for the thesis, following by the list of references in chapter 6.

Our hypotheses for each chapter are listed below:

Chapter 3

1. Across the grassland-cropland edge, land use and soil depth are two main factors controlling basic soil physicochemical properties (i.e., pH, EC, bulk density, aggregate size distributions, TC, TN, TOC, and OC:TN ratio).
2. In topsoil (0–12 cm), soil properties changes across the edge following a pattern: while bulk density decreases from cropland to grassland, TC, TN, TOC, and OC:TN ratio gradually decline from grassland to cropland.

Chapter 4

1. Across the grassland-cropland edge, C and N mineralization increase with increasing temperature regardless of land use and moisture conditions.
2. Grassland is more stable to changes associated with climate change (i.e., temperature and moisture conditions) than cropland and the edge in terms of SOM decomposition.

2. LITERATURE REVIEW

2.1. Soil resilience

Soils provide many ecosystem functions and services other than crop production, but many stresses and disturbances from natural phenomena (e.g., environmental conditions, erosion) or human activities can impact these ecosystem functions and services (Brye and Pirani, 2004). In order to perform effectively, soil must have an ability to resist or recover from both internal and external pressures, which means soil must be resilient (Lal et al., 1997). Originally, resilience is an ecological concept that was first applied to discuss the degree of changes caused by disturbance and the following recovery in species invasions, later the stability aspect was added to this concept (Pimm, 1984). To help address soil ecology and sustainable land use issues, the term "soil resilience" was introduced to soil science in the 90s, and since then soil resilience has been regarded as a fundamental components of soil quality (Seybold et al., 1998). Soil resilience can be defined as the ability of soil to resist the changes, or to recover from the stresses and disturbances in order to function in an effective way (Lal et al., 1997). Based on the degree of resilience, soils can be grouped into different classes (Lal et al., 1997; Seybold et el., 1998). The most resilient class will have high buffering capacities and high rates of recovery; while the fragile class is unstable, cannot recover fully and may lose some of its specific function in the new equilibrium state (Lal et al., 1997).

Factors that affect soil resilience can be divided into intrinsic factors and extrinsic factors. Intrinsic factors are fundamental soil properties (e.g., texture, soil organic matter (SOM) content, and biological characteristic) (Corstanje, 2015). Soil texture can influence both physical and biological resilience since soil texture holds the structure of soil through aggregation, and can control the environment and SOM resources for microbial activities (Gregory et al., 2007; Corstanje, 2015). Soil organic matter is another important factor that can affect soil resilience. During physical recovery such as compressive recovery, SOM may act as a physical string; while in chemical resilience, SOM holds a buffering capacity for soil (Girvan et al., 2005; Griffiths et al., 2008). In addition, SOM also contributes indirectly to soil resilience through interactions with soil texture, which strongly influences the activities of the soil microbial community. However, the relationships between biological factors and soil resilience are very complicated. It is generally hypothesized that soil with larger, more diverse, and functionally richer microbial communities would be more resilient, but evidence is inconclusive (Corstanje, 2015). On the other hand, extrinsic factors are factors related to soil though landscape aspect (e.g., topography, land use or management) that can affect resilience directly and indirectly. Unlike intrinsic factors, it is difficult to observe the effect of a single extrinsic factor on soil resilience, as they are a combination of several factors, both intrinsic and extrinsic (Corstanje, 2015). For example, tillage practices would break down soil structure, release SOM protected within soil aggregates and lead to more activity of microorganisms (Brye and Pirani, 2004). In addition, there are several processes which influence the ability and rate of soil recovery including new soil formation, aggregation, SOM accumulation, nutrient cycling and transformation, leaching of excess salts, and increases in biodiversity (Lal et al., 1997).

Land use and management have a drastic effect on soil resilience, especially in the context of rapidly increasing soil conversion induced by climate change (Lal et al., 1997). Cultivation is considered one of the sources that creates the greatest stress and disturbance to the environment (Seybold et al., 1998). However, appropriate and sustainable land use and crop management can create favorable effects on soil resilience and restore some functions of degraded soil. Using soil according to its capability can help improve soil structure, soil-water relations, erosion management, SOM content maintenance, soil biodiversity regulation, and nutrient cycling (Lal et al., 1997). In contrast, intensive and inappropriate land uses can reduce soil resilience and lead to soil degradation (Power, 2010). Excessive soil practices with the frequent use of heavy farm equipment, and large input of chemical fertilizers can accumulate soil specific constraints, increase ecological stresses, decreases biomass production, and reduce soil quality (Lal et al., 1997). Combining with stresses and pressures caused by climate change, soil can be degraded and lose its functions completely (Corstanje, 2015). Thus, it is important to study soil resilience of different land uses to help maintain soil ecosystem functions and services properly.

2.2. The impacts of agriculture expansion and intensification on soil properties

Food security concerns have accelerated the expansion of cultivation, which causes many negative impacts on soil properties (FAO, 2009). In addition, changes in environmental conditions due to climate change also causes a location shift in agriculture practices to more favorable climate (Barrow, 2009; Purton, 2015). The alternative for cultivation expansion is increase the intensity of agriculture practices, which is even worse for already degraded soil (Lambin et al., 2011; Aguiar, 2019). Ecosystem functions and services provided by soil including crop production, water infiltration, nutrient cycling, erosion control, and habitat for biodiversity have been altered during land use conversion from native land to cropland (Rosenzweig et al., 2016). Cultivation of grasslands have caused an increase in erosion, a decline on SOM, disrupted soil microbial community, accelerated nutrient cycling, reduced nutrient retention, and compacted soil (Rosenzweig et al., 2016; Olson and Gennadiev, 2020).

Agricultural management has a wide range of impacts on many individual soil properties including bulk density, pH, and EC. Bulk density is an important soil property that influences water- and air-filled pore space, biological activity, root penetration, and physical function (Karlen et al., 1997). Brye and Pirani (2004) have found that bulk density in the top 10 cm of soil in Grand Prairie region of east-central Arkansas was significantly higher ($p < 0.05$) under tilled agriculture (1.13 to 1.37 g cm^{-3}) than under native prairie (1.01 to 1.13 g cm^{-3}). For the southwest Saskatchewan area, the bulk density in the top 7.5 cm of cropland was 1.5 times higher than that of native land (Cade-Menun et al., 2017). Land use conversion combined with using heavy farm equipment has led to soil compaction, physical disruption, and loss of soil structure (Hebb et al., 2017). Soil pH and EC are also affected greatly by soil conversion. Generally, when changing from native land to cropland, soil pH will be decreased due to the input of fertilizers, herbicides, and pesticides (Cade-Menun et al., 2017). However, in some situations, soil pH could be increased during crop production. According to Brye and Pirani (2004), continual irrigation of the Grand Prairie has caused an increase in soil pH because of hard, saline groundwater, which has high iron content. In another study, Adingo et al. (2021) found that farmland from Gansu Province in China has significant higher pH compared to abandon farmland and natural grassland from the same area ($p < 0.03$). Both studies also reported lower EC values for native land compared to cropland. Soil EC is an indirect measurement of the ability of soil to transmit electrical current and represents soil salinity (Miller and Curtin, 2008). During crop production, in the area with saline groundwater combined with excessive amount of fertilizer left in soil, EC has increased significantly (Brye and Pirani, 2004). In addition, cropland is frequently disturbed by human, and do not have high surface cover of vegetation all the time, which results in higher EC due to high evaporation and risk of erosion (Adingo et al., 2021).

Furthermore, the conversion of forestland and grassland into cropland has led to a dramatic change in SOM, resulting in many other environment problems (Yannikos et al., 2014). Soils are a major C reservoir, only a small change in the balance between the input and output of the soil C pool would lead to a dramatic change in atmospheric CO_2 (Liang et al., 2017). In 2016, Rosenzweig et al. estimated that 993 Tg of C has been transferred to the atmosphere as CO_2 due to land use conversion to cropland in the US alone. There are many factors that can increase the loss of SOM when changing from native land to cropland including an increase in risk of erosion from deforestation (Olson and Gennadiev, 2020), and SOM inside aggregates exposed to microbial community from using tillage practices and heavy farming equipment (Rosenzweig et al., 2016), and more favorable abiotic conditions for microbial activities enhances SOM decomposition from large input of chemical fertilizers (Baldock and Broos, 2012). In an earlier study, Ellert and Gregorich (1996) found that the transformation of native forests and grasslands into arable agriculture has resulted in a loss of soil organic carbon (SOC) of about 25 to 35%, and the surface layers of cultivated soil had 34% less C. In 1995, Gregorich et al. further argued that most soils lost about 20–30% of SOM due to an increase in mineralization when converting to agricultural land. In a long-term study, David et al. (2009) reported that SOC was decreased by 30–50% due to the conversion of prairies to annual cultivation and artificial drainage. Similarly, Rabbi et al. (2014) found that SOM decomposition rates in crop-pasture rotation were significantly higher compared to that in grassland and woodland. And on the prairie of eastern Kansas, TOC and TON under cultivated fields were 50% lower than that under native land (Rosenzweig et al., 2016). Additionally, Olson and Gennadiev (2020) suggested that after 150 years of converting to cultivation in Kansas, cropland had only maintained 69.4% of total SOC from the initial native timberland, and 30.6% of the SOC was loss due to releasing into the atmosphere or depositing into the water.

2.3. Ecotone and the edge in the Canadian prairies

An ecotone is defined as a transition zone between two adjacent ecological systems with different characteristics (Zhang et al., 2009). Space and time scale, and the strength of the interaction between two adjacent ecological systems are two main factors defining the characters of each ecotone (Pogue and Schnell, 2001). Environmental gradients including edaphic gradients, and gradients conditions that affect species distribution, and directly control ecosystem processes can be found in ecotones (Gosz, 1991). For example, a soil pH gradient has been reported for a prairie-forest ecotone in Saskatchewan (Purton, 2015). Because of its vial function to the energy flux and species harbor, and its close relation to many ecological indicators, ecotones are regarded as a significant ecological factor (Zhang et al., 2009, Yu et al., 2015).

Different from an ecotone, an edge is a sharper transition between two habitat types, and can exist within an ecotone (Burst et al., 2017; Aguiar, 2019). The space of an edge is more defined, and usually marked by changes in vegetation (Ries et al., 2004). An edge can happen naturally, or can be created by human activities including cultivation field, timberland harvesting, road, and urban development (Aguiar, 2019). Edges have unique characteristics because of their constant exchanges with adjacent habitats. In turn, edges can have significant impact on the properties of the surrounding area, and control the resources and energy flow of the area (Ries et al., 2004).

The rapid conversion of forest to grassland at the prairie-forest ecotone in central Canada observed in the paleorecord suggests that this region is sensitive to environmental change (William et al., 2009). The mean annual temperature (MAT) in this region is expected to increase 3^{o}C, and the annual total precipitation is predicted to increase 100–150 mm by 2050 (relative to 1961–1990) (Barrow, 2009). However, the rising of evapotranspiration due to warmer temperature will excess the increase in total precipitation, which will lead to drier conditions with higher moisture stress in the future (Sauchyn and Kulshreshtha, 2008; Barrow, 2009). As a result, the prairie-forest ecotone will be disturbed by both nature phenomena and human activities. A natural rapid shift in dominant species at the ecotone may happen since forest may retreat to the north in response to the increase of temperature and/or draught (Umbanhowar et al., 2006; William et al., 2009; Wyckoff and Bowers, 2010). In addition, agricultural land will expand northward to warmer and cooler climate which is more suitable for crop growth (Perez et al., 2016; King et al., 2018). Since anthropogenic edges can exist within ecotone and it is the actual visible physical location between vegetation habitats (Aguiar, 2019), the current anthropogenic edge between cropland and native land at the prairie-forest ecotones may be the first area in the region to exhibit change due to land use change induced by climate change. The stability over time of anthropogenic edge interface is impacted by land use changes (Harper et al., 2005). In addition, changes in vegetation cover (Raich and Tufekcioglu, 2000; Paré et al., 2006; Purton, 2015), land use management (Yannikos et al., 2014 ;Rosenzweig et al., 2016; Hebb et al., 2017; Olson and Gennadiev, 2020), and temperature and moisture conditions (Davidson and Janssen, 2006; Paré et al., 2006; Wang et al., 2016; Parihar et al., 2019) can alter soil properties and functions. Therefore, it is important to study the soil characteristics of the anthropogenic edge between agriculture and other lands within the prairie-forest ecotone in Central Canada to help predict how soil properties and functions in this area would respond to land use change induced by climate change.

2.4. Soil organic matter stability

Soil organic matter plays an important role in soil (Baldock and Broos, 2012). It can act as an indicator for soil health and soil resilience (Lal et al., 1997), and contribute to food production, mitigation, and adaption to climate change (Lal, 2004). Moreover, SOM has an influence on several soil properties and ecosystem functions such as availability and loss of nutrients, soil structure, water retention and availability, water purification, soil conservation, pesticide efficiency, and decomposition processes in soil (Baldock and Broos, 2012). An adequate amount of SOM needs to be maintained to sustain soil quality and agriculture productivity (Malhi et al., 2003). There is a need to understand the stability of SOM in different land uses, under different climatic conditions. One of the major sources for SOM loss is the exposure of SOM protected within soil aggregates to soil microbial communities (Besnard et al., 1996; Hebb et al., 2017). In addition, the SOM stability is different within different aggregate sizes (Rabbi et al., 2010), and different SOM fractions (Gregorich and Bease, 2008). Other factors such as vegetation cover and changing climatic conditions also can alter SOM stability (Purton, 2015).

2.4.1. Soil organic matter and soil aggregation

There should be a difference in the potential of SOM stabilization into different aggregate size fractions because of the differences in pore geometry of different aggregate sizes (Rabbi et al., 2015). Dexter (1988) argued that larger aggregates have higher porosity compared to smaller aggregates, and SOM content will increase with increasing aggregate sizes. In addition, larger aggregate could have more C content from exchanging with neighbor aggregate, and from C of smaller aggregate retained inside larger aggregates (Guo et al., 2020; Okolo et al., 2020). Many studies have reported a significantly higher amount of SOM in large aggregates compared to smaller aggregates (Guo et al., 2020; Zeraatpisheh et al., 2021). However, SOM in larger aggregates has been reported to have more labile C and higher mineralization rate (Besnard et al., 1996; Okolo et al., 2020), while SOM in smaller aggregates have shown to be more stable and degraded slower than that in larger aggregates (Six et al., 2000; Rabbi et al., 2014). In contrast, Bossuyt et al. (2002) have found a large amount of unprotected SOM within micro-aggregates in

Ultisols. But Razafimbelo et al. (2008) have also found no significant difference in SOM mineralization rates between macro and micro aggregates.

Stabilization of SOM in soil aggregates can be achieved through both physical and physio-chemical protections (Rabbi et al., 2010). The occlusion of SOM into aggregates during its formation can prevent decomposition because pore diameter can influence the accessibility of microbes and enzymes to SOM (Sollins et al. 1996; Young et al., 2008). According to Rabbi et al. (2010), bacteria and fungi cannot approach occluded SOM because of the ratio between dimensions of the microhabitat and the size of the organisms. In addition, high water capacity and slow diffusion of oxygen in micro-aggregates might also contribute to slower SOC decomposition (von Lützow et al., 2006). The interaction between different types of C and clay particles creates the physico-chemical protection for SOM. There are many types of organic matter that can be adsorbed to the clay particles including both simple organic acids and complex bio-macromolecules (e.g., extracellular enzymes, suberins, and DNA). The conformational change of adsorbed SOC can disrupt the enzyme-substrate recognition, therefore enhance the protection against decomposition. The adsorption coverage of clay and the thickness of monolayers are the factors that control its capacity to protect SOC (Rabbi et al., 2010).

The effect of soil conversion from native land to agriculture land on the soil structure, and microbial decomposition have influenced SOM losses by enhancing aggregates disruption, exposing physically protected organic C to soil microorganisms, and creating favorable abiotic conditions (Six et al., 2000; Guo et al., 2020). There is a strong relationship between land use and the amount of SOM stored in macro and micro aggregates (Rabbi et al., 2014; Guo et al., 2020). Land use changes toward cultivation increases the release of inter-aggregates SOC from macro aggregate turnover (Rabbi at al., 2015). This liberation inhibits the formation of micro-aggregates and decreased SOM stabilization. The amount of SOM stored is mostly influenced by the C turnover rate. The breakdown of macro aggregates is sensitive to land use changes (Tisdall and Oades, 1982), and have increased C turnover rate rapidly (Rabbi et al., 2014).

Dry sieving of fresh soil and wet sieving of air-dried soil are two common techniques for separating different soil aggregates size fraction, and both can affect SOM characteristics. Dry sieving involves gentle shaking of fresh soils on top of nest sieves (Helgason et al., 2010), while when wet sieving, soil is air-dried then re-wetted with water to put pressure on the air trapped inside immersed particle pores, followed by vertical strokes in water to create shear forces to separate the soil particles that are initially placed on the top of a nest of subsequently immersed sieves (Blaud et al., 2017). Wet sieving can lead to an overall reduction in soil C concentration in aggregates mainly due to the loss of water-soluble C. In addition, wet sieving also breaks water-dispersible aggregates into smaller fractions; thus, reduced C concentrations typically are associated with macro aggregates (Sarkhot et al., 2007). Furthermore, several studies suggested that dry sieving minimized aggregates disruption, and preserved microbial communities and water-soluble C (Sarkhot et al., 2007; Tiemann and Grandy, 2015). However, it is possible to overestimate C content in the large fraction of soil because macro aggregates preserved during dry sieving might be unstable. Therefore, C content in those macro aggregates might be C associated micro-aggregates inside those macro aggregates. Hence, Bach and Hofmockel (2013) suggested that to examine long-term changes in soil organic matter as well as evaluate stable C in soil, wet sieving is the most useful technique and dry sieving may be useful to capture short term changes. However, dry sieving is faster and more convenient than wet sieving. If there are more than a hundred samples to process, then dry sieving is more practical method to use.

2.4.2. Soil organic matter fractions

Soil organic matter enters soil in particle forms with different sizes and chemical compositions, not as individual molecules (Baldock and Broos, 2012). Soil organic matter is created from a variety of molecular components with different availabilities to soil microbial community (e.g., cellulose, lignin, lipids, proteins). Therefore, the turnover rate of SOM ranges from minutes to millennia (Schimel et al., 1985). To evaluate the effect of soil conversion induced by climate change, it is best to separate SOM into fractions with different decomposition rates.

In many existing studies, SOM is often separated into Light Fraction (LF) and Heavy Fraction (HF) based on density. The light fraction of SOM mainly consists of plant and animal residues at an intermediate state of decomposition (Malhi et al., 2003). It is a precise measure of organic matter changes but due to its sensitivity to management factors, it mainly reflects short-term effects (Janzen et al., 1992). The LF has a wider C:N ratio than that of whole soil but is narrower than that of plant residues (Gregorich and Beare, 2008). The C:N ratio of LF ranges from 17 to 22 for specific gravities of 1.0–1.8 and from 10 to 17 for specific gravities of 1.8–2.2 (Gregorich et al. 2006). However, LF still decomposes faster than the whole organic matter (Malhi et al., 2003). It makes a greater contribution to nutrient cycling and is a source for plant nutrients and substrate for soil microorganisms (Malhi et al., 2003). According to Gregorich and Janzen

(1995), the size of the LF pool in a soil reflects the balance between residue inputs, stabilization, and decomposition rate. The proportion of LF in TOC can be increased by adding more crop residue to the soil (Malhi et al., 2003). However, there are macro aggregates formed around particles of plant residues (Denef et al., 2001), and many soil aggregates have cores of organic particles (Waters and Oades, 1991). These findings have led to two forms of LF with different stability and dynamics due to different spatial location within soil matrix. Free Light Fraction (FLF) are plant residues outside of aggregates with readily accessible to decomposers, therefore have a fast decomposition rate. Conversely, occluded Light Fraction (OLF) is protected within aggregates with slower turnover due to inaccessibility to soil organisms and their extracellular enzymes (Golchin et al., 1998). The HF of SOM is composed of more processed decomposition products and has a strong bonding with soil particles. Therefore, HF has a slow turnover (Christensen, 1992). The HF plays an important role in maintaining soil structure, and contributes to C sequestration (Christensen, 1992).

Many studies have shown results in changing of SOM fractions due to soil conversion. In a study conducted in a Dark Brown Chernozemic soil in Saskatchewan, Malhi et al. (2003) found that the LF mass of cropland were much lower than that of grassland. Similarly, a lower amount of LF has been observed in a cultivated soil compared to soil under hay production and native grasses (Bowman et al. 1990). According to Mahli et al. (2003), the proportion of LF mass in total organic carbon (TOC) of cultivated area was much lower than that of grasslands area. The LF only contributed for 4–5% of TOC mass in cultivated areas but 14–17% in grassland. Also, the decline of C due to cultivation was much higher for LF than for TOC (Malhi et al., 2003). This happened due to LF responding to residue addition before any changes in TOC were apparent. In Australian soils, the loss of LF was 2–11 times greater than that of HF (Dalal and Meyer 1986). In an Ontario soil, since starting maize cultivation, the turnover rate of LF increased over 70% compared to 16% of HF (Gregorich et al., 1995). The reason for this might be because HF was closely associated with clay, therefore, it is harder for decomposers to access the HF. Moreover, the higher the clay content led to the larger difference between these two fractions in rate of loss of organic C (Dalal and Mayer, 1986). Thus, compared to HF and TOC, LF is a more sensitive indicator for soil carbon response to change from grassland to cropland (Malhi et al., 2003).

2.4.3. Factors affecting SOM decomposition

As a major C reservoir, a small change in SOM decomposition in soil would impact atmospheric CO_2 significantly (Liang et al., 2017). And since SOM also plays a vital role in soil quality and soil resilience (Girvan et al., 2005), it is important to look at factors that can influence the decomposition rate of SOM, and understand how these factors control SOM decomposition. Climate change and different land use managements are two important factors that can alter SOM decomposition.

Climate change can alter SOM mineralization through changing temperature and moisture conditions. Soil organic matter decomposition rate depends on root respiration, and microbial activity (Davidson and Janssen, 2006). The rates of both processes are dependent on temperature. Temperature can also alter microbial community composition, enzyme activities (Janssens and Pilegaard, 2003; Wang et al., 2016), and physical and chemical protections of SOM (Gillabet et al., 2010). Soil organic matter decomposition rate can increase exponentially with increasing temperature (Wang et al., 2016). Gillabet et al. (2010) observed cultivated soil mineralized 1.4 times more C at 35^{o}C compared to at 25^{o}C in an incubation study. The percentage of SOC mineralized in a cropland soil under tillage was nearly double when increasing incubation temperature from 27^{o}C to 37^{o}C (Parihar et al., 2019). Moreover, Stanford et al. (1975) found an increase in N mineralized with increasing temperature from 5^{o}C to 15, 25, and 35^{o}C. In addition to temperature, moisture is another climatic factor that can affect SOM decomposition (Ise and Moorcroft, 2006). Like temperature, moisture can control SOM decomposition main processes by controlling water availability for these processes (Davidson and Janssen, 2006). Changes in the thickness of soil water films, and oxygen levels by soil moisture also contribute to altering SOM mineralization (Davidson and Janssens, 2006; Paré et al., 2006). However, the response of SOM to soil moisture is more complicated than that to temperature (Wang et al., 2016). Most of the studies about the impact of climatic conditions on SOM decomposition have focused on temperature. However, both moisture conditions and temperature can affect the same processes in SOM decomposition (Davidson and Janssen, 2006), and it is crucial to study the interacting effects of temperature and moisture conditions on SOM decomposition. For mountain soil in China, Wang et al. (2016) reported a significant effect of the combination of temperature and soil moisture on SOM decomposition ($p < 0.0001$), and the highest SOM decomposition rate occurred at the highest temperature and moisture of incubation. Some reported that the simultaneously variations of temperature and soil moisture could explain 89% of temporal variation in CO_2 efflux (Qi and Xu., 2001), and 91% of temporal variation in soil respiration (Rey et al., 2002).

Similar to climate change, land use can alter SOM decomposition through several mechanisms. Different land use practices affect soil biological and physicochemical properties differently (Arevalo et al., 2012; Sun et al., 2013), leading to different impacts on SOM. One of the common factors is the disturbance of surface soil, leading to the release of SOM within soil aggregates to soil microbial community, which has been discussed above in section 2.4.1. The C:N ratio can control the availability of N to microbial community, and is affected by fertilization (Sun et al., 2013). The amount of N fertilizer added combined with substrate chemistry, and initial N mineralization rate control SOM decomposition (Knorr et al., 2005). In the Aspen Parkland and Moist-Mixed grass ecozone of western Canada, Sun et al. (2013) observed a positive correlation between cumulative CO_2-C and OC content of different land uses, and reported a higher CO_2 efflux in forest land compared to grassland. Vegetation cover is also reported to influence SOM decomposition (Paré et al., 2006). A change in vegetation type would change the quantity and quality of litter input to soil, leading to the change in structure and microclimate of soil, rate of root respiration (Raich and Tufekcioglu, 2000), and soil microbial activities (Paré et al., 2006). However, Raich and Tufekcioglu (2000) argued that the effect of vegetation type on soil respiration was just secondary compared to the effect of environment conditions including temperature. But Paré et al. (2006) did report a significant different among the proportion of labile C pool of soils under different vegetation covers in a 321-day incubation of forest land.

2.4.4. Readily mineralizable carbon and potentially mineralizable nitrogen

One of the most common ways to evaluate the persistent of SOM is to measure the biologically active fractions of soil organic C and N (Purton, 2015). The size of labile pool of SOM which can be mineralized by microbial communities under optimum conditions are reflected by potentially mineralizable C and readily mineralizable N (Haynes, 2005). During incubation for readily mineralizable C, CO_2 evolved is biologically meaningful, and expresses the total metabolic activity of the soil microbiota (Hopkins, 2008). Potentially mineralizable N reflects the mineral N released from active fractions of soil organic N through microbial activity, and the balance between N mineralization and immobilization (Curtin and Campbell, 2008). In addition, the degradability of a soil can be present through readily mineralizable C and potentially mineralizable N per unit mass of SOC and SON, respectively (Baldock and Broos, 2012). However, readily mineralizable

C and potentially mineralizable N are not parallel measurements. While C mineralization show the gross heterotrophic activity of soil microbial community (Purton et al., 2015), net N mineralization reflect the differences between gross mineralization and gross immobilization (Miller and Geisseler, 2018).

3. THE EFFECT OF LAND USE MANAGEMENT ON SOIL PROPERTIES ACROSS AN EDGE BETWEEN GRASSLAND AND CROPLAND IN SASKATCHEWAN

3.1. Preface

Although one of the main sources for C lost due to land use conversion induced by climate change is the exposure of protected SOM within soil aggregates to microorganisms, when studying SOM stability of different land uses, many research studies only considered SOM stability of the whole soil, and fewer studies included SOM stability within different aggregate size fractions. To help understand the underlying processes controlling the biological stability of SOM and the soil itself, this study focused on assessing the effect of land use associated with different vegetation types on soil physiochemical properties, and comparing SOM fractions within different aggregate sizes of soils from an edge between cropland and grassland at an ecotone in Saskatchewan. We further evaluated SOM stability of different aggregate size fractions by measuring readily mineralizable C and potentially mineralizable N of each aggregate size fraction in a short-term incubation. Since the edge between land uses might be the first area to exhibit the impact of land use change induced by climate change, findings from this study could help to understand and predict the stability of soil subject to land use conversion associated with climate change.

3.2. Abstract

It is important to evaluate physicochemical properties and the biological stability of SOM in soils under different land uses to help predict how these land uses might respond to agriculture expansion induced by food security concerns and climate change. The current edge between native land and cropland may be the first area to be impacted by both land use and climatic conditions changes. We investigated physicochemical properties, SOM fractions and biological stability within different aggregate size fractions of soil along transects established at the edge of cropland and grassland at an ecotone in Saskatchewan to examine the resilience of these land uses. Our results indicated that land use and soil depth were the two main factors influencing physiochemical properties of soil. Grassland had the highest bulk density, while cropland had the highest pH. The amount of both TC and TN decreased following the order: edge > grassland > cropland. Grassland also has 1.4 times higher TOC than cropland. Only TOC, TC, TN, and OC:TN ratio in the topsoil (0–12 cm) changed following a distinct pattern across transects. Land use also impacted SOM fractionation within different aggregate size fraction significantly. Cropland had the most SOM in large aggregates (> 2000 µm), while the edge had the most SOM in moderately-sized aggregates (2000–150 µm), which were similar to aggregate size distributions results. All land uses had the same amount of LF due to topsoil redistribution on the hummocky landscape of studied area. The decomposition time of LF is faster than HF reflected in C and N content, and OC:TN results of SOM fractions. For C mineralization, grassland appeared to have higher proportion of decomposable C in SOM pool than cropland. But for N mineralization, our results suggested that cropland was less stable than grassland. Different vegetation covers, the quality and quantity of litterfall, topsoil redistribution, tillage, and fertilizers are a few factors that could impacted soils properties and biological stability of SOM of the soils in our study. It is important to understand these impacts to direct land use management according to soil capability, and restore degraded soils for a sustainable future.

3.3. Introduction

Understanding soil resilience and response to land use conversion induced by climate change is important for developing management strategies for using soil according to its capability, and restoring some functions of degraded soil. The expansion of cultivated land has increased due to food security concerns and climate change (FAO, 2009). Land use change can disturb the soil structure and microbial community (Yannikos et al., 2014), and cause significant change in soil properties including loss of SOM (Davidson and Janssens, 2006), which in turn will contribute more CO_2 to climate change. However, different land uses with different soil properties are likely to have different resilience and response to soil conversion. Thus, to assist in predicting how soil might respond to land use change associated with warming, there is a need to study soil properties of different land uses in areas that are likely to be converted to cultivation due to climate change.

One of the areas that are likely to go through land use conversion due to climate change is the ecotonal transition in the Canadian prairies, especially the anthropogenic edges between cropland and native land that exist within this prairie-forest ecotone. While ecotonal transition is defined as transition zone between biomes, edge is a sharper transition between two habitats and can exist within an ecotone (Burst et al., 2017; Aguiar, 2019). The prairie-forest ecotone is a sensitive area to environmental changes (William et al., 2009). Southern Saskatchewan is predicted to have higher temperatures and drier conditions in the future (Barrow, 2009), which would push agriculture northward to a cooler climate area (William et al., 2009), leading to rapid changes in vegetation cover and land use management. The first area to exhibit changes by this expansion may be the current edges between cropland and native land that exist within the prairie-forest ecotone.

The stability of SOM is one of the indicators that can help to predict how soil might react to land use change induced by climate change in the future. In addition, soil physicochemical properties can both influence SOM decomposition, and combine with SOM to alter soil ability to resist change (Lal et al., 1997). The current land uses can influence soil properties and SOM decomposability through different vegetation covers (Purton, 2015), and land management including agricultural practices (Six et al., 2002; Rosenzweig et al., 2016). Different vegetation type produces litter with different quality and quantity, which would affect SOM composition and decomposition (Raich and Tufekcioglu, 2000). Agricultural activities with heavy farm equipment, chemical fertilizers, and frequent surface disturbances have been observed to have negative impacts on soil properties (Ellert and Gregorich, 1996; Smith et al., 2000; Rosenzweig et al., 2016). Cultivation can disrupt soil aggregation, and release protected SOM to soil microbes, leading to an increase in SOM decomposition (Six et al., 2002; Arevalo et al., 2012).

Our goal was to assess the stability of different land uses at a region of the prairie-forest ecotone that is going to be impacted by land use conversion induced by climate change. To accomplish this goal, we investigated physicochemical properties and SOM fractions within different aggregate size fractions of soils along transects established at a grassland-cropland edge within the prairie-forest ecotone in Saskatchewan. Additionally, the effects of land use and aggregate size fraction on SOM stability were determined through a 28-day incubation of soils from each aggregate size fraction. Then, the differences among soil properties of different land uses were assessed, combined with SOM fractions and stability results, to draw a prediction about the stability of land uses in this edge. Our hypotheses for this chapter are listed below:

1. Across the grassland-cropland edge, land use and soil depth are two main factors controlling basic soil physicochemical properties (i.e., pH, EC, bulk density, aggregate size distributions, TC, TN, TOC, and OC:TN ratio).
2. In topsoil (0–12 cm), soil properties changes across the edge following a pattern: while bulk density decreases from cropland to grassland, TC, TN, TOC, and OC:TN ratio gradually decline from grassland to cropland.

3.4. Material and Methods

3.4.1. Site descriptions

The study site was located within St. Denis National Wildlife Area (SDNWA), about 40 km east of Saskatoon. St. Denis National Wildlife Area lies at the boundary between Aspen Parkland and Moist Mixed Grass eco-regions (Fig. 3.1), and is characterized by undulating to hummocky landscapes with slopes varying from 10 to 15%. The climate is cool and sub-humid with mean annual precipitation of 350 mm and mean temperatures of +25°C in July and -22°C in January (Henderson, 2013). This area was established in 1967 with three main land uses: restored grasslands, native grasslands, and cultivated lands. The dominant soil in the whole site is Dark Brown Chernozemic (Henderson, 2013). The restored grassland area was seeded to grass since 1983, and is characterized as a Gleyed Calcareous Black Chernozem; while the cropland soil was classified as Rego Dark Brown Chernozem and Orthic Black Chernozem.

We sampled at SDNWA in June 2017. Cropland area was seeded with Flax (*Linum usitatissimum* var. CDC Sorrel) for the 2017 growing season in May. Glyphosate was applied to cropland prior to seeding, and granular fertilizer (80 N - 32 P - 15 S lbs/acre) was used at the time of seeding. Grassland at SDNWA were composed of smooth brome (*Bromus inermis* Leyss.), alfalfa (*Medicago stavia* L.), Kentucky bluegrass (*Poa pratensis* L.), and quackgrass (*Elymus repens* L. Gould). Grassland field are cut once a year for hay. The edge is unmanaged, and consists of mostly weed species including Canada thistle (*Cirsium arvense* L. Scop.), cleavers (*Galium* *aparine* L.), flixweed (*Descuriana sophia* L.), and perennial sow thistle (*Sonchus arvensis* L.). The edge did not change in size or move between 2017 and 2018.

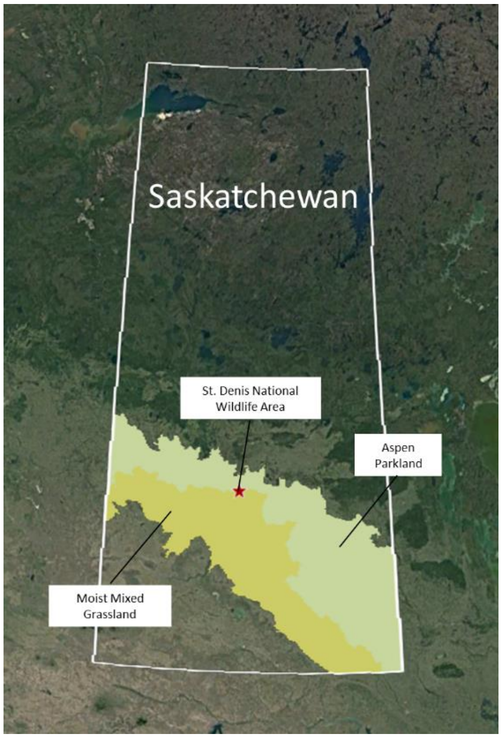

Fig 3.1. St. Denis National Wildlife Area (SDNWA) at the boundary of an eco-region in Saskatchewan

3.4.2. Soil sampling

Three transects were established across the boundary of the cropland and restored grassland at SDNWA (Fig. 3.2). Soil samples were collected at 11 m spacings from the edge (point 0 m, at the centre of the transect) at two depths (0–12 cm and 12–24 cm), with the endpoint at 100 m into the cropland or grassland (Fig 3.3). At point 0 m and 33 m, additional sets of samples were collected at 25 cm, 50 cm, and 1 m (Fig 3.3). Samples were collected using a using a 10 cm diameter punch, and were stored in boxes in a walk-in fridge at 4°C. Another soil core was collected at 0, 11, 33, and 100 m in all 3 transects at both depths using a hammer and a known volume steel ring to determine bulk density.

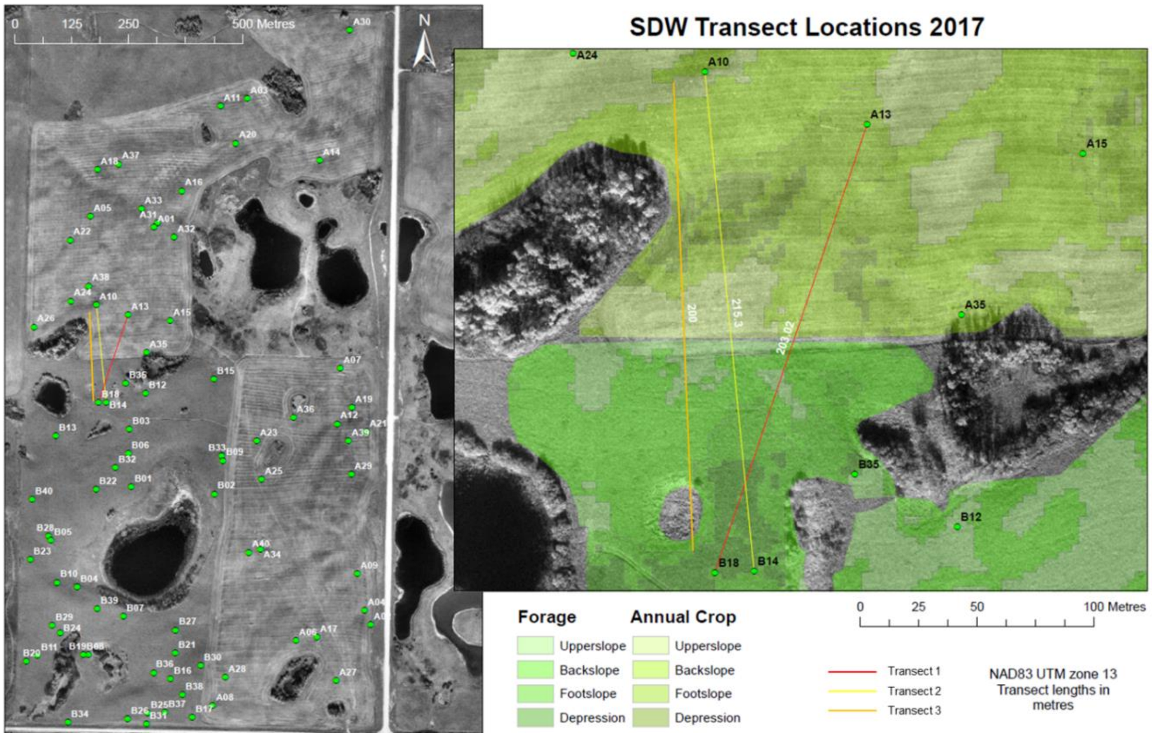

Fig 3.2. Transects map in St. Denis National Wildlife Area

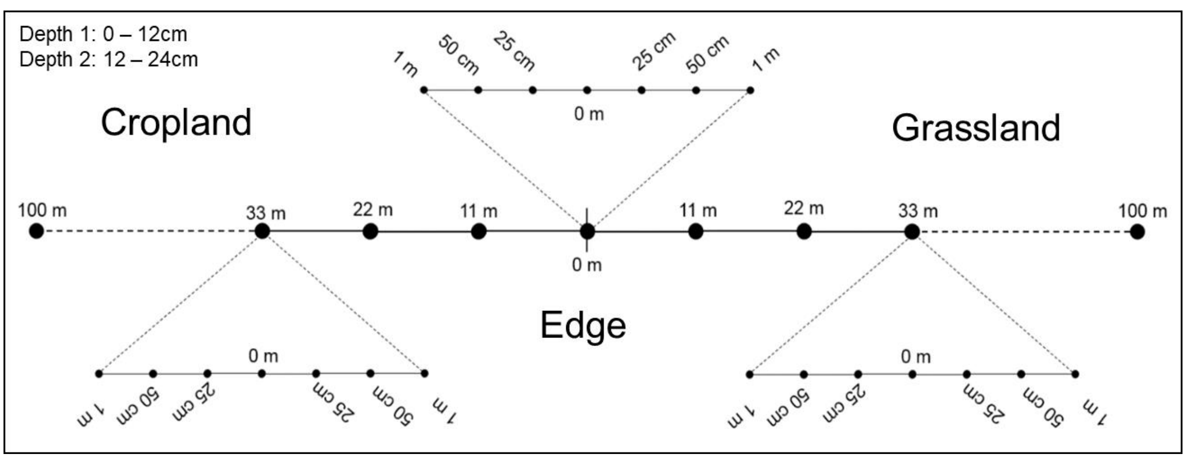

Fig. 3.3. Sampling design at St. Denis National Wildlife Area (SDNWA). Three transects spanning grassland and cropland at SDNWA were established. Soil samples were collected as 11 m spacing from the Edge (point 0) toward both grassland and cropland at two depths 0–12 cm, and 12–24 cm, the end point is 100 m for all transects. Within each transect, nested samples were collected at 25 cm, 50 cm, and 1 m at point 0 m and 33 m in each land use.

3.4.3. Soil properties

A portion of samples (approximately 150 g) were air-dried and ground for soil physicochemical analyses. Total organic C (TOC) was measured by combustion using a LECO CR-12 (LECO Corp, St.Josepth, MI). Samples were acidified with 6% (w/v) sulfurous acid (H_2SO_3) prior to OC measurement to remove inorganic carbonates (Skjemstad and Baldock, 2007); total C and N was measured by combustion using a LECO CNS-2000 (LECO Corp, St.Josepth, MI); soil pH was measured in $CaCl_2$ (Hendershot et al., 2008); EC was measured in 1:5 soil:water suspension (Miller and Curtin, 2008).

For bulk density, the volume of the steel ring used for collecting bulk density sample was calculated and used as soil volume. Dry weight of soil samples was determined after oven-drying at 105^{o}C for 48 hours. Bulk density then was calculated using the equation below:

$$Bulk\ density = \frac{Dry\ soil\ weight\ (g)}{Soil\ volume\ (cm^3)} \qquad \text{Eq. 3.1}$$

For aggregate size distributions, samples were separated into three aggregate size fractions (large: > 2000 µm; moderately-sized: 2000–150 µm; small: < 150 µm) using dry sieving of fresh soil method as described by Helgason et al. (2010). Briefly, 300 grams of field-moist soil was put on top of a nested sieves in a sieving machine (W.S. Tyler's RO-TAP® RX-29). The proportion of each aggregate size was separated and weighed after 7 minutes of sieving on the machine.

3.4.4. Soil organic matter fractions and mineralization within each aggregate size fractions

Since the amount of small aggregate fraction (< 150 µm) was too little for both analyses, only large and moderately size aggregate fractions (> 2000 µm, and 150 – 2000 µm) were chosen for SOM fractionation and mineralization.

Soil organic matter within two main aggregate size fractions (> 2000 µm, and 150 – 2000 µm) was separated into two fractions based on density (light fraction – LF, and heavy fraction – HF) using the procedure described by Gregorich and Beare (2008). Briefly, 35 grams of field–moist soil in each aggregate size fraction was weighed into 40-dram vial with 70 ml of sodium iodide (density 1.8 g cm^{-3}). The vial was capped and shaken for 2 hours at 142 rpm, then left to stand for 48 h at room temperature. Suction was used to aspirate the LF organic matter from the surface of each vial into the filter unit, then 75 mL of calcium chloride (0.01M) and 75 mL of distilled water were used to wash sodium iodide from the LF, and LF that was adhering to the walls of vacuum flask and funnel to the filter paper. The filter was removed, and the LF was washed into a pre-weighed drying tin. If the soil contained a large amount of plant residue, the soil was re-suspended with sodium iodide and all of the filtration steps were repeated. The remaining soil was rinsed several times to remove sodium iodide by shaking with distilled water, settling overnight, and aspirating the supernatant. Heavy fraction and light fraction recovered from the separations were oven-dried at 60°C for 48 hours, then ground to pass through 250 µm sieve. The ground samples were analysed for C and N content using mass spectrometry.

Readily mineralizable C and potentially mineralizable N of soil from large and moderately-sized aggregates were assessed. Surface soil samples (0 -12 cm) taken from point 0 m, 11 m, and 33 m of both grassland and cropland in all three transects were selected for these experiments. There were 18 samples in total, with 9 samples to represent each land uses. The incubation was performed in duplicate, using the procedure described by Hopkins (2008) for readily mineralizable C, and the procedure described by Curtin and Campbell (2008) for potentially mineralizable N, both procedures were modified by Purton (2015).

For readily mineralizable C incubation, 15 g dry-weight equivalent of field-moist soil was weighted into a 30-dram vial, sample moisture was adjusted to 22.5% (w/w). Perforated parafilm was used to cover the vial. The vial then was incubated for 28 days at 25°C, and 85–90% relative humidity. Seven days preincubation was performed to allow equilibration to occur. To determine the weekly mineralization rate, every week the parafilm was removed, the soil vial was put into a mason jar containing a vial of 10 ml deionized water to prevent sample from drying. Background gas samples were taken before the jar was sealed with airtight lid fitted with rubber septa. During sampling, the headspace of each jar was thoroughly mixed, a polypropylene syringe was used to extract ~20 ml of gas and inject into a 12 ml evacuated glass vial (Exetainer®, Labco Ltd.) fitted with silicon and rubber septa. Another gas sample was taken after 6 hours during the first two samplings, and after 24 hours for all the following sampling dates. Carbon dioxide concentrations were analyzed using a Varian CP-4900 Micro Gas Chromatograph (Varian Inc.) with 400 ppm and 2000 ppm CO_2 standards for calibration. Moisture was adjusted weekly with deionized water. Concentrations of CO_2 were converted from ppm to mg CO_2-C using the ideal gas law, with pressure considered constant at 101.3 kPa. Cumulative respiration over the 28 days incubation was calculated from weekly respiration rates according to Paré et al. (2006), with the equation:

$$C_t = C_{t-1} + \frac{(k_p + k_{p-1})}{2} \times (JJ_p - JJ_{p-1}) \qquad \text{Eq. 3.2}$$

C_t: mineralized C (mg CO_2-C g^{-1} TOC) at time t (d)

k: the daily respiration rate (mg kg^{-1} d^{-1}),

p: the incubation period (1–24)

JJ: the Julian day.

The result of C mineralization express per kg soil were included in Appendix A.

For potentially mineralizable N, 15 g dry-weight equivalent of field-moist soil was weighted and mixed with acid washed sand (GRANUSIL®, GHP system, Inc.) (1:1 ratio) into a Buchner funnel that has a glass microfiber pad (type GF/B, Whatman®, GE Co) placed on top of a 30 µm nylon mesh and another glass microfiber pad in the bottom. The Buchner funnel was attached to a Buchner flask, and another glass microfiber pad was placed on top the soil:sand mixture. Samples were pre-leached before incubation. Deionized water was used to bring sample moisture to 22.5% (w/w). The funnel then was covered with perforated parafilm and incubated for 28 days at 25°C, and 85–90% relative humidity, the same condition as readily mineralizable C incubation to establish comparison. Samples were extracted with 100 mL 0.01 M $CaCl_2$ and 25 mL N-free nutrient solution every week. Extracted solution was analyzed for NO_3^- and NH_4^+ using a Technicon AutoAnalyzer (Technicon Industrial Systems). Moisture was adjusted weekly with deionized water. All N mineralization result were converted to mg N g^{-1} TN, and the result expressed per kg soil was shown in Appendix A.

3.4.5. Statistics

Statistical analyses were conducted using SAS® Studio (SAS Institute). Four completely randomized models were tested using Mixed procedure with transect as random effect, and micro scale transects were nested within main transects. Normality of variables were tested using Shapiro-Wilk's test, data with non-normal distribution of residual were log transformed. Post-hoc comparisons were performed using Tukey's HSD to determine the significance of differences between LSmeans ($p < 0.05$).

The first model had a 2-factorial arrangement which was used to test for land use, soil depth, and the combination of land use and soil depth effects on measured physicochemical properties of soil as following:

$$Y = mean + land\ use\ (L) + soil\ depth\ (D) + L*D\ interaction + error \quad \text{Eq. 3.3}$$

Y: soil properties

The second model had a 4-factorial arrangement using to test for the effects of land use, soil depth, SOM fraction pools, aggregate size, and the combined effect of these factors on the fraction mass of SOM fractions as following:

*Y = mean + land use (L) + soil depth (D) + aggregate size (A) + SOM fraction pool (F) + L*D interaction + L*A interaction + L*F interaction + A*D interaction + A*F interaction + L*A*F interaction + L*A*D*F interaction + error* Eq. 3.4

Y: SOM fraction mass

After running the second model, land use and SOM fraction pool appeared to be the two main factors that impacted SOM fraction masses. Thus, a simplified model with 2 factorial arrangements was used to test for the effect of land use, SOM fraction pool, and the combined effects of these two factors on the C and N contents, and C:N ratio of different SOM fractions as following:

*Y = mean + land use (L) + SOM fraction pool (F) + L*F interaction + error* Eq. 3.5

Y: C and N contents, C:N ratio

Lastly, a 2-factorial model was used to test for the effect of land use, aggregate size, and the combined effects these two factors on cumulative C mineralized, and cumulative net N mineralized as following:

*Y = mean + land use (L) + aggregate size (A) + L*A interaction + error* Eq. 3.6

Y: cumulative C mineralized or cumulative net N mineralized

3.5. Results

3.5.1. Soil characteristics across land uses

Land use showed a significant effect on all measured soil properties (Table 3.1). Grassland had the highest pH, while cropland had the highest bulk density ($p < 0.05$, Table A.1). But there were no differences in bulk density among land uses in topsoil (0–12 cm). Electrical conductivity was relatively low across the edge between grassland and cropland ranging from 0.1–1.8 dS m^{-1} (Table 3.1), and declined following the order: grassland > edge > cropland ($p < 0.05$, Table A.1). Overall, the amount of both TC and TN decreased following the order: edge > grassland > cropland ($p < 0.05$). However, for both OC and OC: TN ratio, grassland had the highest values, while cropland had the lowest ($p < 0.05$).

Similar to land use, soil depth also had significant impact on all measured soil properties, except for bulk density. Overall, across the edge between grassland and cropland, pH and EC increased in deeper soil, while OC, TC, TN, and OC:TN ratio decreased in deeper soil ($p < 0.05$).

Although land use and soil depth had significant effects on most measured soil properties, the interaction of these two factors only impacted pH, TC, TN, and OC:TN ratio significantly (Table 3.1). In surface soil, grassland and the edge had the highest amounts of TC and TN, which were 1.3 times higher than the lowest amounts observed in cropland (Table 3.1). In deeper soil, both TC and TN decreased following the order: edge > grassland > cropland ($p < 0.05$). In both depths, the highest OC:TN ratio was observed in grassland, while the lowest was observed in cropland ($p < 0.05$).

Table 3.1. Effects of land use, soil depth, and the combined effects of land use and soil depth on properties of soils across an edge between grassland and cropland at St. Denis National Wildlife Area. Values are means (±SD) and different letters within a column represent significant differences (Type 3 Tests of Fixed Effects, TukeyHSD post hoc, $p < 0.05$). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). L=Land use, D=Soil depth. †Statistic done on Log transformed data.

Soil depth	Land use	pH	EC† dS m^{-1}	Bulk density g cm^{-3}	TOC	TC	TN†	OC:TN
					— g kg^{-1} soil —			
0–12 cm	Grassland	7.0±0.3^{a}	0.7±0.9^{bc}	1.2±0.3^{a}	31.0±5.8^{a}	35.4±6.6^{a}	3.0±0.5^{a}	10.2±1.3^{a}
	Edge	6.8±0.1^{cd}	0.2±0.2^{cd}	0.9±0.1^{a}	30.4±4.6^{a}	35.6±5.5^{a}	3.2±0.6^{a}	9.4±0.7^{ab}
	Cropland	6.7±0.2^{d}	0.1±0.1^{d}	1.3±0.2^{a}	22.5±3.5^{b}	26.3±3.8^{b}	2.5±0.4^{b}	9.2±1.0^{b}
12–24 cm	Grassland	7.1±0.2^{a}	0.9±0.9^{a}	1.2±0.2^{a}	23.3±5.5^{b}	28.3±6.0^{b}	2.3±0.4^{b}	10.7±4.4^{ab}
	Edge	6.9±0.1^{bc}	0.5±0.4^{b}	1.0±0.1^{a}	22.2±4.2^{b}	32.2±2.2^{a}	2.9±0.3^{a}	7.7±1.0^{c}
	Cropland	6.9±0.1^{ab}	0.2±0.1^{cd}	1.2±0.2^{a}	14.7±3.8^{c}	20.2±4.1^{c}	1.7±0.4^{c}	9.1±2.8^{c}

Effect	df	Type 3 Tests of Fixed Effects F value						
L	2	33.52***	28.09***	3.64*	78.39***	109.17***	83.61***	21.02***
D	1	20.87***	27.50***	0.33	165.05***	73.73***	95.34***	34.50***
L*D	2	6.03*	1.96	0.4	0.09	5.29*	7.06*	3.13*

While land use showed a significant impact on all aggregate size distributions, soil depth only influenced the distribution of large and moderately-sized aggregates (> 2000 µm and 2000–150 µm, respectively) ($p < 0.05$) (Table 3.2). Regardless of soil depth The distribution of large aggregates decreased in the following the order: cropland > grassland > edge (Fig 3.4). In contrast, the distribution of moderately-sized and small aggregates declined following the order: edge > grassland > cropland (Fig 3.4). While the proportion of large aggregates increased in deeper soil ($p < 0.05$), proportion of moderately-sized aggregates decreased in deeper soil ($p < 0.05$), and the proportion of small aggregates was the same throughout soil profile ($p > 0.05$, data not shown). The interaction of land use and soil depth was not significant (Table 3.2).

Table 3.2. The influences of land use, soil depth, and the combined influences of land use and soil depth on aggregate size distributions of soils across an edge between grassland and cropland at St. Denis National Wildlife Area. Values are F values. Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). L=Land use, D=Soil depth.

Effect	df	Aggregate size distribution		
		> 2000 µm	2000–150 µm	< 150 µm
L	2	33.55***	21.15***	29.43***
D	1	4.20*	10.12*	0.53
L*D	2	0.11	0.25	0.02

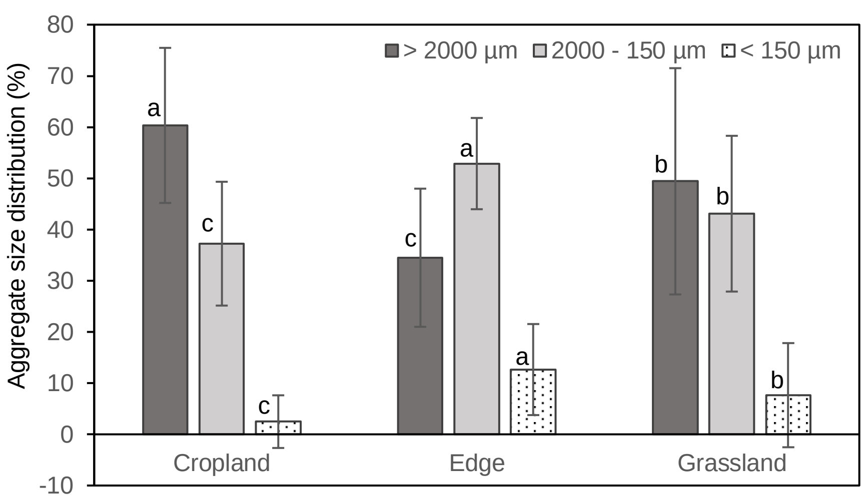

Fig 3.4. Means of aggregate size distributions across land uses. Soil samples originated from an edge between grassland and cropland at St. Denis National Wildlife Area. Means with the different letter on the same column color and pattern are significantly different (TukeyHSD post hoc, $p > 0.05$). Bars are standard deviations.

The ranking of TC, TN, and OC were highest in the grassland and lowest in the cropland with a transition occurring at the edge (Fig 3.5). Soil pH dropped abruptly at 33 m in cropland (from rank 7 at 22 m to rank 1 at 33 mm), and increased sharply in grassland (from rank 1 at 22 mm to rank 8 at 33m). Overall, soil properties sampled at nested sampling locations at 33 m in the grassland were higher than that of cropland, except for the proportion of large aggregates (> 2000 µm).

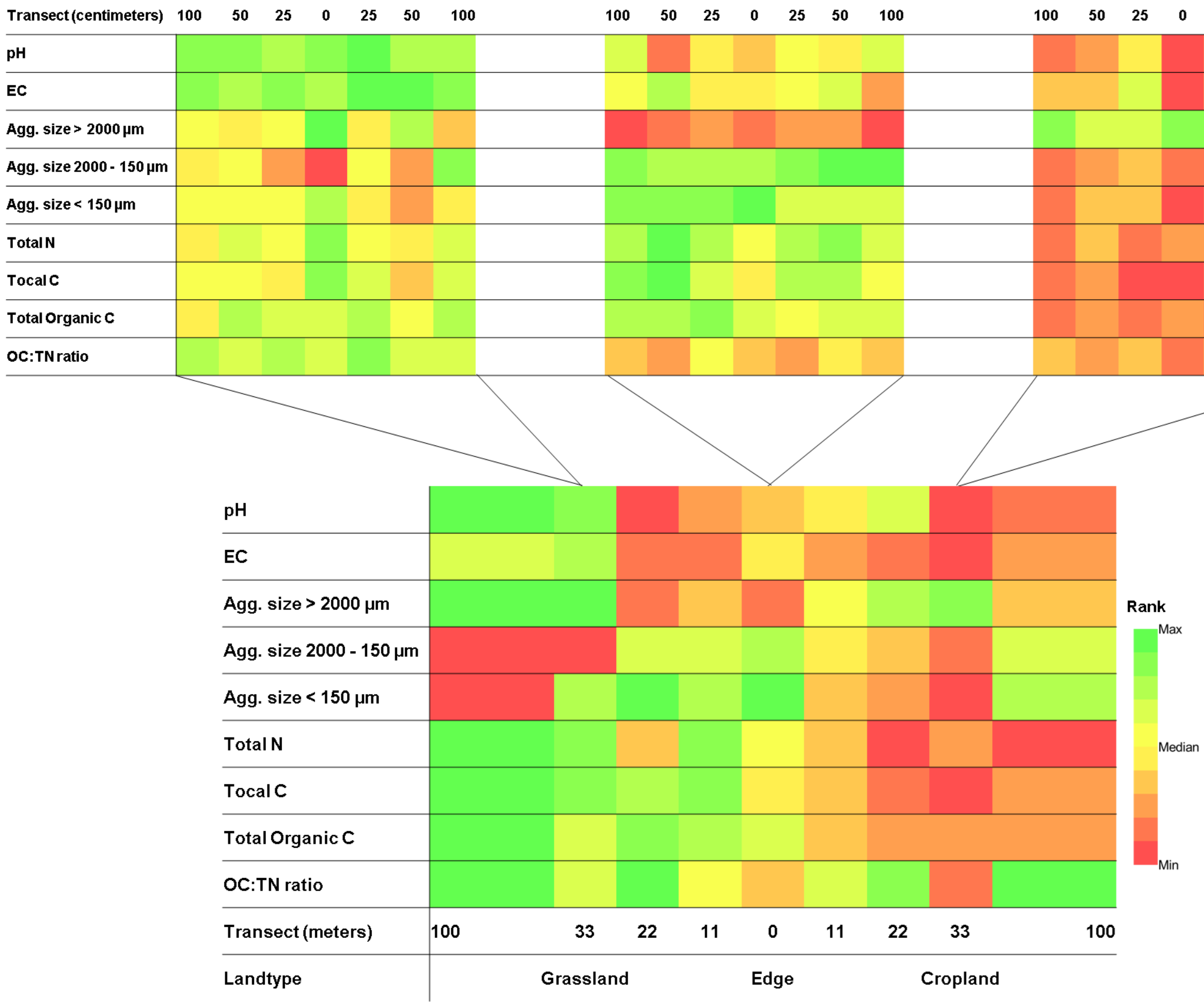

Fig 3.5. Ranking of soil properties changes across the edge between grassland and cropland at St. Denis National Wildlife Area. The edge was located at 0m at the center of the transect (0m), with the transects extending up to 100 m into grassland and cropland. At 0 and 33 m, another set of soil samples were nested within the transects (0, 25, 50, 100 cm) to represent each land use.

3.5.2 Soil organic matter fractions within different aggregate size fraction

While land use and SOM fraction pool impacted mass of both LF and HF significantly ($p < 0.0001$), soil depth and aggregate size did not show any significant effect on fraction masses ($p > 0.05$) (Table 3.3). Overall, cropland had the highest total amount of LF and HF from both aggregate sizes (249.6 g kg^{-1} soil) compared to the edge and grassland (205.19 and 204.13 g kg^{-1} soil, respectively; $p < 0.05$,). In addition, the mass of HF was nearly 10 times higher than the mass of LF (407.76 and 40.94 g kg^{-1} soil, respectively).

Although aggregate size did not have any significant effect on mass of both LF and HF, the combination of land use and aggregate size, and the combination of land use, aggregate size, and SOM fraction pool did ($p < 0.0001$) (Table 3.3). In addition, the combined effect of land use and SOM fraction pool on the mass of LF and HF was also significant ($p < 0.05$). Cropland had the highest amount of HF compared to grassland and the edge, while LF mass was similar across land uses (Fig 3.6). Overall, the mass of HF from large aggregates declined as the following order: cropland > grassland > edge (547 > 399 > 276 g kg^{-1} soil), with the amount of HF in croplands nearly 2 times higher than that of the edge. However, the edge had the highest HF mass from moderately-sized aggregates (474 g kg^{-1} soil), which was 1.4 times higher than cropland and grassland (350, and 347 g kg^{-1} soil, respectively) ($p < 0.05$) (Fig 3.6b).

Table 3.3. The influences of land use, soil depth, aggregate size, SOM fraction pool, and the combined influences of these factors on mass of SOM fractions (heavy fraction, and light fraction) within two different aggregate size fractions (> 2000 μm, and 2000–150 μm) at two depths (0–12 cm, and 12–24 cm) across land uses. Soil samples were taken from an edge between grassland and cropland at St. Denis National Wildlife Area. Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***).

Effect	df (num)	F Value
Land use (L)	2	10.18^{***}
Soil depth (D)	1	0.06
Aggregate size (A)	1	0.91
SOM fraction (F)	1	1244.23^{***}
L*D	2	0.32
L*A	2	33.11^{***}
L*F	2	4.46^{*}
A*D	1	0.02
A*F	1	0.54
L*A*F	2	24.49^{***}
L*A*D*F	8	0.55

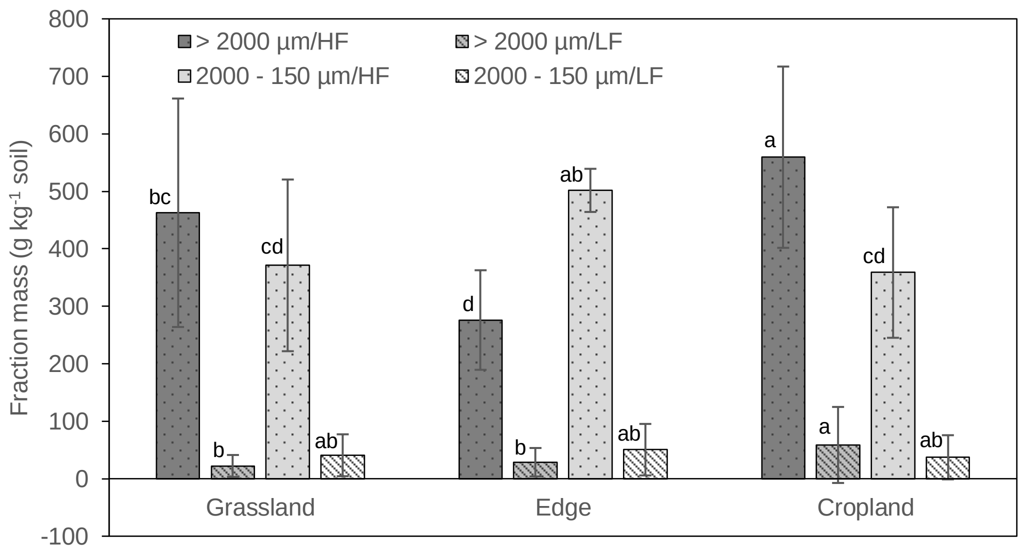

Fig 3.6. Total mass of heavy fraction (HF) and total mass of light fraction (LF) from two different aggregate size fractions (> 2000 μm and 2000–150 μm) at two depths (0–12 cm and 12–24 cm) across land uses. Soil samples were taken from an edge between grassland and cropland at St. Denis National Wildlife Area. Means with the different letter on the same column pattern are significantly different (TukeyHSD post hoc, $p > 0.05$). Bars are standard deviations.

Land use and SOM fraction pool were the two main factors impacting SOM fractions (Table 3.3); therefore, differences in C and N content, and C:N ratio of LF and HF were examined with land use and SOM fraction pool, and the combined effects of land use and SOM fraction pool (Table 3.4). Similar to SOM fraction mass, both land use and SOM fraction pool had individual significant impact on C content of LF and HF from moderate and large aggregate size fractions (Table 3.4). However, the interaction of land use and SOM fraction pool did not impact C content of LF and HF significantly. Carbon content of LF was 4.4 times lower than that of HF. The highest amount of C was observed in the edge, which was 1.2 times higher than the lowest C content observed in grassland ($p < 0.05$).

Unlike SOM fraction mass and C content results, only SOM fraction pool showed significant effect on N content of LF and HF (Table 3.4). Overall, N content of LF was 6 times lower than that of HF ($p < 0.05$).

Although land use had no significant impact on C:N ratio, SOM fraction pool and the interaction of land use and SOM fraction pool influenced OC:TN ratio significantly (Table 3.4). Similar to SOM fraction mass, and C and N content, C:N ratio was higher in HF compared to LF ($p < 0.05$). However, when considering the combined effect of both land use and SOM fraction pool, only the edge had a significant difference between C:N ratio of LF and HF ($p < 0.05$), and C:N ratio was not significantly different between the LF and HF from grassland and cropland ($p > 0.05$).

Table 3.4. Means (±Standard deviation) of C and N contents (g kg^{-1} soil), and C:N ratio of soil organic matter fractions (LF=Light fraction, and HF=Heavy fraction) within two different aggregate size fractions at two soil depths across land uses. Soil samples originated from an edge between grassland and cropland at St. Denis National Wildlife Area. Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). L=Large aggregates, M=Moderately-sized aggregates.

Factors	Soil depth			0–12 cm			12–24 cm		
	Land use			Grassland	Edge	Cropland	Grassland	Edge	Cropland
Agg. size fraction	L	LF	C	1.3±1.0	2.5±2.3	2.1±2.3	1.4±2.3	1.2±0.9	1.4±1.5
			N	0.1±0.1	0.3±0.2	0.2±0.2	0.1±0.2	0.1±0.1	0.1±0.1
			C:N	11.8±1.9	10.6±1.0	10.6±1.7	13.3±6.8	10.6±0.7	13.2±4.5
		HF	C	9.8±5.4	5.9±1.9	6.6±3.0	8.7±7.2	6.4±4.3	8.6±2.9
			N	0.8±0.5	0.4±0.3	0.6±0.3	0.6±0.5	0.5±0.5	0.7±0.3
			C:N	11.4±1.6	11.0±1.1	10.6±1.1	13.1±4.5	10.5±0.7	12.0±3.9
	M	LF	C	3.0±2.7	1.8±1.3	1.6±0.3	1.3±1.3	2.0±1.3	1.0±0.9
			N	0.3±0.2	0.2±0.1	0.2±0.0	0.1±0.1	0.2±0.1	0.1±0.1
			C:N	13.0±2.8	19.3±11.2	13.5±5.3	15.2±11.7	19.2±13.1	15.8±9.8
		HF	C	6.9±3.1	12.9±2.6	7.5±3.9	6.0±5.0	8.1±3.5	4.7±2.7
			N	0.6±0.3	1.2±0.3	0.7±0.3	0.5±0.3	0.8±0.4	0.4±0.2
			C:N	11.2±1.4	10.9±1.7	10.9±1.3	12.5±6.3	11.1±1.8	14.9±11.9

Type 3 Tests of Fixed Effects

Effect	df	C	N	C:N
		F value		
Land use (L)	2	3.15*	2.52	0.12
SOM fraction (F)	1	428.03***	316.94***	8.38*
L*F	2	0.7	0.55	4.62*

3.5.3. Readily mineralizable carbon within different aggregate size fraction

While land use had a significant effect on mineralized C starting from the second week of incubation, aggregate size fraction and the combination of land use and aggregate size fraction did not have a significant effect on cumulative mineralized C during 28-day incubation (Table 3.5). Overall, grassland produced more C-CO_2 per g TOC than cropland throughout the incubation ($p < 0.05$). The amount of C produced by both major aggregate size fractions of surface soil in grassland was 1.4–1.6 times greater than that in cropland (Fig 3.7).

Table 3.5. Effects of land use, aggregate size, and the combined effects of land use and aggregate size on cumulative C mineralized (mg CO_2-C g^{-1} TOC) throughout a 28-day incubation at 25°C and 22.5% (w/w) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area. Values are means (±SD) and different letters within a column represent significant differences (Type 3 Tests of Fixed Effects, TukeyHSD post hoc, $p < 0.05$). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). L=Land use, A=Aggregate size.

Aggregate size fraction	Land use	Day 7	Day 14	Day 21	Day 28
Large (> 2000 μm)	Grassland	0.7±0.4[a]	2.0±1.0[a]	5.0±1.8[a]	9.7±2.8[a]
	Cropland	0.4±0.1[a]	1.1±0.3[b]	2.6±0.6[b]	4.7±1.0[c]
Moderately-sized (2000–150 μm)	Grassland	0.6±0.3[a]	1.9±0.9[ab]	4.6±1.3[a]	8.6±1.8[ab]
	Cropland	0.6±0.1[a]	1.6±0.2[ab]	3.5±0.8[ab]	6.2±1.5[bc]
Effect	df	Type 3 Tests of Fixed Effects			
		F Value			
L	1	3.88	10.59*	20.42***	31.00***
A	1	0.75	0.08	0.46	0.56
L*A	1	1.40	3.79	2.62	3.08

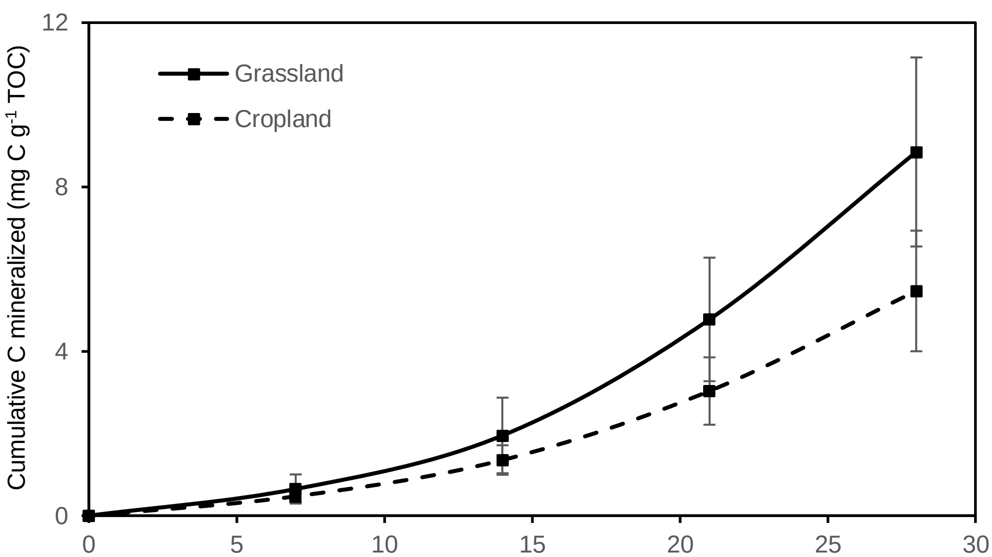

Fig 3.7. Cumulative carbon mineralized of surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area throughout 28 days of incubation at 25°C and 22.5 % (w/w) moisture condition. Lines represent means of 18 subsamples for each land use. Bars are standard deviations.

3.5.4. Potentially mineralizable nitrogen within different aggregate size fraction

Unlike C mineralization, land use did not show any significant effect on cumulative net N mineralized at anytime during a 28-day incubation, but aggregate size, and the combination of land use and aggregate size did throughout the 28-day incubation (Table 3.6). Overall, large aggregate fraction mineralized more N per g TN than moderately-sized aggregate fraction (Fig 3.8). While grassland had 1.4–1.5 times less cumulative net N mineralized than cropland in large aggregate fraction ($p < 0.05$), grassland and cropland mineralized similar amount of cumulative net N mineralized in moderately-sized aggregate fraction ($p > 0.05$) (Table 3.6).

Table 3.6. Effects of land use, aggregate size, and the combined effects of land use and aggregate size on cumulative net N mineralized (mg N g^{-1} TN) throughout a 28-day incubation at 25°C and 22.5% moisture condition (w/w) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area. Values are means (±SD) and different letters within a column represent significant differences (Type 3 Tests of Fixed Effects, TukeyHSD post hoc, $p < 0.05$). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). L=Land use, A=Aggregate size.

Aggregate size fraction	Land use	Day 7	Day 14	Day 21	Day 28
Large (> 2000 μm)	Grassland	5.1±2.0^{b}	6.1±1.9^{b}	6.7±1.9^{b}	7.6±2.2^{b}
	Cropland	7.1±2.3^{a}	9.2±3.1^{a}	10.5±3.7^{a}	11.5±3.6^{a}
Moderately-sized (2000–150 μm)	Grassland	3.7±3.0^{c}	5.3±2.8^{b}	6.6±3.2^{b}	8.5±3.8^{b}
	Cropland	1.7±0.6^{c}	3.1±1.5^{b}	3.9±1.4^{b}	4.8±1.8^{b}
Type 3 Tests of Fixed Effects					
Effect	df	F Value			
L	1	0.90	0.36	1.73	0.62
A	1	52.65***	19.24**	24.33***	16.71**
L*A	1	8.25*	11.01*	13.08**	15.02**

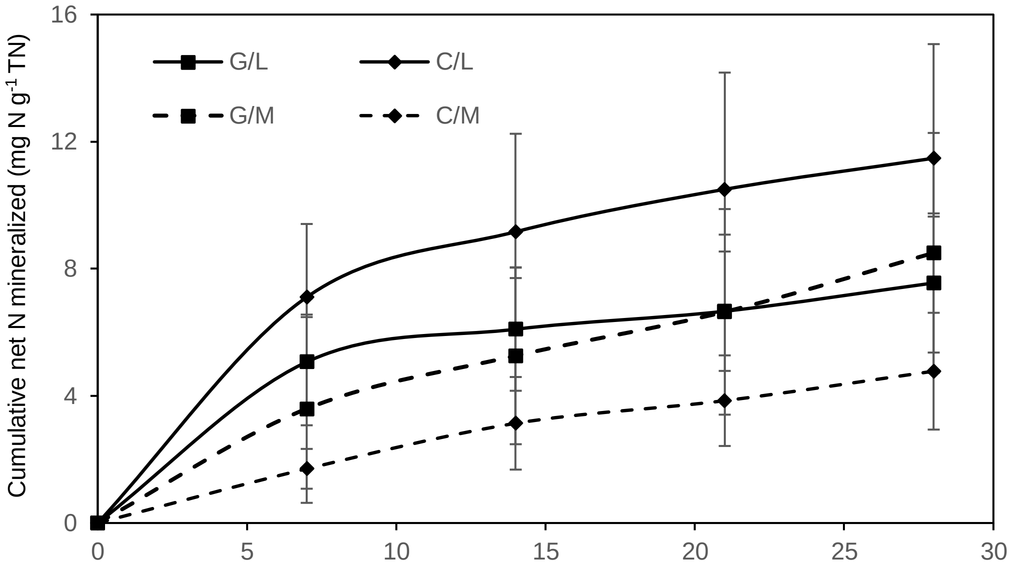

Fig 3.8. Cumulative net nitrogen mineralized of different aggregate size fraction of surface soils surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area throughout 28 days of incubation at 25^{o}C and 22.5 % (w/w) moisture. Lines represent means of nine subsamples. Bars are standard deviations. C=Cropland, G=Grassland, L=Large aggregates (> 2000 µm), M=Moderately-sized aggregates (150 – 2000 µm).

3.6. Discussion

At the edge between grassland and cropland at SDNWA, land use was the primary factor controlling soil physiological properties, SOM fractionation, and C mineralization. In topsoil, TOC, TC, TN, and OC:TN gradually decline from grassland to cropland across the edge. Beside land use, soil depth was another main factor beside land use that affected soil physiological properties significantly. Which supported our first hypothesis that land use and soil depths were the two primary factors influencing basic soil properties. For HF of SOM, cropland had more HF mass overall and mainly within the large aggregate size fraction, while the edge had the most HF mass in the moderately-sized aggregate fraction. These results were similar to aggregate size distributions results. As for LF, all soils and aggregate fractions had the same amount of LF mass. Since LF is decomposed faster than HF, all soils had more C and N content, and higher OC:TN ratio in HF than LF. In terms of C mineralization, the two main aggregate size fractions of grassland seemed to be more decomposable than that of cropland. Land use did not have significant influence on N mineralization individually, but through the combination of land use and aggregate size instead. Large aggregate fraction of all soils mineralized more N per g TN than moderately-sized aggregate fraction. In addition, grassland was more stable than cropland in terms of N mineralization.

3.6.1. Physicochemical properties across land uses

Land use and soil depth were the two main factors controlling pH, but only land use impacted bulk density significantly in our study. Grassland had the highest pH among all land uses, and the same pH throughout both depths. While both cropland and the edge had higher pH in deeper soils. The input of fertilizers, herbicides, and pesticides could decrease soil pH in cropland (Cade-Menun et al., 2017). Cropland in our study was fertilized with granular fertilizer at rate of 80lbs N $acre^{-1}$. Long-term application of N fertilizer can decrease pH by promoting acidification through increasing nitrification and H+ production (Rosenzweig et al., 2016). Deeper soil is not impacted by fertilizer as much as the topsoil, therefore deeper soil in our study had higher pH than topsoil. For bulk density, it was not surprising that cropland had the highest bulk density since the use of heavy farm equipment in crop production has compacted soil and disrupted soil structure (Hebb et al., 2017). However, grassland was also impacted by heavy equipment during hay removal process; therefore, bulk densities of all land uses were similar in topsoil (0–12 cm). Half of our second hypothesis (i.e., bulk density of topsoil decreased from cropland to grassland) was rejected.

Even though land use influenced all physicochemical properties significantly, only the changes of TOC, TC, TN, and OC:TN ratio in the topsoil (0–12 cm) followed a distinct pattern, gradually declined from grassland to cropland across the edge. Thus, half of our second hypothesis was supported. Our results were consistent with previous studies that cropland had the lower amount of C and N compared to grassland. Ellert and Gregorich (1996) reported cultivated land had 34% less C for Ontario soils. In Saskatchewan, cropland had 31–43% and 23–47% lower C and N compared to grassland, respectively (Malhi et al., 2003). The decomposition of SOM was enhanced by tillage practices and heavy farm equipment through exposing SOM protected inside aggregates to soil microbial communities (Baldock and Broos, 2012). Cropland at SDNWA was previously tilled then converted to no-till, the time of conversion is unknown, but they continue to use heavy farm equipment. The input of fertilizer can also impact the amount of C and N in soil by changing soil microbial activities through C:N ratio (Sun et al., 2013). Despite being applied with N fertilizer, TN of cropland at SDNWA was still 1.3 times lower than that of grassland, which was not surprising since cropland appeared to have lower OC:TN ratio than grassland. Another factor that can impact the amount of C and N in soil through microbial activity is the quantity and quality of litter (Paré et al., 2006). Grassland in SDNWA had plant species that can produce high quality litter with high amount of C and N (Aguiar, 2019). The major of above ground biomass is removed every year in cropland area at SDNWA. The removal of above ground biomass can decrease the amount of C and N overtime since this process disrupts the natural cycles (Aguiar, 2019).

Beside land use, soil depth is another main factor controlling C and N content of soils, and the combined effect of land use and soil depth also impacted C and N content significantly in our study. Gregorich et al. (1995) argued that the differences in C and N content were greatest at topsoil and declined with increasing depth. They found that in soils from Ontario, the TOC of cropland in 0–10 cm and 10–25 cm were nearly 3 times and 1.23 times lower than forest land. Similarly, our study found that cropland had lower C and N, and OC:TN ratio than grassland at both depths. However, the differences in C and N among land uses at SDNWA increased in deeper soils. At SDNWA, annual roots system of cropland is much smaller than perennial roots system of grassland (Aguiar, 2019), combing with the redistribution of topsoil due to undulating landscape could lead to the bigger differences in C and N content among land uses in deeper soil. Overall, all soils had higher amount of C and N in topsoil compared to deeper soil since topsoil received more C and N every year through litterfall.

Like other physicochemical properties, land use impacted the distribution of all aggregate sizes, but soil depth only had significant impact on the distributions of large and moderately-sized aggregates. Overall, cropland had the highest distribution of large aggregates, while the edge had the highest distribution of moderately-sized and small aggregates, and the distribution of all aggregate sizes of grassland remained in between cropland and the edge. Most of the previous studies found that cropland had lower large aggregate distribution than grassland because agricultural practices has disrupted soil structure and break down macro aggregates (Tisdall and Oades, 1982; Haynes et al., 1991; Six et al., 2002; John et al., 2005). Our study found that cropland had higher large aggregate distribution, which might be because we used dry-sieving technique, therefore large aggregates of cropland might not be water-stabled. In addition, cropland might have higher clay particle and binding substances, combing with smaller aggregates to form large amounts of large aggregates (Ge et al., 2019). Especially in the winter, topsoil of all land uses is covered in snow. But while grassland and the edge still have plant covers, all above ground biomass of cropland was removed. This might create a favorable condition to bind smaller aggregates into large aggregates in cropland, resulting in an increase of large aggregate distribution but decrease in smaller aggregate distribution. Similarly, Zhao et al. (2017) found that comparing to grassland, paddy soils, which has a special water management system, had significant higher distribution of large aggregates. In addition, cropland might have the highest large aggregate size distribution but most of it came from deeper soil since the highest large aggregate size distribution among all land uses and soil depths was observed in cropland at 12–24 cm. While in topsoil, both grassland and cropland had similar large aggregate size distribution ($p < 0.05$).

3.6.2. Soil organic matter factions of two main aggregate size fractions across land uses

For SOM fractionation of two main aggregate size fractions, land use and SOM fraction pool were the two primary factors that influenced the mass of SOM fractions, not aggregate size nor soil depth. However, the combination of land use and aggregate size, and the combination of land use, aggregate size, and SOM fraction pool did have a significant impact on SOM fraction masses. Overall, HF masses of all soils were nearly 10 times higher than LF masses. Which was consistent with previous studies since LF consists of easily decomposed plant and animal residues, so LF will be mineralized faster than HF, resulting in higher HF masses in all land uses compared to LF masses (Dalal and Meyer 1986; Janzen et al., 1992; Gregorich et al., 1995; Malhi et al., 2003). In addition, decomposers also have limited access to HF due to the close association between HF and clay particles (Gregorich et al., 1995). Many previous studies also found that grassland had more LF mass than cropland since grassland has more frequent residue input than cropland, and agriculture practices have accelerated SOM decomposition of LF (Dalal and Meyer 1986; Malhi et al., 2003). However, in contrast to these studies, our study found that LF masses in all land uses, and all aggregate size fractions were the same at SDNWA. The reason behind this might be because SDNWA has an undulating landscape which leads to soil erosion and the redistribution of topsoil. In addition, since the mass of LF was really small, it could be lost during SOM fractionation processing in the lab. Some of LF mass might also still be protected inside aggregate and was not released during lab procedure. For HF mass, large aggregates of cropland had the highest HF masses, while moderately-sized aggregates of cropland had the lowest HF masses among land uses. The opposite happened to the edge. While grassland had the same amount of HF in both large and moderately-sized aggregates. Since all land uses had similar LF masses, suggesting that most SOM of cropland was from large aggregates, and most SOM of the edge was from moderately-sized aggregates, and there was a balance in SOM from two aggregate fractions for grassland. Since SOM in smaller aggregates is more stable and decomposed slower than that in larger aggregates (Rabbi et al., 2014), these findings were consistent with the result that the edge had higher TC and TOC than cropland.

Even though both the individual effects of land use and SOM fraction pools on SOM fractions masses of two main aggregate size fractions across land uses were significant, land use only impacted C content of these SOM fractions significantly. While SOM fraction pools remained a primary factor affecting C and N content, and OC:TN ratio of these SOM fractions. Like SOM fraction masses, all HF had higher C and N content than LF. Which were not surprised since HF mass of all land uses was nearly 10 times higher compared to LF masses. In addition, OC:TN ratio of HF was also higher than that of LF. Since LF decomposes faster than HF (Malhi et al., 2003), OC:TN ratio of LF is more balanced. Despite having similar amounts of OC, the edge had more C content in SOM fractions of two main aggregate size fractions than grassland, suggesting that grassland might have more C content in small aggregate size fraction than the edge.

3.6.3. Readily mineralizable C of different aggregate sizes fractions across land uses

In our study, land use was the main factor regulating C mineralization, with the two main aggregate size fractions of grassland mineralized more C per g TOC than that of cropland starting from week two of the incubation. The effect of land use got stronger as time went on. Neither aggregate size nor the combination of land use and aggregate size affected cumulative C mineralized significantly. This result suggested that SOC of grassland was more degradable than SOC of cropland, especially when cropland had higher HF mass compared to grassland. Land uses with different vegetation covers had different SOM quantity, quality, and decomposition rates (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013), which would influence root respiration, and microbial activities differently (Raich and Tufekcioglu, 2000). In addition, soil microbial activities, nutrient cycling, and soil structure were disrupted due to agriculture activities in cropland, leading to an increase in C mineralization (Ronsenzweig et al., 2016). Therefore, most of the more decomposable C in cropland had been mineralized in the field due to cultivation. Fertilization also contributes to the differences in C mineralization between land uses (Sun et al., 2013). As mentioned above, cropland in SDNWA were previously tilled, and currently under impact of heavy farm equipment and N fertilizer. Our results also showed that cumulative C mineralized were correlated positively with OC, TC, and OC:TN throughout all 28 days of incubation (Table A.4). Total OC, TC, and OC:TN ratio were also significant different among land uses, suggesting that the amount of C and OC:TN ratio were the two main soil properties that controlling C mineralization in our study. Overall, in terms of C mineralization, the two main aggregate sizes of grassland were less stable than that of cropland.

3.6.4. Potentially mineralizable N of different aggregate sizes fractions across land uses

In contrast to C mineralization, aggregate size was the main factor controlling N mineralization of two main aggregate size fractions, not land uses. In addition, the combination of land use and aggregate size also impacted cumulative net N mineralized significantly. Regardless of land use, large aggregates mineralized 1.4–2.5 times more N per g TN than moderately-sized aggregates, and the differences decreased as time went on in the incubation. Suggesting that the proportion of decomposable N in the N pool of large aggregates size fraction was higher than that of moderately-sized fraction, but it was quickly mineralized at the beginning of the incubation. If the incubation was longer, there might not be any differences between aggregate sizes fraction after a couple weeks of incubation. This result is consistent with previous finding that larger aggregates mineralized more SOM than smaller aggregates (Dexter, 1988; Besnard et al. (1996); Franzluebbers and Arshad, 1997; Rabbi et al., 2014). Larger aggregates have higher porosity, creating more favorable condition for aerobic microorganism activities (Dexter, 1988). High water capacity and slow diffusion of oxygen in smaller aggregates contribute to the slow SOM decomposition (von Lützow et al., 2006). There were no differences in cumulative net N mineralized between moderately-sized aggregates of cropland and grassland, suggesting that both land uses had similar proportions of easily transform N in the N pool of moderately-sized aggregate fraction. However, for large aggregates, cropland mineralized more N per g TN than grassland, despite having less TN. Which was not surprised since LF of cropland had significantly lower C:N ratio than that of grassland ($p < 0.05$). In addition, cropland also had lower TN in whole soil compared to grassland. Therefore, overall, cropland was less stable in terms of N mineralization compared to grassland. Cumulative net N mineralized in our study only correlated with TN for the first 14 days of incubation ($p < 0.05$, Table A.4) since land use was only secondary factor controlling N mineralization compared to aggregate size.

3.7. Conclusion

In conclusion, land use and soil depth were the two primary factors influencing soil physicochemical properties at the edge between grassland and cropland at SDNWA, which supported our first hypothesis. However, in the topsoil (0–12 cm), only TOC, TC, TN, and OC:TN ratio declined gradually from grassland to cropland across the edge, bulk density did not show any differences between land uses. Therefore, only half of our second hypothesis was supported. Land use management has altered soil properties significantly since the use of farm equipment has disrupted soil structure, breakdown soil aggregates, and expose SOM protected within to microbial communities. Removal of above ground biomass can also decrease SOM overtime. However, cropland surprisingly had the highest distribution of large aggregates overall despite being affected by tillage in the past, and heavy farm equipment. There are several reasons that could lead to this result including the dry-sieving technique, high amount of clay particle and biding substances, and snow cover on bared topsoil. In addition, most of the differences in large aggregate size distribution of cropland compared to other land uses came from deeper soil, while both grassland and cropland had similar proportions of large aggregates in topsoil.

For SOM fractionation of two main aggregate sizes fractions, land use continued to impact SOM fraction mass and C content of these fractions significantly, but SOM fraction pool was the main factor controlling everything including fraction masses, C and N contents, and OC: TN ratio of these fractions. All land uses had the same amount of LF due to topsoil redistribution on the undulating landscape of SDNWA, lost during lab processing, and some OLF was not released during lab procedure. Cropland appeared to have the most amount of SOM in large aggregate fraction, while the edge had the most SOM in moderately-sized aggregate fraction, and grassland had a balance in SOM distribution among two main aggregate size fractions. Since LF is decomposed much faster than HF, all land uses had higher amounts of HF than LF, this was also reflecting in C and N content, and OC:TN ratio results.

Land use was the primary factor controlling C mineralization of two main aggregate size fractions, but aggregate size was the main factor controlling N mineralization of those. The proportion of decomposable C in SOM pool of two main aggregate size fractions of grassland was higher than that of cropland. There are a few land use management factors that can impact C mineralization including different vegetation covers, the quality and quantity of litterfall, substrate availability, tillage, applying fertilizers, and the use of heavy farm equipment, all can impact the activities of soil microbial communities to mineralize SOM. Regardless of land uses, large aggregates mineralized more N per g TN than moderately-sized aggregates since large aggregates had higher porosity which created more favorable conditions for microbial activities. Despite having lower TN in whole soil, large aggregate fraction of cropland decomposed more N per g TN than that of grassland, and moderately-sized aggregate fraction of both land uses mineralized similar amounts of N per g TN, suggesting that cropland was less stable than grassland in terms of N mineralization.

4. Biological stability of soil organic matter and its relation to soil resilience to climate change at a Grassland-cropland edge in Saskatchewan

4.1. Preface

The previous chapter has demonstrated that there were differences in soil properties associated with different land use type along transects established on a grassland-cropland edge at SDNWA, Saskatchewan. Land use has a significant effect on readily mineralizable C of two main aggregate size fractions in a 28-day incubation. However, we still did not know which land use would be more stable to cope with climatic conditions change (i.e., changing temperature and moisture conditions). Since biological stability of SOM is one of the main indicators for soil resilience, in this chapter we aim to access the biological stability of SOM within each land use under different climatic conditions to answer the question above. We measured the SOM biological stability in short-term aerobic laboratory incubations under different temperature and moisture conditions. Findings from this study will help to determine the effect of climate and land use on SOM stability and to improve predictions about soil resilience and changes in C and N storages at the grassland-cropland edge that exist within the prairie-forest ecotone in Saskatchewan.

4.2. Abstract

Soil organic matter is an important indicators of soil quality and resilience that can be altered by many factors including land use management and climate. There is a need to evaluate the stability of SOM from different land use management in a warmer climate scenario to help predict the recovery of soils that may undergo agriculture conversion in the future. The current edge between native land and cultivated land may the first area to be converted to cultivation under warming climate. To determine which land uses at this edge would be more stable under land use change induced by climatic conditions change, we evaluated the individual and combination effect of land use, temperature, and moisture conditions on readily mineralizable C, and potentially mineralizable N through a series of 28-day incubations at 2 different temperature (25 and 30^{o}C), and 3 moisture conditions (25, 35, and 75% of water-filled pore space) of soils from an edge between grassland and cropland in Saskatchewan. Our results showed that both C and N mineralization were susceptible to temperature and moisture conditions. Regardless of land use and moisture conditions, all soils mineralized more C per g TOC and N per g TN at higher temperature throughout the incubation. Soil moisture was only secondary factor influencing C mineralization compared to temperature. We found that 35% WFPS level was the optimum moisture conditions for microbial activities, and substrate availability for C mineralization in our studies. Similarly, optimum moisture condition ranged from 35% to 50% WFPS level for N mineralization at 25^{o}C. Land use was another primary factor controlling C mineralization beside temperature, but only secondary factors influencing N mineralization compared to climatic conditions. The edge and grassland appeared to be less stable than cropland in terms of C mineralization, but cropland was less stable than grassland and the edge in terms of N mineralization. Our results also suggested that the edge was more susceptible to temperature than grassland and cropland, since only the edge had significant differences between C mineralized per g TOC at 25^{o}C and that at 30^{o}C. Both climatic conditions and land uses can alter SOM biological stability through various mechanics involve altering root respiration, and microbial decomposition rate and conditions. Understanding these mechanisms, couple with evaluating SOM biological stability, would help maintaining adequate amount of SOM to sustain soil health and resilience.

4.3. Introduction

Understanding SOM responses to climate change is important for predicting and maintaining adequate SOM to sustain soil quality and ecosystem functioning. Global MAT is predicted to increase within the range of 0.9–1.8°C for the period of 2021–2025 (relative to 1850–1990; WMO, 2021). This change in the global temperature would increase C and N mineralization (Purton, 2015), leading to changes in the soil C pool balance. In addition, there are many factors that can influence the stability of SOM. Therefore, there is a need to evaluate the persistence of SOM in a warmer climate regardless of these factors to make a better prediction of future SOM stocks, especially in regions that are likely to go through soil conversion due to climate change.

The edge boundaries between land uses within prairie-forest ecotone in the Canadian prairies are one of those regions that are likely to be converted to cultivation due to climate change. While the global MAT will increase at least 1°C for the period of 2021–2025 (relative to 1850–1990) with 90% chance of at least one year during this period will become the warmest year on the record (WMO, 2021), the MAT in southern Saskatchewan is expected to increase 3°C by 2050 (relative to 1961–1990), with seasonal temperature change range from 2–4.5°C (Barrow, 2009). In addition, the annual total precipitation is predicted to increase 100–150 mm by 2050 (relative to 1961–1990), with seasonal change ranging from 2–17% (Barrow, 2009). Although, the total precipitation is expected to increase in the future, the evapotranspiration will likely rise due to warmer temperatures which would overwhelm the effect of increased precipitation (Sauchyn and Kulshreshtha, 2008; Barrow 2009). Overall, the climate in this region will be drier leading to higher moisture stress. As a result, agricultural practices would shift northward to cooler climate areas, which may lead to a rapid shift in dominant plant species (Williams et al., 2009). The current ecotone and current anthropogenic edges between cropland and native land will shift with the agriculture.

Soil organic matter decomposition can be influenced by both climate and land use. Climate can alter SOM stock through changing temperature and moisture conditions. Root respiration and microbial decomposition are the two mains SOM decomposition processes in soil. Both processes are temperature dependent and can be affected by water limitation (Davidson and Janssens, 2006). In addition, temperature also controls the rate of chemical processes related to SOM stabilization, changes the adsorption of SOM to mineral surface, and regulates enzyme production. Moisture condition can affect the substrate availability for decomposition through changing the thickness of soil water films, and controlling oxygen diffusion (Davidson and Janssens, 2006; Paré et al., 2006). Studies about the effect of climate on soil mineralization mostly focus on temperature, a few focus on moisture conditions. However, there are conflicts among these studies in both lab and field experiments because there are other environmental conditions that can affect soil mineralization as well (Conant et al., 2011). Many studies have suggested that SOM stabilization also depends on vegetation types (Paré et al., 2006). Different vegetation types would create different carbon input into the soil and alter the microbial community compositions and diversity which are critical to SOM decomposition process (Raich and Tufekcioglu, 2000). Land use can influence SOM pool not only through changing vegetation type but also through surface disturbances which lead to differences in soil structures (Six et al., 2002). A loss in SOM due to land use conversion has been reported by many researchers (eg., McGill et al., 1988; Campbell and Souster, 1982; Ellert and Gregorich, 1996; Smith et al., 2000)

Our goal was to determine which land use at a grassland-cropland edge would be more stable under changing temperature and moisture conditions using the biological stability of SOM. To accomplish this goal, we evaluated the effects of land use, temperature, and moisture conditions on SOM biological stability (i.e., readily mineralizable C and potentially mineralizable N) of soil across a grassland-cropland edge in moist-mixed grassland and aspen parkland ecozone in Saskatchewan. Characterizing the susceptibility of SOM labile pool to degradation for each land use will assist with making predictions regarding soil resilience across land uses under climate change. Our hypotheses for this chapter are listed below:

1. Across the grassland-cropland edge, C and N mineralization increase with increasing temperature regardless of land use and moisture conditions.
2. Grassland is more stable to changes associated with climate change (i.e., temperature and moisture conditions) than cropland and the edge in terms of SOM decomposition.

4.4. Material and methods

4.4.1. Site and sampling design

Soil samples were collected at St. Denis National Wildlife (SDNWA) within the boundary between Aspen Parkland and Moist Mixed Grass eco-regions, about 40 km east of Saskatoon. Mean temperatures of this area are + 25°C in July and -22°C in January, with mean annual precipitation of 350 mm (Henderson, 2013). There are 3 main land uses in SDNWA: restored grassland, native grassland, and cultivated land. Soil samples from the cropland area were classified as Rego Dark Brown Chernozem and Orthic Black Chernozem, and soils from grassland area was classified as Gleyed Calcareous Black Chernozem.

The samples were taken at SDNWA in June 2017, after cropland area was seeded with Flax (*Linum usitatissimum* var. CDC Sorrel) for the 2017 growing season in May. Glyphosate was applied to cropland prior to seeding, and granular fertilizer (80 N - 32 P - 15 S lbs/acre) was used at the time of seeding. Restored grassland area was seeded in 1983 with non-native mixtures for hay harvesting once a year. The mixture consisted of mostly smooth brome (*Bromus inermis* Leyss.), alfalfa (*Medicago stavia* L.), Kentucky bluegrass (*Poa pratensis* L.), and quackgrass (Elymus repens L. Gould). The edge is unmanaged, and consists of mostly weed species including Canada thistle (*Cirsium arvense* L. Scop.), cleavers (*Galium aparine* L.), flixweed (*Descuriana sophia* L.), and perennial sow thistle (*Sonchus arvensis* L.). The edge did not move or change in size between 2017 and 2018.

Three transects spanning grassland and cropland were established at SDNWA. For the experiments in this chapter, we chose surface soil samples (0–12 cm) collected at point 11 m, 33 m, and 100 m from the center of each transect (point 0 m, the edge) symmetrically toward both sides to represent cropland, and grassland (Fig. 3.3). To represent the edge, we selected surface soils taken at the center of each transect and at point 25 cm from the edge toward both sides. There were 27 surface soil samples in total (3 samples/transect * 3 land uses * 3 transects), with 9 surface soil samples to represent each land use. To determine bulk density, another soil core was collected at point 0 m, 11 m, 33 m, and 100 m in all 3 transects.

4.4.2. Soil properties

Approximately 150 g of samples were air-dried and ground prior to physicochemical analyses. Total carbon (TC) and total nitrogen (TN) were measured by combustion using LECO CNS-2000 (LECO Corp, St. Josepth, MI). Total organic carbon (TOC) was measured by combustion using LECO CR-12 (LECO Corp, St. Josepth, MI), samples were acidified 6% (w/v) sulfurous acid (H2SO3) to remove inorganic carbonates prior to the analysis (Skjemstad and Baldock, 2007). Soil pH was measured in 1:2 soil:$CaCl_2$ 0.01M suspension (Hendershot et al., 2008), and EC was measured in 1:5 soil:water suspension (Miller and Curtin, 2008). Field-moist soil samples were separated into 3 aggregate size fractions (large: > 2000 µm; moderately-sized: 2000–150 µm; small: < 150 µm) using a sieving machine (W.S. Tyler's RO-TAP® RX-29) for 7 minutes.

4.4.3. Readily mineralizable carbon and potentially mineralizable nitrogen under different temperature and moisture conditions

Readily mineralizable C and potentially mineralizable N from soils from the edge, grassland, and cropland under different temperature and moisture conditions were assessed using the procedures described by Hopkins (2008), and Curtin and Campbell (2008) (respectively), and both procedures were modified by Purton (2015). There were 2 temperatures (25°C and 30°C) and 3 water-filled pore space levels (WFPS) (25%, 35%, and 50%), providing a total of 6 different sets of experimental conditions. Porosity was calculated from bulk density.

For readily mineralizable C, 15 dry-weight equivalent grams of field-moist soil were placed into a 30-dram vial, and then adjusted to according WFPS levels using deionized water. The vial was covered with perforated parafilm, and incubated for 28 days at according temperature mentioned above and 85 to 90% relative humidity. Samples were preincubated for 7 days to allow reaching the equilibrium. Throughout 28 days of incubation, to determine weekly respiration rate, each week we removed the parafilm, and put the soil vial into a mason jar with 10 ml vial of deionized water. Immediately after sealing the jar, we took the background gas sample using a polypropylene syringe, and transfer the gas into a 12 ml evacuated glass Exetainer® vial fitted with silicone and rubber septa (Labco Ltd., U.K.). Another gas sample was taken after 6 hours of incubating in a sealed jar for the first two week of incubation, and after 24 hours for the rest. We then analyzed CO_2 concentration of the gas using a Varian CP-4900 Micro Gas Chromatograph (Varian Inc.). Moisture was adjusted to according WFPS levels weekly with deionized water. We used ideal gas law to convert CO_2 concentration from ppm to mg CO_2-C, pressure was considered constant at 101.3 kPa. Cumulative respirations during 28-day incubation were calculated using Eq. 3.2 (Paré et al., 2006). All results were standardized to per g OC, and unstandardized results (mg CO_2-C per kg^{-1} soil) were included in Appendix A.

For potentially mineralizable N, we mixed 15 dry-weight equivalent grams of field-moist soil with acid-washed GRANUSIL® sand (GHP system, Inc.,) at a 1:1 ratio. In a Buchner funnel, we put a Whatman® type GF/B glass microfiber pad (GE Co.,) on top of 30 µm nylon mesh and another layer of the same glass microfiber pad. The soil:sand mixture was then placed on top of the filter assembly in the funnel and another GF/B glass microfiber pad was placed on top of the mixture. We then attached the Buchner funnel to a Buchner flask, and pre-leached the sample before incubation. We used deionized water to adjust the sample moisture to according WFPS levels. Perforated parafilm was used to cover the funnel during 28 days incubation at according temperature mentioned above and 85 to 90% relative humidity, which are the same conditions used for the readily mineralizable C incubation. We extracted the sample with 100 mL 0.01 M $CaCl_2$ and 25 mL N-free nutrient solution every week. A Technicon AutoAnalyzer (Technicon Industrial Systems) was used to measure NO_3- and NH_4^+ in the extracted solution. Moisture was adjusted to according WFPS levels weekly with deionized water. All results were standardized to per g TN, and unstandardized results (mg N per kg^{-1} soil) were included in Appendix A.

4.4.4. Statistics

The statistical analyses were conducted using SAS® Studio (SAS Institute). The effect of land use on physicochemical properties was assessed using the Mixed procedure, and micro scale transects were nested within main transects. Turkey's HSD was used for post-hoc comparisons ($p < 0.05$). Shapiro-Wilk's test was used to test for the normality of variables, and log transformation was used to transform data with non-normal distribution of residual.

The completely randomized design model with 3 factorial arrangements was used to test for land use, temperature, and soil moisture effects on cumulative C mineralized, and net N mineralized as following:

*Y = mean + land use (L) + temperature (T) + soil moisture (M) + T*M interaction + L*T interaction + L*M interaction + L*T*M interaction + error* Eq. 4.1

Y: cumulative C mineralized or net N mineralized after 28 days incubation

Mixed procedure of SAS® Studio (SAS Institute) was used to analyze this model with transect as random effect, and micro scale transects were nested within main transects. Normality of variables were tested using Shapiro-Wilk's test, data with unnormal distribution of residual were transformed prior to analysis using log transformation. Post-hoc comparisons were performed using Tukey's HSD to determine the significance of differences between LSmeans ($p < 0.05$).

4.5. Results

4.5.1. General soil characteristics

The differences between land uses were evident for surface soils including TOC, TC, TN, the distribution of large and moderately-sized aggregates, pH, and bulk density (Table 4.1). Land use had a significant effect on TOC ($p < 0.001$), with the highest amount observed in the grassland. Similarly, land use also impacted TC and TN significantly, with the amount of TC declining in following the order: grassland > edge > cropland. For aggregate size distributions, grassland had the highest distribution of large aggregates ($p < 0.05$), which was 1.9 times greater than the lowest distribution of large aggregates size observed in the edge. In contrast, the distribution of moderately-sized aggregates in the edge were 1.7 times greater than that in grassland ($p < 0.05$). In addition, pH was also significantly different between land uses ($p < 0.05$), following the same order as TOC (Table 4.1). The OC:TN ratio, small aggregates size distribution, and EC were not significantly different among land uses.

Table 4.1. The effect of land use on properties of surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area. Values are means (±SD) and different letters within a row represent significant differences (TukeyHSD post hoc, $p < 0.05$). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). †Statistic done on Log transformed data.

Land use	TOC	TC	TN	OC:TN	> 2000µm	2000 - 150µm	< 150µm	pH	EC	Bulk density
		— g kg^{-1} —				Aggregate size distribution (%)			dS m^{-1}	g cm^{-3}
Grassland	34.6±6.0^{a}	40.0±7.8^{a}	3.4±0.3^{a}	10.1±1.4^{a}	59.9±26.7^{a}	33.7±21.0^{b}	7.5±8.2^{a}	7.1±0.4^{a}	0.29±0.32^{a}	1.18±0.26^{ab}
Edge	31.0±6.9^{b}	36.2±8.3^{b}	3.3±0.9^{a}	9.4±0.8^{a}	31.4±10.6^{b}	55.9±6.7^{a}	12.7±9.2^{a}	6.7±0.2^{b}	0.28±0.25^{a}	0.92±0.10^{b}
Cropland	23.2±2.8^{b}	27.0±3.2^{c}	2.4±0.4^{b}	9.8±1.5^{a}	52.1±16.3^{ab}	43.3±11.1^{ab}	4.5±6.8^{a}	6.7±0.2^{b}	0.1±0.1^{a}	1.3±0.2^{a}
Statistical Analysis						F value				
Land use effect	12.19*	28.58***	6.82*	0.44	5.12*	5.41*	2.51	5.95*	1.77	4.31*

4.5.2. Readily mineralizable carbon

Land use, temperature, and soil moisture all impacted cumulative C mineralized significantly throughout a 28-day incubation (Table 4.2). Overall, except for day 14, the edge and grassland produced similar amount of C-CO_2 per g TOC throughout the incubation time ($p > 0.05$), which was 1.5–1.6 times higher than the lowest amount of cumulative C mineralized observed in cropland. Additionally, under all moisture conditions, all soils mineralized more C per g TOC at higher temperature ($p < 0.05$) during the incubation (Fig 4.1). Higher amounts of mineralized C were also observed at the highest moisture condition (50 % WFPS), which were 1.2–1.3 times higher than the amount of mineralized C at the lowest moisture conditions (25% WFPS) ($p < 0.05$). However, the difference between the amount of C mineralized at 50% WFPS and that at 25% WFPS was reduced over the course of the incubation. At the end of the incubation, C mineralized at different moisture conditions was not significantly different ($p > 0.05$) (Table 4.2).

Although land use and temperature are two main factors controlling C mineralization, the combination of land use and temperature did not have a significant effect on cumulative C mineralized during the 28-day incubation (Table 4.2). However, the combined effect of temperature and moisture condition on cumulative C mineralized was significant for the first week of incubation ($p < 0.05$). In addition, the interaction of land use and moisture condition also impacted C mineralization significantly for the first two weeks of incubation ($p < 0.05$). While the edge produced the highest amount of C at 35% WFPS ($p < 0.05$), cropland produced the least amount of C at 25% WFPS ($p < 0.05$), and grassland produced similar amount of C at all WFPS levels ($p > 0.05$) (Fig 4.1). Lastly, the interaction of all three factors (land use, temperature, and moisture conditions) only influenced cumulative C mineralized significantly at week 2 of the incubation (Table 4.2). At 25^oC, all soil produced similar amount of CO_2-C per g TOC at all moisture conditions ($p > 0.05$) (Fig 4.1). Similar results were observed for 30^oC and 50% WFPS condition. But at 30^oC and 25 or 35% WFPS level, cumulative C mineralized at the edge was higher than that of cropland ($p < 0.05$).

Table 4.2. Mean (±Standard deviations) of cumulative C mineralized (mg CO_2-C g^{-1} TOC) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25^{o}C and 30^{o}C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***). †Statistic done on Log transformed data.

WFPS level (M)	Land use (L)	Temperature (T)							
		25^{o}C				30^{o}C			
		Day 7	Day 14	Day 21†	Day 28	Day 7	Day 14	Day 21	Day 28
25%	Grassland	0.7±0.3	1.6±0.6	3.6±1.2	6.7±2.6	2.6±0.9	7.8±2.5	17.1±6.7	28.4±12.6
	Edge	0.6±0.3	1.5±0.8	3.1±1.9	5.8±3.6	2.9±1.0	8.7±2.6	16.5±5.1	26.0±8.2
	Cropland	0.3±0.1	0.7±0.4	1.5±0.9	3.0±1.6	2.0±0.5	6.3±1.5	12.0±2.9	18.3±4.6
35%	Grassland	0.6±0.1	1.7±0.4	4.0±1.1	7.3±2.4	3.0±1.1	8.8±3.0	17.5±5.4	27.8±8.8
	Edge	0.5±0.2	1.6±0.8	3.8±2.0	7.0±3.9	3.8±1.5	10.8±3.8	19.8±6.9	29.8±10.2
	Cropland	0.4±0.2	1.0±0.6	2.4±1.0	4.4±2.1	2.5±0.6	7.6±1.5	14.9±3.2	23.5±5.8
50%	Grassland	0.8±0.2	2.1±0.5	5.0±1.5	9.5±3.4	3.3±1.4	9.3±3.6	18.0±6.6	28.1±9.5
	Edge	0.5±0.3	1.6±0.8	4.6±2.8	9.3±5.8	2.9±0.9	8.3±3.1	15.4±7.0	23.9±10.7
	Cropland	0.4±0.2	1.2±0.6	3.0±1.7	5.5±3.4	3.3±0.7	9.9±2.1	18.0±4.9	26.4±7.1

Type 3 Tests of Fixed Effects

Effect	df	F value			
		Day 7	Day 14	Day 21	Day 28
L	2	9.34**	11.02***	9.70**	13.88***
T	1	789.75***	862.48***	581.18***	507.69***
M	2	6.45*	4.88*	3.75*	2.35
T*M	2	5.01*	2.21	0.76	0.95
L*T	2	1.17	0.75	0.09	0.12
L*M	4	2.68*	3.19*	1.59	1.29
L*T*M	4	2.13	2.58*	1.66	1.64

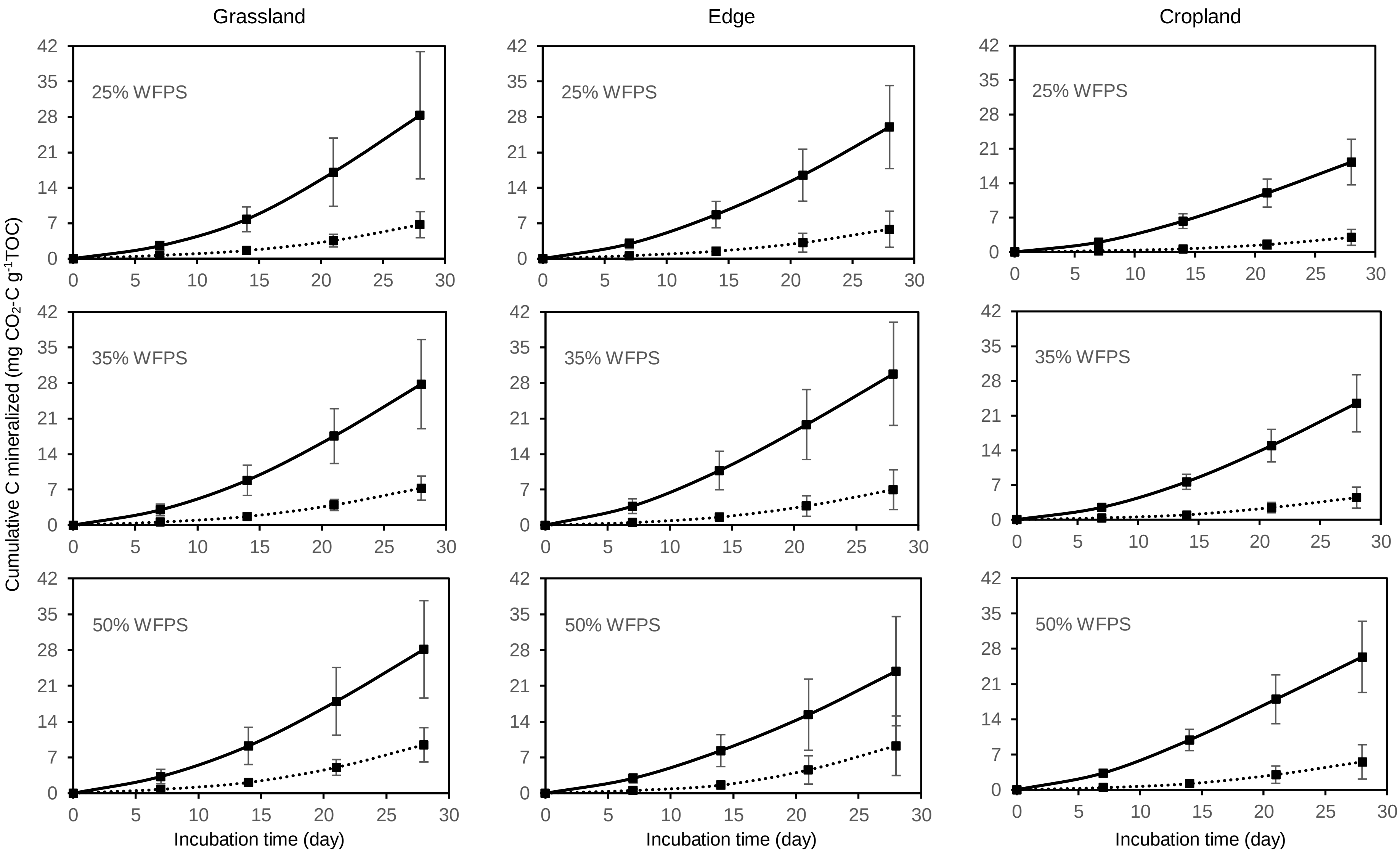

Fig 4.1. Cumulative C mineralized of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25^{o}C and 30^{o}C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level). Lines represent mean of nine subsamples. Bars are standard deviations. Full line and dots line represent 30^{o}C and 25^{o}C, respectively.

4.5.3 Potentially mineralization nitrogen

Similar to C mineralization, land use, temperature, and moisture conditions all had a significant impact on N mineralization throughout a 28-day incubation (Table 4.3). However, land use only influenced cumulative N mineralized significantly for the first two weeks of incubation ($p < 0.0001$, and $p < 0.05$, respectively). In contrast to C mineralization, cropland mineralized the most amount of N per g TN ($p < 0.05$) for the first two weeks of incubation, while the edge and grassland mineralized similar amounts of N per g TN ($p > 0.05$). But in agreement with C mineralization, the highest amount of N mineralized overall was observed at the highest temperature (Fig 4.2) throughout the incubation. Additionally, the least amount of N mineralized was also observed in the lowest moisture condition (25% WFPS level) during all 28 days of incubation, with the highest amount of N mineralized observed at 35% WFPS level for the first two week, and at 50% WFPS level for the rest of the incubation ($p < 0.05$) (Fig 4.2).

The combination of land use and temperature showed a significant influence on N mineralization during the entire time of the incubation, and the combination of all three factors did not have any significant effect on N mineralization (Table 4.3). At 30^{o}C, all soils mineralized the same amount of N per g TN. But at 25^{o}C, cropland and grassland mineralized similar amount of N per g TN, which was 1.6–2.4 times higher and the lowest amount of N mineralized observed in the edge ($p < 0.05$) (Fig 4.2). In addition, only the edge mineralized more N per g TN at 30^{o}C than at 25^{o}C significantly throughout all 28 days of incubation, with grassland displaying similar result only in the last two weeks of incubation ($p < 0.05$).

The combined effects of temperature and soil moisture on N mineralization was significant for the first three weeks of incubation ($p< 0.05$), and the interaction of land use and soil moisture also impacted N mineralized significantly for the first two weeks of incubation (Table 4.3). At 25^{o}C, the amount of cumulative N mineralized was lowest at 25% WFPS, while a similar amount of cumulative N mineralized was observed across all WFPS level at 30^{o}C (Fig 4.2). N mineralized was significantly different between land uses only at 35% WFPS level, with cropland mineralizing 1.2–1.9 times more N per g TN than the edge ($p < 0.05$). While at 25 and 50% WFPS levels, all land uses had a similar amount of cumulative N mineralized (Fig 4.2).

Table 4.3. Means (±Standard deviations) of cumulative net N mineralized (mg N g^{-1} TN) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25°C and 30°C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level). Significant p-value at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***).

WFPS level (M)	Land use (L)	Temperature (T)							
		25°C				30°C			
		Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
25%	Grassland	6.7±3.8	11.0±4.6	12.4±5.1	14.5±5.4	14.6±5.9	17.6±6.9	22.3±8.4	26.1±9.8
	Edge	5.2±1.8	8.2±2.3	8.9±2.6	11.0±3.1	25.8±13.6	27.2±14.1	29.1±14.8	34.2±16.4
	Cropland	8.7±8.4	10.8±8.3	11.4±8.3	13.2±8.4	21.6±10.2	23.3±10.7	26.4±11.8	28.3±12.7
35%	Grassland	14.3±4.5	20.6±3.6	22.0±4.4	24.3±5.4	19.0±8.4	21.5±9.2	24.2±9.7	31.7±16.4
	Edge	4.4±1.5	8.4±1.7	9.7±2.4	13.5±4.2	19.2±8.4	21.8±7.2	25.8±8.1	32.6±9.4
	Cropland	24.5±13.8	27.8±13.8	28.6±13.9	30.3±14.2	24.8±11.7	26.1±12.2	27.7±12.8	32.4±13.7
50%	Grassland	10.5±5.3	14.6±4.8	15.7±5.1	21.1±5.7	11.0±5.4	14.6±6.0	24.0±7.0	30.2±8.2
	Edge	9.9±6.8	10.6±6.8	13.6±6.4	18.9±6.5	17.3±10.2	21.8±10.8	30.0±13.9	36.4±16.9
	Cropland	18.2±9.6	25.7±9.5	26.1±9.7	29.2±10.3	17.5±7.7	20.1±8.8	26.9±11.4	31.4±13.2

Type 3 Tests of Fixed Effects

Effect	df	F value			
		Day 7	Day 14†	Day 21†	Day 28
L	2	10.07***	7.13*	3.02	0.46
T	1	45.01***	35.92***	68.39***	58.18***
M	2	4.95*	6.92*	9.27**	10.32***
T*M	2	10.29***	7.86**	7.07*	2.79
L*T	2	4.92*	15.37***	11.9***	7.54**
L*M	4	2.72*	3.09*	2.23	0.83
L*T*M	4	1.29	1.12	1.65	0.95

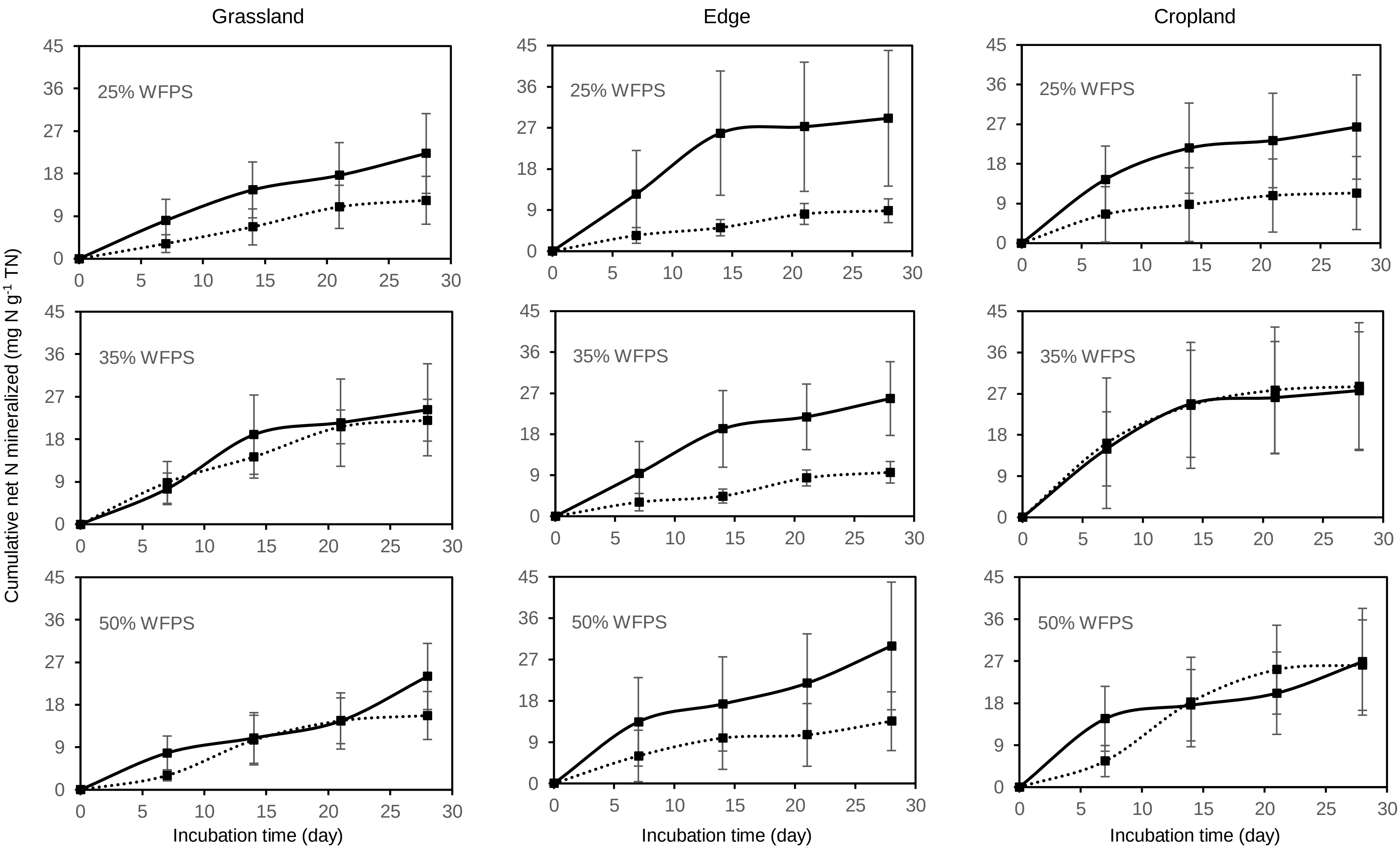

Fig 4.2. Cumulative net N mineralized of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25ºC and 30ºC) and three different moisture conditions (25, 35, and 50% of water-filled spore space level). Lines represent mean of nine subsamples. Bars are standard deviations. Full line and dots line represent 30ºC and 25ºC, respectively.

4.6. Discussion

Land use and temperature appeared to be the two main factors that control both C and N mineralization of soils at the edge between grassland and cropland at SDNWA, while the moisture effect was different between C and N mineralization. Soil moisture was only a secondary factor that impacted C mineralization, but was a primary factor that influenced N mineralization. Among all three land uses, cropland had the lowest amount of TOC and lowest amount of cumulative C mineralized per g TOC. Even though, the edge had similar amount of TOC compared to cropland, the edge still mineralized similar amount of C per g TOC compared to grassland. In contrast to C mineralization, cropland had the lowest amount of TN but mineralized the most N per TN throughout all 28 days of incubation. For climatic condition changes, soils incubated at higher temperature or higher moisture condition mineralized more C and N. Overall, N mineralization is more susceptible to climactic conditions than C mineralization. However, readily mineralizable C and potentially mineralizable N are not parallel measurements. The incorporation of the immobilization process in net N measurement but not C mineralization might create the differences in the reaction of C and N mineralization to climate conditions change (Purton et al., 2015). Increasing temperature could increase microbial activity or microbial growth. Therefore, depending on the substrate availability of the soil, increasing temperature could increase N mineralization and/or N immobilization (Miller and Geisseler, 2018), making it difficult to compare between net N mineralized and C mineralized.

4.6.1 Carbon mineralization response to climatic and land use changes

Temperature had a significant effect on cumulative C mineralized throughout all 28 days of incubation ($p < 0.0001$), higher temperature produced higher cumulative C mineralized. Regardless of land uses and soil moisture conditions, our study showed that cumulative C mineralized per g TOC increased 5.8–10 times when increasing incubation temperature from 25^{o}C to 30^{o}C. Many previous studies have reported the same results and agree that increasing temperature would increase decomposition rate, but to what degree is dependent on other factors of the studies. In a 364-day incubation, C respiration rate of both wheat and grass soils increased 1.58–1.88% when increasing incubation temperature from 25 to 35^{o}C (Conant et al., 2008). Similarly, Wang et al. (2016) found an exponential increase in SOM decomposition rate when increasing incubation temperature. Parihar et al. (2019) also reported that SOC mineralized nearly doubled when increasing temperature from 27^{o}C to 37^{o}C. Since temperature can control SOM decomposition directly by altering root respiration and microbial decomposition (Davidson and Janssens, 2016) or indirectly through changing physical and chemical protections of SOM (Gillabet et al., 2010), and microbial composition, and enzyme activities (Jassens and Pilegaard, 2003; Wang et al., 2016), temperature is certainly one of the main factors that controls C mineralization. Thus, half our first hypothesis was supported, C mineralization increases with increasing temperature, regardless of other factors.

Rather than temperature, moisture is another principal environmental factor that controls SOM decomposition (Ise and Moorcroft, 2006). Like temperature, soil moisture can alter SOM decomposition by various mechanics including changing oxygen diffusion, soil water films thickness, and substrate availability (Davidson and Jassens, 2016). The important role of soil moisture on microbial decomposition is also well recognized (Parihar et al., 2019). Our results showed that there was an average increase of 1.2–1.3% in cumulative C mineralized per g TOC with increasing WFPS level at all temperatures and land uses. However, cumulative C mineralized only increased significantly when increasing from 25% WFPS level to higher WFPS level ($p < 0.05$), there was no difference in C mineralization between 35% and 50% WFPS level ($p > 0.05$). Therefore, 35% WPFS level might be within the optimal moisture condition for microbial activities, and substrate availability for C mineralization of our studied soils. But at the end of the incubation, moisture condition did not affect C mineralization significantly, suggesting that the effect of temperature, and the effect of land use on C mineralization were stronger than the effect of moisture condition on C mineralization. Similarly, Wang et al. (2016) also found while the response of SOM decomposition to temperature of all soil samples were apparent, SOM decomposition rate responded to soil moisture was more complicated depending on vegetation covers. Thus, compared to temperature and land use, moisture condition was only a secondary factor controlling C mineralization.

Since both climatic conditions impacted C mineralization significantly, it is crucial to also look at the combined effect of these factors on C mineralization. The combined effects of both temperature and moisture conditions only influenced C mineralization significantly at the first week of incubation ($p < 0.05$). Wang et al. (2016) found that SOM decomposition rate at the highest temperature and moisture was approximately 2.6 times higher than that at the lowest temperature and moisture conditions for mountain soils in a 14-day incubation. Previous studies also reported that the simultaneously variations of temperature and soil moisture could explain

89% of temporal variation in CO_2 efflux (Qi and Xu., 2001), and 91% of temporal variation in soil respiration (Rey et al., 2002). However, these studies were either short-term or only used soils from one land use management type, suggesting that land use effect on C mineralization was also stronger than the combined effect of temperature and soil moisture. Additionally, there was potential that longer term incubation might be better to demonstrate the combined effect of temperature and soil moisture since the variation in soil C pool sizes and decomposability to temperature and soil moisture could cause a mistake in interpreting CO_2 fluxes in short-term incubations (Knorr at al., 2005).

Land use also had a significant effect on C mineralization for all 28 days of incubation ($p < 0.001$, and $p < 0.0001$), but the combined effect of land use and other factors did not. Even though the edge and cropland had the same amount of TOC, the edge mineralized more C per g TOC than cropland during the incubation ($p < 0.05$), regardless of climatic conditions. Similarly, grassland also mineralized more C per g TOC than cropland. These results suggested that the proportion of labile C in SOC pool of the edge and grassland was higher than that of cropland. Therefore, in terms of SOC biological stability, the edge and grassland were not as stable as cropland. Land use can impact C mineralization through different vegetation covers (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013). Changing vegetation type would change the quality and quantity of litter supplied to soil, the structure and microclimate of soil, and rate of root respiration (Raich and Tufekcioglu, 2000). A change in the quality and quantity of litterfall would also impact the soil microbial community (Paré et al., 2006), which would affect C mineralization. In a 321-day incubation, Paré et al. (2006) found that even among forest soils, soils with different covers respired different amount of C, with balsam fir soil mineralized 3–6 times more C than sugar maple soil. However, when analyzing existing data on soil respiration rate under different vegetation covers, Raich and Tufekcioglu (2000) found no predictable differences in soil respiration rate between grassland and cropland, contrasting to our results. Based on their data set, they concluded that the effect of vegetation type on soil respiration was just secondary compared to the effect of environment conditions including temperature. But Arevalo et al. (2012) argued that when accessing C mineralization of different land managements, the type, intensity, and duration of the management should also be taken into the evaluation. Raich and Tufekcioglu (2000) did not include the land management as a factor, rather they combined data from both managed and unmanaged grassland to run the comparison analysis. In addition, most of their data were from field studies, not incubation studies, fluctuating in temperature and soil moisture conditions out in the field could also contribute to differences in measurements. With land use management, the conversion of grassland to cultivation can increase SOM decomposition rate by disrupting soil microbial activities, accelerating nutrient cycling, reducing nutrient retention, and compacting soil (Ronsenzweig et al., 2016). In addition, soil structure would be broken down, and physically and chemically protected organic C would be exposed to soil microbes creating favorable abiotic conditions for mineralization (Six et al., 2000; Yannikos et al., 2014). Therefore, most of the more decomposable C in cropland of our study has been mineralized in the field due to cultivation. Fertilization can also contribute to the differences in SOM decomposition in different land use by altering soil microbial community through C:N ratio (Sun et al., 2013). The amount of N fertilizer added combined with substrate chemistry, and initial N mineralization rate control SOM decomposition (Knorr et al., 2005). Cropland area of our study location was fertilized with granular form fertilizer at the rate of 80lbs N acre^{-1}. This could have neutral or negative effect on C mineralization depending on substrate chemistry of the soils. Our results indicated that rather than only one soil property having a strong influence on C mineralization within each land use, all the soil properties were working together and simultaneously controlling SOM decomposition. Overall, land use proved to be one of the main factors that control C mineralization.

4.6.2 Nitrogen mineralization response to climatic and land use changes

Similar to C mineralization, temperature had significant effect on cumulative net N mineralized throughout all 28 days of incubation ($p < 0.0001$). Our study found that when increasing incubation temperature from 25 to 30^{o}C, cumulative net N mineralized per g TN of all land uses increased 1.5–1.7 times, which supported the other half of our first hypothesis. Our results are consistent with previous findings. The rate constant k of N mineralization was reported to be double for every 10-degree raising in temperature in a 24-week incubation, and 35^{o}C was the optimal temperature for N mineralization in North America (Stanford et al., 1973). Campbell et al. (1981) also found a similar result with cumulative net N mineralized and the mineralization rate constants of Australia soils increased with increasing temperature. They assumed the optimal temperature for N mineralization was 40^{o}C for these subtropical soils. Along with similar results observed in Chile soils by Oyanedel and Rodriguez (1977), Campbell et al. (1981) argued that the effect of temperature on the decomposability of SOM is universal in arable soils. Similarly, in a 126-day incubation of forest and grassland soils from the same Aspen Parkland and Moist Mixed

Grassland eco-regions as our study, Sun et al. (2013) reported that cumulative dissolve organic N of forest land increased 2.5 times and of grassland increase 2.7 times when incubation temperature increased 10 degrees. Temperature can control N mineralization through altering microbial activities like C mineralization (Sun et al., 2013). One of the limitations in N mineralization is the release of dissolved organic nitrogen (DON), increasing temperature would help increase microbial activities to release more DON (Chapin et al., 2011). However, N mineralization also depends on the quality of SOM, when C:N ratio of soils is high, soil microbes would immobilize N for their own growth (Cookson et al., 2007), leading to N-limited decomposition (Chapin et al., 2011). But if C:N ratio is low, N may not be limited, and microbes would release N to the soil (Cookson et al., 2007). In our study, the cumulative net N mineralized during 28-day incubation did not correlate with OC:TN ratio ($p > 0.05$, Table A.7) at both 25 and 30^{o}C. We did observe a trend of increasing net N mineralized with decreasing OC:TN ratio at both temperatures, suggesting that OC:TN ratio might have an influence on the effect of temperature on N mineralization. But since our study consisted of three different land uses, there are other factors rather than OC:TN ratio that have stronger influences on the relationship between temperature and N mineralization.

Like temperature, soil moisture plays an important role in N mineralization activities of soil microorganisms (Miller and Geisseler, 2018). Our results showed soil moisture had a significant effect on N mineralization, and the effect got stronger by the end of the incubation (from $p < 0.05$ to $p < 0.0001$). This finding suggested that the microbial communities of our soils were trying to get used to the new moisture condition at the beginning of the incubation, by the end these microbial communities has stabilized, thus the effect of soil moisture on N mineralization became more apparent. Similarly, in short-term incubation of various soils, Stanford and Epstein (1974) found that cumulative N mineralized increased with increasing soil moisture. However, they also reported that 80–90% of WFPS level was the optimum moisture condition for N mineralization. But our result suggested that 35–50% WFPS level was the optimum range of moisture condition for N mineralization of our soils. There were other factors in our study that could interfere with the influence of moisture condition on N mineralization including different temperature and land uses. Stanford and Epstein (1974) only used one temperature in their study (35^{o}C), and did not included soils from different land uses in their incubation. In addition, an increase in denitrification and decrease in activities of aerobic were observed when more than 40–50% of total porosity was filled with water (Rasiah and Kay, 1997), but the percentage of WFPS that set off denitrification varied among soils (Weier et al., 1993).

The combination of temperature and moisture conditions also affected cumulative net N mineralized significantly for the first 21 days of incubation, and the effect got weaker as time went on (from $p < 0.0001$ in the beginning to $p < 0.05$ at day 21). At 25ºC, all soils mineralized the least amount of N per g TN at 25% WFPS level. But at 30ºC, all soils mineralized the same amount of N per g TN at all WFPS levels. Suggesting that the optimal range of moisture conditions for N mineralization was around 35–50% WFPS at 25ºC like the overall result. As for 30ºC, the microbial community in our soils appeared to be more responsive to temperature than soil moisture for N mineralization, and 30ºC was closer to the optimum temperature for N mineralization of North America soils, which is 35ºC (Stanford et al., 1973). Thus, at 30ºC, the effect of moisture conditions on N mineralization was only secondary compared to temperature. By the end of the incubation, the temperature effect completely took over, therefore there was no significant effect from the combination of temperature and soil moisture.

Unlike C mineralization, land use was only a secondary factor controlling N mineralization compared to climatic conditions in our study. Land use only showed significant effect on cumulative net N mineralized for the first 14 days of incubation ($p < 0.0001$, and $p < 0.05$). For those 14 days, cropland had the highest amount of cumulative net N mineralized per g TN despite having the least amount of TN. Suggesting that even though grassland and the edge had a bigger N pool than cropland, the proportions of decomposable N in SOM pool of cropland was higher than that of grassland and the edge. Therefore, cropland was less stable than grassland and the edge in terms of N mineralization. In contrast to our result, in a 24-week incubation of soils along an ecotonal climosequence, Purton et al. (2015) found a trend that grassland decomposed more N than cultivated land and forest land, but there were no statistical differences among cumulative net N mineralized of these land uses. Similar to C mineralization, changes in soil properties due to land managements (Arevalo et al., 2012; Sun et al., 2013), and differences in vegetation covers are how land use controls N mineralization (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013). According to Aguiar (2019), grassland in our study location had some species produced litter that are more decomposable, high in N content, and low C:N ratio (eg., *B. inermis*, *M. stavia,, and M. officinalehave*). This would certainly change the quality of the litter leading to an increase in N mineralization in grassland. Although cropland in our study location is productive, but annual above ground removal has disrupted the nature cycles here and change the litter quality and quantity of the area, which would impact both N mineralization and immobilization processes (Sun et al., 2013). Additionally, cropland was also applied with N fertilizer prior to sampling which would certainly impact N mineralization and immobilization processes in cropland. We also found that cumulative N mineralized per g TN of our soils were strongly negatively correlated with OC, TC, and TN ($p < 0.0001$, Table A.7), but not with OC:TN ratio. There was also an increase in N mineralization when increasing small aggregate size distribution at the last 14 days of incubation ($p < 0.05$), suggesting that small aggregate size fraction contained more decomposable N than large and moderately-sized aggregate size fractions.

Unlike C mineralization, the combination of land use and temperature influenced cumulative net N mineralized significantly throughout all 28 days of incubation (Table 4.3). Similar to the combined effect of temperature and moisture conditions, all soil mineralized the same amount of N per g TN at 30^{o}C, which was consistent with the argument from Stanford et al. (1973) that when reaching an optimum temperature, all soils mineralized the same amount of N regardless of land uses. The optimum temperature of their soil was 35^{o}C, in our case it appeared to be 30^{o}C. On the other hand, at 25^{o}C, cropland continued to decompose more N per g TN than the edge despite having lesser amount of TN, therefore cropland was less stable than the edge at 25^{o}C in terms of N mineralization. However, only the edge had significant differences between the amount of cumulative net N mineralized per g TN at 25^{o}C and 30^{o}C ($p < 0.05$), indicating that the edge was more sensitive to temperature than grassland and cropland. Thus, half of our last hypothesis was supported, grassland is more resistant to climatic conditions than the edge. The edge at SDNWA had the highest plant richness and diversity due to an increase in annual weeds compared to cropland and grassland in the area (Aguiar, 2019). As a result, the edge would have more diversity in SOM quality than grassland and cropland, which would make the edge more sensitive to climatic conditions changes (i.e., temperature and moisture conditions).

4.7. Conclusion

Assessing the resilience of different land uses at a grassland-cropland edge revealed that both readily mineralizable C and potentially mineralizable N of all soils were susceptible to climatic conditions, and temperature was the main factor that controlled both processes. However, soil moisture was just a secondary factor influencing C mineralization, but acted as a main factors impacting N mineralization process. Regardless of moisture conditions and land uses, C and N mineralization tended to increase with increasing temperatures, which supported our first hypothesis.

In contrast to soil moisture, land use was a main factor controlling C mineralization, but only secondary factors controlling N mineralization compared to climatic condition. For C mineralization, the edge and grassland mineralized more C per g TOC than cropland or all 28 days of incubation, proving that the edge and grassland was less stable than cropland, in terms of SOC decomposition. Our results suggested that the effect of land use on C mineralization of soils in our study was mostly coming the combined effects of all soil properties, and the differences in litter quality and quantity in the study location. As for N mineralization, despite having the least amount of TN, cropland mineralized the most amount of N per g TN, indicating that cropland was less stable than grassland and the edge in terms of N mineralization.

Despite that both land use and temperature were two main factors that controlled C mineralization in our soils, the combined effect of these two factors did not impact C mineralization significantly. Since no evidence was found, half of our last hypothesis that grassland is more stable to climatic conditions changes than cropland, was rejected. However, for N mineralization, even though land use effect was only secondary to temperature effect, the combination of land use and temperature impacted cumulative net N significantly throughout all 28 days of incubation. And the edge was found to be less stable to changing temperature and moisture conditions than grassland and cropland, which supported the other half of our last hypothesis.

5. Synthesis and Conclusion

5.1. Overview

Agriculture has been expanded and intensified to improve food security (FAO, 2019), with consequences on the environment including decreased ability of soil to resist or recover from stresses and disturbances (Conrstanje, 2015). In Canadian prairies, the prairie-forest ecotone is particularly sensitive to environmental change (William et al., 2009). Warming conditions created by climate change will push agriculture to expand northward in this region (Barrow, 2009; King et al., 2018). The first area to show the impact by both land use and climatic conditions changes within this region may be the current anthropogenic edge between native land and cultivated land. Soil resilience in this area will be affected significantly by land use change induced by climate change since both land use management and climatic conditions can alter soil physicochemical and biological properties through several different mechanisms (Paré et al., 2006; Davidson and Jassens, 2016).

Soil organic matter is an important indicator of soil resilience and soil quality (Corstanje, 2015, Ludwig et al., 2017). Agricultural practices have affected soil structure leading to differences in SOM stabilization between cultivated land and native land (Six et al., 2002; Hebb et al., 2017). In addition, temperatures and moisture conditions can also alter SOM decomposition (Paré et al., 2006; Ise and Moorcroft, 2006). Previous studies mostly focused on evaluating the differences in soil properties between land uses (Hebb et al., 2017; Aguiar, 2019; Adingo et al., 2021), SOM loss due to cultivation (Gregorich et al., 1998; Rosenzweig et al., 2016; Olson and Gennadiev, 2020), and temperature effect on SOM decomposition (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013). Fewer studies have evaluated soil resilience using SOM fractions within different aggregate size fraction, and the biological stability of SOM under different climatic conditions including both temperature and moisture.

In this MSc thesis, we explored the question of which land use at the anthropogenic edge between cropland and native land that exist within the prairie-forest ecotone would be more stable under land use change induced by changing temperature and moisture conditions. Since different land uses with different soil properties respond differently to land use change, we evaluated physicochemical properties, and SOM fractionation and biological stability within different aggregate size fraction of soils from an edge between grassland and cropland at SDNWA, Saskatchewan, to create the base line of soil properties in the area. To further determine how soil under different land uses with these base line properties would respond to changes in climatic conditions, we investigated changes in the biological stability of SOM under different temperatures and moisture conditions.

5.2. Summary of findings

At the edge between grassland and cropland at SDNWA, land use and soil depth appeared to be primary factors controlling soil physicochemical properties, which supported our first hypothesis for chapter 3. Half of our second hypothesis for chapter 3 was rejected since cropland had the highest bulk density overall, but there was no difference between bulk density of different land uses in the topsoil (0–12 cm). Unlike physical properties, TOC, TC, TN, an OC:TN ratio of topsoil declined gradually from grassland to cropland across transects, which support the other half of our second hypothesis in chapter 3. Our results also showed that grassland had higher TC, TN, and OC than cropland overall, which were consistent with previous studies (Ellert and Gregorich, 1996; Malhi et al., 2003; Aguiar, 2019). Agricultural practices including tillage, using heavy farm equipment, fertilizer applications increase SOM decomposition in cropland by exposing SOM protected within aggregates to microbial community (Baldock and Broos, 2012), and altering decomposition conditions through changing C:N ratio (Sun et al., 2013). Vegetation cover is another factor that impacts C and N content of soil (Paré et al., 2006). At our study location, grassland had plant species that produce high quality litter with higher amounts of C and N, and the major of above ground biomass of cropland is removed every year (Aguiar, 2019). Although grassland has more litter fall than cropland in both quality and quantity, the differences in C and N contents among land uses increased in deeper soil. The reasons behind these results might be because annual roots system of cropland at SDNWA has been reported to be much smaller than perennial roots systems of grassland (Aguiar, 2019), and our studied location has an undulating landscape which could lead to topsoil redistribution. But overall, topsoil of all land uses still have significant higher amounts of C and N contents compared to deeper soil ($p < 0.0001$).

Land use also impacted SOM fractionation and SOC stability of two main aggregate size fractions significantly, but not N mineralization. Even though grassland has greater plant residue input than cropland, and cultivation has increased LF decomposition (Dalal and Meyer, 1986;

Malhi et al., 2003), all soils in our study had the same amounts of LF, which might happen because of topsoil redistribution in a undulating landscape. Additionally, OLF might not all be released during lab procedure, and LF mass can also be lost during transferring from filtration unit to tin foil cup for drying. Unlike LF, among all land uses, cropland had the highest HF mass in large aggregate fraction, while the edge had the highest HF mass in moderately-sized aggregate fraction. Therefore, our results also indicated that most of SOM of cropland comes from large aggregates, and most of SOM of the edge comes from moderately-sized aggregates, which similar to aggregate size distribution results. As for SOC stability, even though cropland in our studies were applied with N fertilizer, compacted by heavy farm equipment, and previously tilled, SOC from two main aggregate size fractions of grassland was more degradable than cropland, which was consistent with the result that cropland has more HF in these aggregate size fractions. In addition, most of more labile C in cropland has been decomposed in the field during cultivation. Although land use did not impact N mineralization significantly, the combination of land use and aggregate size did. Large aggregates of cropland mineralized more N than moderately-sized aggregates, and more than large aggregates of grassland since C:N ratio of LF in cropland was lower than that of grassland. And since both aggregate size fractions of grassland mineralized the same amount of N, cropland is potentially less stable than grassland in terms of N mineralization. Our result also showed that large aggregate fraction of all soils mineralized more N than moderately-sized aggregate fraction, which are consistent with previous findings that large aggregates mineralized more SOM due to having higher porosity (Dexter, 1988, Rabbi et al., 2014), while smaller aggregates have higher water capacity and slow diffusion of oxygen (von Lützow et al., 2006).

Both temperature and land use were the primary factors influencing C mineralization of soils at the edge between grassland and cropland at SDNWA, while moisture condition was only a secondary factor. Regardless of moistures conditions and land uses, all soils produced higher cumulative C mineralized at higher temperatures, which proved our first hypothesis of chapter 4 is true. Many previous studies reported the same results that SOM decomposition rate increased with increasing temperature, but to what degree is depending on other factors of the study (Conan et al., 2008; Pirahar et al., 2009; Gillabet et al., 2010; Wang et al., 2016). For moisture conditions, our results indicated that 35% WFPS level might be within the optimal moisture condition for C mineralization of soils in our study since cumulative C mineralized of all soils only increased significantly when moisture conditions increased from 25% to 35% or 50% WFPS level ($p < 0.05$), and there was no difference in C mineralization between 35% and 50% WFPS level ($p > 0.05$). However, at the end of the incubation, temperature and land use effects have taken over, and moisture conditions did not influence C mineralization significantly. Among all three land uses, cropland had the lowest amount of TOC and lowest amount of cumulative C mineralized per g TOC. Even though the edge had the lowest amount of TOC similar to cropland, the edge still mineralized similar amount of C compared to grassland, and even mineralized more C than grassland at the second week of incubation. Our results indicated that the edge and grassland has more labile C in SOC pool compared to cropland, especially the edge. The biological stability of SOC can be influenced by land uses through different vegetation covers producing different quantity and quality of litterfall (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013), and different land management type resulting in different soil physicochemical properties (Six et al., 2000; Yannikos et al., 2014; Resenzweig et al., 2016). As mentioned above, cropland in our study location has lower litter quality and quantity than grassland, and is applied with N fertilizer which could have neutral or negative effect on C mineralization depending on subtract availability of soils at time of application. Especially, most of labile C in cropland was mineralized in the field due to cultivation, while more C was reserved in grassland and the edge. Since grassland had more decomposable C than cropland, it is recommended that the farmers should continue to minimize the disturbance in grassland area to prevent releasing more CO_2 to the atmosphere.

Unlike C mineralization, both temperature and moisture conditions appeared to be primary factors impacting N mineralization, land use was only secondary factor. But the combined effect of land use and temperature on N mineralization was significant for all 28 days of incubation. All soils produced higher cumulative net N mineralized at higher temperature, and these results are consistent with previous findings (Campbell et al., 21981; Stanford et al., 1973; Cookson et al., 2007; Sun et al., 2013). Moisture condition effect became stronger by the end of the incubation suggesting that at the beginning of incubation, microbial communities were trying to get used to the new conditions and were stabilized by the end. The optimum moisture conditions for N mineralization in our study ranged from 35% to 50% WFPS level. Previous studies also suggested that the percentage of WFPS that set off denitrification varied among soil (Weiner et al., 1993), but denitrification will increase when more than 40–50% of total porosity was filled with water (Rasiah and Kay, 1997). Both temperature and moisture conditions can alter N mineralization through similar mechanisms controlling C mineralization (Sun et al., 2013). In addition, N mineralization can also be limited by the releasing of DON, soil microorganism releases more DON at higher temperature (Chapin et al., 2011). Despite having the least amount of TN, cropland produced the highest amount of cumulative net N mineralized per g TN regardless of temperature. Thus, cropland is more degradable than grassland and the edge in terms of N mineralization. Even though grassland in our location had some species produced litter that are more decomposable, high in N content, and low C:N ratio (Aguiar, 2019). But annual biomass above ground removal and N fertilizer application can also change both N mineralization and immobilization processes in cropland of our study location (Sun et al., 2013). Among all three land uses, the edge is more sensitive to temperature than cropland and grassland since only the edge had significant differences in the amount of cumulative net N mineralized of 25°C and 30°C. The edge at SDNWA had the highest plant richness and diversity compared to other land uses in the area (Aguiar, 2019). In addition, the edge also constantly exchanges material with surrounding habitats (Ries et al., 2004). Therefore, the edge would be more sensitive to climatic changes due to more diversity in SOM quality.

Overall, N mineralization is more susceptible to climactic conditions than C mineralization. However, we must keep in mind that readily mineralizable C and potentially mineralizable N are not parallel measurements. While C mineralization show the gross heterotrophic activity of soil microbial community (Purton et al., 2015), net N mineralization reflect the differences between gross mineralization and gross immobilization (Miller and Geisseler, 2018). Purton et al. (2015) argued that the differences in response of C and N mineralization to climatic change might relate to the present of N immobilization process in N mineralization. Increasing temperature could increase microbial activity or microbial growth. Therefore, depending on the substrate availability of the soil, increasing temperature could increase N mineralization or N immobilization (Miller and Geisseler, 2018), making it difficult to compare between net N mineralized and C mineralized. Our results did show a strong positive correlation between cumulative C mineralized and cumulative net N mineralized throughout all 28 days of incubation ($p < 0.0001$, Table A.7). Generally, the edge and grassland are more degradable than grassland in terms of C mineralization, which is consistent with the results of SOC biological stability of two main aggregate size fractions. While in terms of N mineralization, cropland is more degradable compared other land uses, and the edge is more sensitive to climatic changes. Therefore, half of our last hypothesis in chapter 4 that grassland is more stable to climatic change than the edge was supported, while the other half about grassland being more stable to climatic change than cropland was rejected. The edge at SDNWA is currently unmanaged but since SOC of the edge was less stable than cropland and N mineralization of the edge was more sensitive to temperature than other surrounding land uses the farmers should plant some flower stripes or hedgerow along the edge to prevent runoff, increase more C sequestration, and reduce invasive ruderal plant species that are currently competing with crop in the area.

5.3. Future research

Since soil quality and resilience can be measured by changes in soil capacity to functions relative to a baseline condition (Seybold et al., 1998). We can use the results of soil properties in this thesis as a baseline for future research on soil resilience in this area. Furthermore, since the edge proved to be more sensitive to climatic change than other land uses, future research should expand on other edges and locations including other crops with different land use managements, grazed grassland, and forestland, which will assist us to understand how sensitive the edge can be with different surrounding habitats and at different locations. In addition, the edge has higher diversity than other land uses due to constantly exchanges material with surrounding habitats (Ries et al., 2004). And both climatic conditions and land uses can alter SOM decomposition through changing microbial activities (Sun et al., 2013). Thus, determining both microbial community functions and composition would be helpful in explaining the underlying mechanisms influencing SOM biological stability in the area. Measuring the quantity and composition of litterfall, as well as SOM chemistry composition could also improve our understanding of C and N mineralization. The quality and quantity of litterfall can impact SOM decomposition significantly (Raich and Tufekcioglu, 2000; Paré et al., 2006; Sun et al., 2013), and the N mineralization and immobilization processes are limited by SOM quality (Cookson et al., 2007; Chapin et al., 2011).

Other ways to expand future research are longer term incubation with more variety of temperature and moisture conditions in the lab as well as in the field, and more research focus on the interact effect of temperature and moisture conditions. Although temperature and moisture conditions are two climatic conditions which can both alter SOM decomposition through similar mechanisms, not many studies have focused on the interaction of these two factors. In this study, we only chose two temperature 25oC and 30oC since it fits the scenario for climate change at our study location for the near future. Many studies have shown that all soils decomposed more SOM with increasing temperature (Conan et al., 2008; Pirahar et al., 2009; Gillabet et al., 2010; Wang et al., 2016). Therefore, it is important to include more variety and higher temperatures conditions in longer incubation studies with multiple land uses for a more holistic understanding of how stable to climatic change these land uses can be. Furthermore, all incubations in our study were short duration, some of the effects was not apparent in the short-term incubation due to not enough time for microbial community to stabilize in the new conditions. In addition, the variation in sizes and decomposability of C pool also can cause a mistake in interpreting CO_2 fluxes in short-term incubation (Knorr et al., 2005). Most of the decomposable C in cropland was mineralized due to cultivation (Conant et al., 2008), and less labile C were sensitive to temperature (Conant et al., 2008; Malhi et al., 2013, Parihar et al., 2019). Therefore, long term incubation might be better to access the impact of land uses and climatic conditions on C and N mineralization. However, when looking at the impact of microbial community on C and N mineralization, short-term incubation is appropriate considering the short life cycle of soil microorganisms. The next step for this would be field incubation. But we have to keep in mind that the fluctuating of temperature and moisture conditions in the field would certainly impact the results of field studies.

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APPENDIX A. SOIL PROPERTIES, INCUBATION RESULTS PER KG SOIL, AND PEARSON CORRELATION RESULTS

Table A.1. Comparison of soil physicochemical properties at two depths across an edge between grassland and cropland at SDNWA. Values are means (±SD), different letters within each row represent significant differences, lower letter case for land uses and upper letter case for soil depth. (TukeyHSD, $p < 0.05$). Agg.=Aggreagte.

Soil properties			Land uses			Soil depth	
			Grassland	Edge	Cropland	0 – 12 cm	12 – 24 cm
pH			$7.0±0.3^{a}$	$6.8±0.2^{b}$	$6.8±0.2^{b}$	$6.8±0.3^{B}$	$6.9±0.2^{A}$
EC		dS m^{-1}	$0.8±0.9^{a}$	$0.3±0.3^{b}$	$0.2±0.1^{c}$	$0.4±0.6^{B}$	$0.5±0.7^{A}$
Bulk density		g cm^{-3}	$1.2±0.2^{ab}$	$1.0±0.1^{b}$	$1.3±0.2^{a}$	$1.2±0.2^{A}$	$1.2±0.2^{A}$
TOC		g kg^{-1} soil	$27.1±6.8^{a}$	$26.3±6.0^{a}$	$18.6±5.3^{b}$	$27.7±6.2^{A}$	$19.8±6.0^{B}$
TC		g kg^{-1} soil	$31.8±7.2^{b}$	$33.9±4.4^{a}$	$23.3±5.0^{c}$	$32.1±7.0^{A}$	$26.3±6.7^{B}$
TN		g kg^{-1} soil	$2.7±0.6^{b}$	$3.1±0.5^{a}$	$2.1±0.6^{c}$	$2.9±0.6^{A}$	$2.2±0.6^{B}$
OC:TN			$10.5±3.2^{a}$	$8.5±1.2^{b}$	$9.1±2.1^{b}$	$9.6±1.2^{A}$	$9.3±3.4^{B}$
Agg. size	> 2000 µm	%	$52.3±24.0^{b}$	$34.5±13.5^{c}$	$60.3±15.1^{a}$	$48.2±19.6^{B}$	$53.1±22.4^{A}$
	2000 – 150 µm	%	$40.4±17.8^{b}$	$52.9±8.9^{a}$	$37.2±12.1^{c}$	$45.2±14.4^{A}$	$39.7±15.5^{B}$
	< 150 µm	%	$7.5±10.0^{b}$	$12.6±8.9^{a}$	$2.4±5.1^{c}$	$6.6±8.3^{A}$	$7.3±9.9^{A}$

Table A.2. Comparison of cumulative C mineralized (mg CO_2-C kg^{-1} soil) of two different aggregate size classes (> 2000 µm and 2000 – 150 µm) from surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area throughout a 28 – day incubation at 25°C and 22.5% (w/w). Values are means (±SD) and different letters within a column represent significant differences (TukeyHSD, $p < 0.05$).

Aggregate size class	Land use	Day 7	Day 14	Day 21	Day 28
Large (> 2000 µm)	Grassland	20.6±11.5^{a}	63.6±26.9^{a}	156.6±44.4^{a}	290.7±70.4^{a}
	Cropland	8.7±3.4^{ab}	25.7±8.2^{c}	59.9±18.4^{b}	109.0±32.5^{b}
Moderately – sized (2000 – 150 µm)	Grassland	20.8±10.1^{a}	61.3±27.9^{ab}	150.0±44.7^{a}	278.3±68.3^{a}
	Cropland	12.8±2.2^{b}	36.3±5.3^{bc}	78.4±11.3^{b}	140.3±20.3^{b}

Table A.3. Comparison of cumulative net N mineralized (mg N kg^{-1} soil) of two different aggregate size classes (> 2000 µm and 2000 – 150 µm) from surface soils across an edge between grassland and cropland at St. Denis National Wildlife Area throughout a 28 – day incubation at 25°C and 22.5% (w/w). Values are means (±SD) and different letters within a column represent significant differences (TukeyHSD, $p < 0.05$).

Aggregate size class	Land use	Day 7	Day 14	Day 21	Day 28
Large (> 2000 µm)	Grassland	15.2±5.0^{ab}	18.5±5.0^{a}	20.3±5.0^{a}	23.1±6.2^{a}
	Cropland	17.8±6.9^{a}	22.6±8.2^{a}	25.8±9.7^{a}	28.2±9.5^{a}
Moderately – sized (2000 – 150 µm)	Grassland	10.8±8.1b^{c}	15.9±7.6^{a}	20.3±9.1^{a}	26.2±11.0^{a}
	Cropland	4.2±1.7^{c}	7.6±3.4^{b}	9.4±3.4^{b}	11.6±4.2^{b}

Table A.4. Pearson correlation between cumulative C and net N mineralized of two different aggregate size classes (> 2000 µm and 2000 – 150 µm) throughout 28 days of incubation, light fraction measurements of those two aggregate size classes, and soil properties of the bulk soil. These surface soils originated from an edge between grassland and cropland at St. Denis National Wildlife Area. Only significant correlations are reported[†]. Agg.=Aggregate.

Parameters		Cumulative C mineralized				Cumulative net N mineralized			
		Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Cumulative C mineralized	Day 7	1	0.98^{***}	0.88^{***}	0.81^{***}	NS	NS	NS	NS
	Day 14	0.98^{***}	1	0.94^{***}	0.87^{***}	NS	NS	NS	NS
	Day 21	0.88^{***}	0.94^{***}	1	0.99^{***}	NS	NS	NS	NS
	Day 28	0.81^{***}	0.87^{***}	0.99^{***}	1	NS	NS	NS	NS
Cumulative net N mineralized	Day 7	NS	NS	NS	NS	1	0.95^{***}	0.92^{***}	0.86^{***}
	Day 14	NS	NS	NS	NS	0.95^{***}	1	0.97^{***}	0.91^{***}
	Day 21	NS	NS	NS	NS	0.92^{***}	0.97^{***}	1	0.97^{***}
	Day 28	NS	NS	NS	NS	0.86^{***}	0.91^{***}	0.97^{***}	1
Light fraction	Mass	NS	NS	NS	NS	-0.41^{*}	NS	NS	NS
	C	NS	NS	NS	NS	NS	NS	NS	NS
	N	NS	NS	NS	NS	0.36^{*}	NS	NS	NS
Soil properties	Agg.size	NS	NS	NS	NS	0.60^{**}	0.55^{**}	0.49^{*}	0.40^{*}
	OC	NS	NS	NS	NS	NS	NS	NS	NS
	TC	NS	NS	0.35^{*}	0.41^{*}	NS	NS	NS	NS
	TN	NS	NS	NS	NS	NS	NS	NS	NS
	ratio	NS	NS	NS	NS	NS	NS	NS	NS

[†]Significant correlation at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***).
NS=Not significant.

Table A.5. Mean (±Standard deviations) of cumulative C mineralized (mg CO_2-C kg^{-1} soil) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25°C and 30°C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level).

WFPS level	Land use	Temperature							
		25°C				30°C			
		Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
25%	Grassland	22±7	54±16	121±38	227±83	85±21	260±59	567±180	939±349
	Edge	17±6	42±16	89±38	165±73	87±15	258±41	487±86	766±140
	Cropland	6±3	15±10	36±24	71±44	47±15	148±45	283±88	431±138
35%	Grassland	21±5	59±20	137±45	251±94	100±32	296±83	587±138	927±209
	Edge	15±4	45±16	108±40	200±76	109±22	316±54	581±97	874±145
	Cropland	9±7	24±17	58±32	107±63	58±18	178±45	348±94	547±162
50%	Grassland	26±9	73±23	172±51	319±102	108±30	306±76	598±144	940±216
	Edge	16±5	45±17	129±54	263±113	87±21	245±68	448±149	695±227
	Cropland	10±5	28±17	71±45	132±90	78±24	231±67	419±138	613±185

Table A.6. Means (±Standard deviations) of cumulative net N mineralized (mg N kg^{-1} soil) of surface soils across an edge between grassland and cropland at St. Denis National Wildlife throughout 28 days of incubation at two different temperatures (25°C and 30°C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level).

WFPS level (M)	Land use (L)	Temperature (T)							
		25°C				30°C			
		Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
25%	Grassland	22.7±12.9	36.9±14.3	41.7±16.1	49.1±16.9	48.6±17.8	59.1±21.7	75.0±27.7	88.1±32.5
	Edge	16.4±5.6	25.7±5.1	27.7±5.2	34.2±5.3	80.1±26.4	84.4±27.6	90.2±28.5	106.4±30.8
	Cropland	20.3±17.7	24.9±17.0	26.2±16.8	30.4±16.6	49.7±19.8	53.8±20.8	61.0±23.2	65.4±24.5
35%	Grassland	48.5±15.9	69.7±11.5	74.2±12.0	81.8±13.6	63.3±24.7	71.7±26.2	80.9±26.8	106.0±32.5
	Edge	15.6±8.9	27.9±9.0	31.7±9.6	43.4±10.8	64.7±30.4	71.8±26.0	82.8±22.4	104.4±23.4
	Cropland	56.8±26.9	64.5±25.7	66.3±25.6	70.2±25.6	56.6±20.9	59.6±22.0	63.2±23.0	74.6±25.9
50%	Grassland	35.6±18.1	49.2±15.6	52.9±15.6	70.8±15.8	36.7±16.5	48.8±17.5	80.9±21.9	101.7±24.4
	Edge	31.9±21.6	34.0±21.6	43.0±19.9	59.9±18	53.3±20.7	67.6±20.5	93.1±24.1	113.0±27.3
	Cropland	41.7±19.5	58.9±19.4	61.0±18.4	68.0±17.9	40.7±15.1	46.6±17.0	62.1±21.6	72.4±24.3

Table A.7. Pearson correlation between cumulative C and net N mineralized of surface soils throughout 28 days of incubation at two different temperatures (25^{o}C and 30^{o}C) and three different moisture conditions (25, 35, and 50% of water-filled spore space level), soil properties of those surface soils, and climatic conditions. Soil samples originated from an edge between grassland and cropland at St. Denis National Wildlife Area. Only significant correlations are reported†. Agg.=Aggregate.

Parameters			Cumulative C mineralized				Cumulative net N mineralized			
			Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Cumulative C mineralized	Day 7		1	0.99^{***}	0.97^{***}	0.95^{***}	0.34^{***}	0.37^{***}	0.47^{***}	0.58^{***}
	Day 14		0.99^{***}	1	0.99^{***}	0.96^{***}	0.37^{***}	0.34^{***}	0.49***	0.56^{***}
	Day 21		0.97^{***}	0.99^{***}	1	0.99^{***}	0.37^{***}	0.34^{***}	0.49^{***}	0.56^{***}
	Day 28		0.95^{***}	0.96^{***}	0.99^{***}	1	0.35^{***}	0.34^{***}	0.48^{***}	0.55^{***}
Cumulative net N mineralized	Day 7		0.34^{***}	0.37^{***}	0.37^{***}	0.35^{***}	1	0.97^{***}	0.92^{***}	0.88^{***}
	Day 14		0.33^{***}	0.34^{***}	0.34^{***}	0.34^{***}	0.97^{***}	1	0.96^{***}	0.92^{***}
	Day 21		0.47^{***}	0.49^{***}	0.49^{***}	0.48^{***}	0.92^{***}	0.96^{***}	1	0.98^{***}
	Day 28		0.54^{***}	0.56^{***}	0.56^{***}	0.55^{***}	0.88^{***}	0.92^{***}	0.98^{***}	1
Climatic conditions	Temperature		0.84^{***}	0.86^{***}	0.84^{***}	0.81^{***}	0.37^{***}	0.31^{***}	0.43^{***}	0.47^{***}
	Moisture		NS	NS	NS	NS	NS	NS	NS	0.20^{*}
Soil properties	TOC		NS	NS	NS	NS	-0.35^{***}	-0.36^{***}	-0.34^{***}	-0.32^{***}
	TC		NS	NS	NS	NS	-0.36^{***}	-0.37^{***}	-0.36^{***}	-0.33^{***}
	TN		NS	NS	NS	NS	-0.38^{***}	-0.49^{***}	-0.41^{***}	-0.38^{***}
	ratio		NS	NS	NS	NS	NS	NS	NS	NS
	Agg. size	> 2000 μm	-0.17^{*}	-0.15^{*}	-0.16^{*}	-0.18^{*}	NS	NS	NS	NS
		2000 – 150 μm	NS	NS	NS	NS	NS	NS	NS	NS
		< 150 μm	0.19^{*}	0.18^{*}	0.20^{*}	0.23^{*}	NS	NS	0.16^{*}	0.21^{*}

†Significant correlation at $p \leq 0.05$ (*), $p \leq 0.001$(**), and $p < 0.0001$ (***).

NS=Not significant.

APPENDIX B. EDGE PHOTOGRAPH

Fig B.1. Edge at the sampling location at SDNWA, 2017