LAND USE EFFECTS ON SOIL BIOLOGICAL PROPERTIES IN TWO TOPOGRAPHICALLY VARIABLE AGROECOSYSTEMS IN SASKATCHEWAN

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ABSTRACT

Soil biological properties tend to be under utilized as indicators in soil health tests. Novel measures - those seldom used in such metrics - such as microbial abundance and biomass, community structure, and enzyme activity are directly related to soil resource availability, organic matter decomposition, and nutrient cycling, which are affected by agricultural land use. The inclusion of biological properties in soil health tests may allow land managers to compare the effects of management practices faster than if they relied solely upon changes in chemical and physical properties over time. The objective of this project was to measure how enzyme activity, microbial abundance, and community structure are affected by annual and perennial cropping systems at different landscape positions, over different depths, and across the growing season at two different locations within the province, [St. Denis National Wildlife Area (SDNWA), and the Conservation Learning Centre (CLC)]. The SDNWA and CLC were chosen due to their topographic variation and presence of adjacent perennial and annual land use. Samples were taken at different landscape positions at different time points within the growing season to account for both location and seasonal effects on soil function. Time within the growing season influenced enzyme activity differently between perennial vs. annual cropping systems at both sites. However, seasonal dynamics in the annual system differed between the two sites due to differences in crop rotation. For example, SDNWA had elevated enzyme activity in the annual cropping system early in the growing season, a trend that did not exist at CLC. The effects of perennial cover were found to often buffer the effects of topography in the perennial agroecosystems, which affected not only enzyme activity but also PLFA abundance, particularly fungal signatures. Finally land use effects were moderated by both topographic and depth effects at CLC in a complex interaction which affected enzyme activity and PLFA abundance, while the same interaction was not seen at

SDNWA. These findings indicate that novel soil biological properties respond to changes in management factors often interconnectedly with land use, and environmental factors such as topography, depth, and season, and that these biological properties could be valuable additions to soil health indices.

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LIST OF ABBREVIATIONS

SOC	Soil Organic Carbon
SOM	Soil Organic Matter
MBC	Microbial Biomass Carbon
PLFA	Phospholipid Fatty Acid Analysis
NMDS	Non-metric Multidimensional Scaling
BG	β-Glucosidase Enzyme
NAG	N-Acetyl-Glucosaminidase Enzyme
Phos	Phosphatase Enzyme
TP	Total PLFA
GB	General Bacteria
GF	General Fungi
G+	Gram Positive Bacteria
G-	Gram Negative Bacteria
AMF	Arbuscular Mycorrhizal Fungi
AB	Actinobacteria
B:F	Bacterial:Fungal Ratio
SDNWA	St. Denis National Wildlife Area
CLC	Conservation Learning Centre
DNA	Deoxyribonucleic Acid
GC	Gas Chromatograph
ANOSIM	Analysis of Similarity
PERMANOVA	Permutational multivariate analysis of variance
D	Depth
LU	Land use
LP	Landscape Position

1.0 INTRODUCTION

Soil health has become a topic of interest among farmers, land managers, policy makers, and the public, as the pressure on global soils to produce food, fibre, and building material continues to increase in the face of climate uncertainty, and an ever-growing human population. One definition, identified by Doran & Parkin (1994) and that has evolved over time, states that soil health is "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health." The ability of soils to function properly is directly linked to their ability to provide what society demands of them. As a result, the ability to quantify a soil's health is of a high importance when it comes to making decisions associated with its management.

Despite a resurgence of interest and demand from land managers and the public for both education, and tools to quantify soil health, the means to do so remain convoluted due to the different lenses through which soil quality is viewed (de Paul Obade & Lal, 2016; Bünemann, Bongiorno, & Bai, 2018; Norris et al, 2020). Historically, soil quality was a measure of a soil's ability to produce goods rather than provide a suite of ecosystem services, a lens still applied in many agricultural settings today (de Paul Obade & Lal, 2016; Norris et al., 2020). Norris et al. (2020) states that quantifying and understanding soil health is also contextual to its inherent properties, as well as individual functional capacities across a landscape, which vary greatly with geography.

At the end of the 20th century land managers were demanding information on management strategies that would improve soil health (Doran & Doran, 2002). In response researchers around the world began to develop tools that could evaluate soil health through numerous methods,

employing both laboratory based analysis as well as visual evaluation tools that could be easily used by land managers directly (Bünemann et al., 2018). The tools and metrics that were developed had to integrate soil property measurements that were easy to measure and sensitive to changes in management and soil function, as well as sensitive to inherent soil properties (de Paul Obade & Lal, 2016; Norris et al., 2020). Tests such as the Soil Management Assessment Framework (SMAF), Cornell's Comprehensive Assessment of Soil Health (CASH) and the Haney Soil Test, were among such tools developed to attempt to quantify soil health, and are used by land managers to make informed decisions about soil management within their landscape by evaluating select suites of soil physical, chemical, and biological properties commonly referred to as indicators (Bünemann et al., 2018; Norris et al., 2020).

While these tests include a broad range of chemical and physical properties, the use of biological properties within these assessments are limited. A review of soil quality assessments indicated that chemical and physical soil properties were measured at a higher incidence than biological properties in studies examining soil quality and health indices (Bünemann et al., 2018). In the past, lack of inclusion of soil biological properties in soil health assessments has stemmed from a limited ability to measure them, whether due to a lack of technology, high time investment in analysis, or financial limitations (Bünemann et al., 2018). With new technology available, both decreasing analysis time and lowering analysis cost, collecting information about soil biological properties has become more accessible and financially viable than it was previously. While previous soil health testing frameworks were often limited to measurements of microbial biomass C and soil respiration (Andrews, Karlen, & Cambardella, 2004; Haney, Haney, Smith, Harmel, & White, 2018), more recent initiatives are including biological properties such as extracellular enzyme activities—as indicators of nutrient and carbon cycling—and phospholipid fatty acid

(PLFA) analyses to determine soil microbial community abundance and structure (Bünemann et al., 2018; Norris et al., 2020). These novel biological properties are becoming more accessible and including these measurements in soil health tests is resultantly more feasible.

The inclusion of soil biological properties in soil health tests helps to create a complete picture of soil function at a landscape level when included with soil physical and chemical properties. Indeed, soil biota are integral to soil function, playing a central role in nutrient cycling, soil organic matter (SOM) formation, and soil organic carbon (SOC) stabilization. Biological properties are more responsive to immediate changes in environmental conditions whether it be natural or as a product of management, than chemical and physical soil properties (Lehman et al., 2015; Bünemann et al., 2018). The carbon (C) cycle, as well as other nutrient cycles such as nitrogen (N) and phosphorus (P), are strongly influenced by soil microbial community activity (Norris et al., 2020), and can vary quickly as a result of changes that affect the community's ability to cycle nutrients, which can be seen long before any changes to soil organic matter content or soil organic carbon stores can be detected (Bünemann et al., 2018).

When evaluating soil health, management is often of the end goal, as well as the cause of existing conditions. Soil management on agricultural sites varies with land use type. In Saskatchewan, most agricultural sites can be split into two management system categories: perennial land cover comprising grazed pasture or hay lands; and annual crop production, often following a pulse – oil seed – grain rotation. Management differs between the two, with annual systems often having more intensive management regimes than perennial systems (Smyth & Dumanski, 1995; Del Galdo et al., 2003; Kiani et al., 2017). Both management factors of these systems as well as inherent properties affect soil function through the response of the soil microbial community (Sohlenius, 1990; Williams & Hedlund, 2014).

Several studies have examined the effects of land use on soil biological properties; some found distinct correlations between land use and soil properties such as microbial activity and microbial community structure (Hedlund, 2002; Wallenius et al., 2011; Tischer, Blagodatskaya, & Hamer, 2015; Kiani et al., 2017). Wallenius et al. (2011) found that soil enzyme activity for multiple enzymes including the C catalyzing enzyme β-glucosidase, was significantly higher in a perennial meadow than in an annual crop field. Another study by Kiani et al. (2017) found that even different management decisions for annual systems such as complex crop rotations that included perennials had an effect on soil biological response. They found differences in microbial community composition and microbial biomass C (MBC) between simple and complex crop rotations, with MBC 1.5 times higher in soils under complex rotations than those under simple rotations (Kiani et al., 2017).

In studies of soil health, topographic variation is not often taken into consideration in sampling campaigns. Of the soil tests reviewed by Norris et al. (2020), the SMAF, CASH, and Haney soil tests do not include any mention of the effects of topography on soil health. Similarly, in a recent study by Wu & Congreves. (2021), soil samples from across Saskatchewan were collected to assess soil health; while soil health scores were differentiated among soil zones, there was no indication of whether topography was accounted for in the sampling design. Finally, in the list of global soil tests gathered and reviewed by Bünemann et al. (2018), only a visual soil test from Germany - The Muencheberg Soil Quality Rating - included slope as part of its assessment.

Due to its role in soil formation, topography can have a strong effect on all soil properties (Bedard-Haughn et al., 2006; Block & Van Rees, 2006; Helgason, Konschuh, Bedard-Haughn, & VandenBygaart, 2014), which includes the structure and function of the soil microbiome (Dengiz, Kizilkaya, Gol, & Hepsen, 2007; Helgason et al., 2014; Wickings, Grandy, & Kravchenko, 2015).

For this reason, topographic variation is important to account for in landscape scale studies, particularly those focusing on land use and associated management. Since topography can alter soil properties from one slope position to another, a composite sample may not be enough to capture variability in resource availability or soil microbial response across a site. For example, Pennock, Anderson, & de Jong, (1994) and Slobodian, Van Rees, & Pennock, (2002) indicate the effects of cultivation resulted in uneven distribution of SOC in hummocky landscapes in Saskatchewan, with net losses of SOC in upper slope positions and net gains in lower slopes. These studies indicate that sampling across the landscape, with representative samples of topographic position tells a more complete story of what is happening in the soil across the site, and is indicative that soil health ratings themselves may change depending on where soil samples are collected within a landscape.

This thesis research is a subproject of a broader collaborative study "Understanding Resilience in Agroecosystems: Landscapes in Transition" led by Dr. Angela Bedard-Haughn. The project developed an index of soil resiliency and delineated functional management zones based on a suite of soil properties and plant metrics at two field sites in Saskatchewan (Bedard-Haughn, unpublished, 2021; Smith, 2020). The two field sites, St. Denis National Wildlife Area and the Conservation Learning Centre, are located in the transition zones between the Moist Mixed Grassland and the Aspen Parkland ecoregions and the Aspen Parkland and Boreal Transition ecoregions, respectively. Members of other subprojects collected data on various soil chemical and physical properties, which are included in the Appendix and provide support to the soil biological property data I collected. Specifically, I focused on characterizing the soil microbial community and extracellular enzyme activities important to nutrient and carbon cycling.

The purpose of this thesis is to examine the response of a set of novel soil biological properties, including extracellular enzyme activity and PLFA profiles, to differences in land use and landscape variability, comprised of landscape position, seasonal variation, and depth, at two field sites in Saskatchewan. Results from my research will inform whether these novel soil biological properties could be considered for use in soil health tests and frameworks based upon the sensitivity of their response to land use change in topographically variable landscapes. I hypothesize that microbial community composition and abundance as well as enzyme activity differ under differing land use, and that landscape position, depth, and sampling time will modulate the effects of land use.

2.0 LITERATURE REVIEW

2.1 Soil biological processes in agricultural systems

Soil microorganisms make up one of the most diverse pools of life on the planet. They are classified into the domains of bacteria, fungi, and archaea and each of these domains contain numerous phyla composed of groups of species that perform a multitude of soil functions (Fierer, Allen, Schimel, & Holden, 2003; Wertz et al., 2007; Bowles, Acosta-Martínez, Calderón, & Jackson, 2014). Of the three domains, bacteria and fungi are of particular importance when it comes to driving soil carbon sequestration, soil organic matter formation, and nutrient cycling within soils (Kibblewhite, Ritz, & Swift, 2007).

The sequestration of carbon (C) within soils is an essential function that is driven by the soil microbial community. Soil microbes participate directly in the C cycle in both the release and sequestration of C to and from the atmosphere, through generation of microbial products and necromass that are stabilized with the mineral soil matrix in the form of SOM, and within aggregates as well as the release of C in the form of CO_2 through respiration (Kallenbach & Grandy, 2011; Gougoulias, Clark, & Shaw, 2014; Lehman et al., 2015; Liang, Schimel, & Jastrow, 2017; Kästner, Miltner, Thiele-Bruhn, & Liang, 2021; Bhattacharyya, Ros, Furtak, Iqbal, & Parra-Saldívar, 2022). The presence and stability of soil organic matter (SOC) is therefore controlled by the viability of soil microbial community, which is related to the quality and quantity of soil organic matter (SOM) (Bhattacharyya et al., 2022). Thus, the total amount of soil C (i.e., stocks) sequestered is governed by the balance of inputs from plant litter and losses through respiration of CO_2, which largely depends on future carbon fluxes, metabolization, and the time spent stabilized within the soil matrix (Bhattacharyya et al., 2022).

Along with soil C, soil microorganisms are important in the cycling of nutrients such as nitrogen (N) and phosphorus (P). While N is an abundant element in our atmosphere, most remains in an inert form (N_2). In this state, N is inaccessible to most organisms that require it for metabolic function and this results in N being one of the most common limiting resources in the soil ecosystem (Ross, Izaurralde, Janzen, Robertson, & McGill, 2008). Some soil organisms, however, can independently or symbiotically fix N_2 gas through nitrogen fixation, integrating N into the terrestrial system. Soil organisms also mineralize organic N forms into bioavailable NH_4^+, that they and primary producers can then utilize for their metabolic function (Dharmakeerthi, Kay, & Beauchamp, 2005). Soil microorganisms are also responsible for converting NH_4^+ to NO_3^- during nitrification as well as transforming bioavailable forms of N back into the inert N_2 gas when denitrification is complete (Dharmakeerthi et al., 2005). A separate product of the denitrification process is the production of N_2O a well-known greenhouse gas (GHG) which soil microorganisms also take part in producing.

Unlike C and N, P does not have an atmospheric stage within its cycle, rather, the primary source of natural P into soil ecosystems is the weathering of P-containing bedrock. As a result, many soils have a finite amount of P that tends to decrease over time due to the water solubility of P, unless soils are exposed to redistribution of P rich sediment through flooding (Richardson & Simpson, 2011). Phosphorus is a necessary component for the formation of DNA and RNA in cells, and aids in the formation of phospholipids required to build cell membranes, making it an invaluable nutrient to the soil microbial community (Filippelli, 2008). The microbial community is involved in the solubilization, mineralization, and immobilization of P, through turning organic and inorganic forms of P into biomass, or mineralizing organic P to inorganic P in the soil solution (Filippelli, 2008; Richardson & Simpson, 2011).

2.2 Measures of soil microbial communities and biological processes

There are multiple ways in which soil microorganisms and biological processes can be measured. Traditional cultivation techniques, such as species isolation using agar plates, to quantify the structure of the microbial community, has largely given way to genomic techniques such as DNA isolation and sequencing (Dunbar, Takala, Burns, Davis, & Kuske, 1999; Insam, 2001; Mocali & Benedetti, 2010; Geisen et al., 2019). Soil microbial biomass, commonly determined through fumigation-extraction techniques to quantify biomass C, is often used as a measure of microbial response to environmental or management change (Degens, 1998; Griffiths et al., 2000; Zhou, Wang, Zheng, Jiang, & Luo, 2017) and represents a labile pool of soil carbon (Biederbeck, Janzen, Campbell, & Zentner, 1994).

Phospholipid fatty acid (PLFA) analysis is a molecular analysis that uses fatty acid signatures in the cell walls of soil microbes as a method of identification (Quideau et al., 2016). It is a useful tool because it is a culture independent analysis which increases the number of microbes that can be analyzed (Willers, Jansen van Rensburg, & Claassens, 2015). The extracted fatty acids are identified through gas chromatography, and the specific biomarkers can be assigned to microbial groups such as different types of bacteria and fungi, or into groups specific to nutrient or carbon cycles and analyzed using multivariate statistics (Frostegård, Tunlid, & Bååth, 2011). While PLFA cannot be used to measure soil community diversity like genomic techniques because it cannot resolve microorganisms at the species level (Frostegård et al., 2011), it can be used to simultaneously determine viable microbial biomass and abundance as well as community structure (Lewe et al., 2021), and has been shown to be sensitive to land use and management change (Bossio, Scow, Gunapala, & Graham, 1998; Bardgett & McAlister, 1999; Helgason, Walley, & Germida, 2010; Arcand, Helgason, & Lemke, 2016). Thus, PLFA has advantages over genomic

techniques as it allows the researcher to identify groups of microbes by a simpler more cost-effective method to characterize microbial community structure and biomass (Quideau et al., 2016). Such analysis may provide insight into new indicators that could be used in place of or alongside existing biological indicators in current soil health monitoring indices (Hartmann, Frey, Mayer, Mäder, & Widmer, 2015; Hermans et al., 2017; Norris et al., 2020).

There are several methods used to assess soil biological processes in order to quantify soil microbial community function and activity. Soil respiration is one such method, used as a proxy for the activity of the living biomass. Soil respiration is a measure of the CO_2 released as a function of the total metabolic activity of soil microbes, roots and mycorrhizae (Raich & Schlesinger, 1992). While respiration is a useful tool for quantifying metabolic activity it is also useful in quantifying soil C fluxes and is thus important to understanding soil carbon stores ((Fierer et al., 2003; Bending, Turner, Rayns, Marx, & Wood, 2004).

Another biological process that can provide useful information on microbial activity and carbon and nutrient cycling is the production of microbial enzymes. Microbes produce enzymes as a way of both retrieving and transforming nutrients within the soil to meet carbon and nutrient requirements. Soil microbes produce a variety of enzymes that transform nutrient compounds by severing the chemical bonds that hold nutrients in biologically unavailable forms (Caldwell, 2005; Colberg, 2007; Averill & Finzi, 2011). These enzymes are also produced to catalyze reactions that break down C compounds within soil and are used by microbes to obtain energy necessary for their metabolic function (Bowles et al., 2014; Wickings et al., 2015). These metabolic processes break down organic matter and transform nutrients and C compounds into forms that are available for both microbial and plant uptake (Bowles et al., 2014).

Soil microbes produce two kinds of enzymes, intra and extracellular. Extracellular enzymes are excreted into the soil solution; once there, the enzyme can catalyze the transformation of the complex nutrient and carbon compounds into simpler forms. As a result, extracellular enzymes are essential in soil nutrient cycling and the break down of C, N, and P compounds in organic matter (Burns, 1982). Assessment of enzymes such as β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), and phosphatase (Phos), are commonly used to assess C, N, and P cycling in soils, respectively, by providing potential rates of turnover under ideal conditions (Tarafdar & Jungk, 1987; Taylor, Wilson, Mills, & Burns, 2002; Stott, Andrews, Liebig, Wienhold, & Karlen, 2010; Tischer et al., 2015). These enzymes can also provide an indication of the nutrient demands of the microbial community (Sinsabaugh & Moorhead, 1994). Therefore, BG, NAG, and Phos are enzymes that were chosen in my study because they carry out specific transformations associated with the catalyzation of C, N and P compounds, respectively.

β glucosidase (BG) is a common hydrolytic enzyme studied in soil microbiology (Hayano, 1973; Taylor et al., 2002; Stott et al., 2010). It plays a key role in the degradation of cellulose and is the most easily detected of the enzymes that perform this function (Debosz, Rasmussen, & Pedersen, 1999; Turner, Hopkins, Haygarth, & Ostle, 2002; Stott et al., 2010). Changes in chemical properties such as SOC in agricultural systems take time to develop in response to management and land use, and it can take years for measurable amounts of change to occur (Lal, Negassa, & Lorenz, 2015). Enzymes such as BG can be used as a proxy measurement to detect changes in soil function that would affect soil organic carbon (SOC) long term, before changes to these specific soil resources are detectable in the lab (Tischer et al., 2015; Creamer et al., 2016). This is particularly useful when evaluating soil management practices that affect organic matter inputs like plant residues and root exudates (Stott et al., 2010).

N-acetyl-glucosaminidase (NAG) is responsible for the hydrolysis of fungal chitin and bacterial peptoglycan which are linked to decomposition and turnover of organic material in soil (Tischer et al., 2015). NAG is an essential enzyme in the function of N and C cycles in soil associated with the turnover of OM which can be affected by management decisions over time. NAG has been used in previous studies with similar enzymes like BG, whose activity levels can be used to determine microbial sensitivity to land use change (Bending et al., 2004; Brockett, Prescott, & Grayston, 2012).

Phosphatase (Phos) plays a role in the mineralization of organic P through the hydrolysis of phosphoric acid (Nakas, Gould, & Klein, 1987). Phosphatase enzymes include two types, acid and alkaline, the former of which is produced by bacteria, fungi, arbuscular mycorrhizal fungi (AMF), and plant roots. This results in Phos activity often being enhanced with plant communities and root zones in surface soils. Since the Phos enzyme is sensitive to added P inputs in annual agricultural systems, with additions of synthetic P causing activity levels to decrease, activity of Phos is often part of measures of soil function in studies in perennial ecosystems, where it performs its role with less interference from intensive management inputs (Eivazi & Tabatabai, 1977; Karamanos, Robertson, Puurveen, & Domier, 2013; Yang et al., 2014)

2.3 Agricultural land use effects on soil biological processes and properties

In Saskatchewan, land use change has drastically altered the function of the soils across the landscape(Slobodian et al., 2002). In agricultural landscapes, crop productivity and economic profitability is often the sole driver for management decisions. As a result, the function of soil and the biota therein, have a less significant role in agricultural landscapes where human intervention in natural cycles is more consistent. In natural systems however, soil biota often play a much more significant role by regulating soil resources and cycles which are allowed to occur with little to no interference from humans. Saskatchewan's agricultural landscape is currently made up of a patchwork of intensively managed annual systems used to grow grains, pulses, and oil seed, and extensively managed perennial systems used for grazing or hay production. These two types of systems are examples of the differences in soil function through different types of management.

While both annual and perennial agroecosystems produce economically important products, the soil ecosystems differ under each type of management. The soils in annual agroecosystems have been exposed to initial conversion through tillage, with the associated erosion of material from upper to lower slopes, as well as the ongoing effects of tillage until no-till systems were widely adopted in the 1980's (Slobodian et al., 2002; DuPont, Culman, Ferris, Buckley, & Glover, 2010; Pennock, Bedard-Haugh, & Viaud, 2017). Annual systems typically have shallow rooting zones, and are planted in monocrop fashion unlike perennial systems which host a comparatively diverse plant community with correspondingly deeper and denser root zones (Carpenter-Boggs, Stahl, Lindstrom, & Schumacher, 2003). Nutrients are applied annually to these annual systems and soil carbon inputs are reduced compared to those of perennial agroecosystems (Janzen et al., 1998; Kiani et al., 2017).

Due to the differences in soil resource availability and soil C between annual and perennial agroecosystems, the soil microbial activity and community abundance can also be affected. A common finding is that perennial agricultural systems support higher microbial abundance than annual systems (Bossio et al, 1998; Allison, Miller, Jastrow, Matamala, & Zak, 2005; Williams & Hedlund, 2014). Conversely, a study by Sohlenius (1990) found that bacterial abundance did not differ between annual crop and perennial leys; however, this study did not include measurements of fungi. It has been documented by other studies that many species of fungi found in soils are more sensitive to disturbance than bacteria (Carpenter-Boggs et al., 2003; Kabir, 2005). Had Sohlenius (1990) examined fungal biomarkers as well as bacteria, their findings may have been more in line with the findings of Bossio et al. (1998), Allison et al. (2005), and Williams & Hedlund. (2014). Since annual systems are subject to soil disturbance of varying severity, both through historical management decisions like tillage and summer fallow, as well as current management like seeding, application of herbicide, pesticide, and fungicide, and harvest, it is possible that any microbial community abundance discrepancies between annual and perennial agroecosystems are in part caused by these physical and chemical disturbances.

Keeping in mind the culminating effects of land use history within these agroecosystems, Bossio et al. (1998) found that land use history and time play a much more significant role in microbial community abundance and composition than any short-term management decision. On the other hand, when examining distinct management types among annual cropping systems Helgason et al. (2010) found that community composition, did not change between no-till and conventional-till sites across three locations on the Canadian prairies and concluded that physical disturbance alone may not play as much of a role in determining community composition. In the same study, however, it was found that while there appeared to be no change in the structure of

the microbial community, like the findings of Bossio et al. (1998) microbial abundance was sensitive to management differences (Helgason et al., 2010).

Another study found a strong positive correlation between viable microbial biomass and the concentration of SOC, and that the community composition was dependent on the amount of C present (Helgason et al., 2014). In addition, Arcand et al. (2016) found that extracellular enzyme activities and microbial abundance were highest in treatments with greater SOC and nutrient concentrations. Perennial systems typically have higher concentrations of SOC as well as a greater prevalence of available nutrients than annual agroecosystems. However, nutrient concentrations can fluctuate greatly throughout the growing season in both types of systems in response to factors such as early season fertilizer applications in annual systems, and the seasonal fluctuation of plant productivity in the perennial system (Sarathchandra, Perrott, Boase, & Waller, 1988; Sohlenius, 1990; Dharmakeerthi et al., 2005; Kotroczó et al., 2014). Land use can compound effects of seasonal variability through those same pathways that fundamentally differentiate perennial from annual agroecosystems as previously mentioned. In a species-diverse perennial system for example, peak plant productivity may occur at a different point within the growing season than in a single species annual system.

Extracellular enzymes are particularly sensitive to SOM, SOC, and nutrient availability as these are what drive their production (Burns, 1982); therefore, they are particularly responsive to changes that occur amongst these soil resources. In a study by Kotroczó et al. (2014) it was found that C inputs through litter additions did not affect enzyme activity, but removal of roots did. It was also found that these same enzymes had higher activity levels in the spring likely associated with increased soil moisture from spring meltwater and higher levels of root activity from plants coming out of winter dormancy (Kotroczó et al., 2014). The root zone is subject to significant

changes in moisture, nutrient, and C inputs throughout the growing season. As a result, the microbial communities are driven by the same seasonal fluctuations in response to the changes in resources provided by seasonal variability (Allison & Vitousek, 2005).

2.4 Landscape effects on soil biological processes and properties

Landscape position plays a crucial role in the distribution of soil on a landscape and so directly affects the soil properties along a catena. This is important to account for when trying to understand biological soil dynamics within studies on landscapes with significant topographical variation. Typically, landscape influences erosion risk, the distribution of water through precipitation and seasonal runoff, the soil structure, pH, and resulting distribution of soil nutrients and C (Pennock et al., 1994; Wickings et al., 2015). Erosion leads to a decrease in A horizon thickness, increased runoff, and lower levels of soil C and nutrients as well as lower plant productivity in upper slope positions, while eroded soil material, moisture, concentrations of soil C and nutrient and plant productivity increase down the catena into the lower slopes (Pennock et al., 1994; Bedard-Haughn et al., 2006; Block & Van Rees, 2006).

The varying distribution of soil resources within and across agricultural landscapes directly affects soil microbial communities and function. A study by Wickings et al. (2015) saw landscape position affect both microbial biomass as well as enzyme activity, with higher BG and NAG activity and microbial biomass in the depressions than in the summits and upper slopes. Similarly, Helgason et al. (2014) found that PLFA concentration increased in depositional slope positions corresponding with redistributed A horizon material from upslope. Changes in microbial community abundance and function have been shown to positively correlate with SOC, SOM, nutrient availability, and soil moisture, all of which were also affected by landscape position

(Dengiz et al., 2007; Helgason et al., 2014; Wickings et al., 2015). Soil moisture was found to be higher in footslopes than in upper slopes in a study on topographic effects on wheat root dynamics by Block & Van Rees (2006). In a related study, Slobodian et al. (2002) examined belowground biomass and SOC and found that both increased moving downwards to footslopes within a landscape. Including topographic effects is essential since topography can affect the thickness of surface horizons where the root zone is most dense (Block & Van Rees, 2006). Understanding how topography influences soil development also aids in understanding topographic effects on microbial communities, since microbial activity is often most concentrated in the root zone across a landscape.

Soil biological activity is often highest in the surface A horizon, which can vary in thickness across a topographically variable landscape. Given this, soil depth also becomes important in understanding how soil microbial communities function in these landscapes. Soil microbial communities often become less diverse and less active with depth coinciding with a decrease in the quantity and quality of the SOM and soil carbon inputs and nutrient cycling (Dengiz et al., 2007; Helgason et al., 2014; Loeppmann, Blagodatskaya, Pausch, & Kuzyakov, 2016; VeVerka, Udawatta, & Kremer, 2019). Ross et al. (1999) found significant relationships between soil depth and the distribution of nutrients and substrates such as available N and SOC in the profile. Due to differences in soil disturbance and mixing of crop residues and nutrients into the soil profile, tillage systems can have a distinct soil depth effect on microbial community composition (Helgason et al., 2010). Further, Helgason et al. (2014) found that microbial abundance positively corresponded with SOC at all depths, including in buried A horizons.

When compounded with land use in agroecosystems, effects of landscape position and depth grow complex. One example from Slobodian et al. (2002) reported significant differences in A

horizon thickness between upperslope, footslope, and depression positions in an annual crop system but little to no significant differences in the corresponding perennial system. This may be due to the buffering effect of a thicker root zone typical of perennial systems, which may offset the effects of soil disturbance such as tillage, which breaks up the rooting zone that holds soil in place, leaving it at greater risk to the effects of wind and water erosion as well as gravitational translocation of material in highly variable landscapes (Block & Van Rees, 2006; Helgason et al., 2014). As for these complex effects on the soil microbial activity and nutrient cycling potential, Wickings et al. (2015) found that there were significant landscape position and management interactions for soil extracellular enzymes, with higher acid phosphatase activity in upper slopes in low input management sites than in the conventionally managed agricultural fields. In light of the complexities of soil biological response at a landscape scale this study aims to measure the effects of land use as well as landscape level factors such as topography, depth, and seasonality, on enzyme activity and PLFA profiles at two different field sites in Saskatchewan.

3.0 MATERIALS AND METHODS

3.1 Site descriptions

3.1.1 St. Denis National Wildlife Area

The St. Denis National Wildlife Area (SDNWA) is the first of two sites used in this study. The site is located at approximately 40 km east of Saskatoon SK, (Latitude: 52.206133, Longitude: -106.104991), between the Moist Mixed Grassland and the Aspen Parkland ecoregions, capturing a transition in soil, vegetation, and climatic conditions. SDNWA is characterized by hummocky terrain on unsorted till parent material (Acton & Ellis, 1978) and the presence of ephemeral and permanent kettle wetlands across the entire protected area. Located in the Dark Brown Soil Zone, the soils present are typically Orthic Dark Brown Chernozems, with Calcareous Dark Brown Chernozems, Orthic Regosols, and Orthic Gleysols associated with landform (Acton & Ellis, 1978). Two adjacent quarter sections in the west block of SDNWA were chosen due to the presence of both perennial and annual cropping systems in the same agricultural landscape (Figure 3.1). The field in the annual cropping system during the study period was planted to flax, *Linum usitatissimum* (Linn.) in 2017 and barley, *Hordeum vulgare* (Linn.) in 2018 with the flax stubble piles being burned *in situ* in October of 2017. The current crop rotation on the site is variable with the producer maintaining a legume-oil seed-grain mix. Canola, *Brassica napus* (Linn.) is not permitted in a rotation within the boundaries of the SDNWA, due to restrictions within the National Wildlife Area on neonicotinoid coated seed, thus the producer uses alternate oilseed species, including flax. The perennial system consists of a mixture of introduced and native grass and forb species. It was cut for hay once in late July in each of the 2017 and 2018 growing seasons. The annual site has been under management by the same producer since 2009.

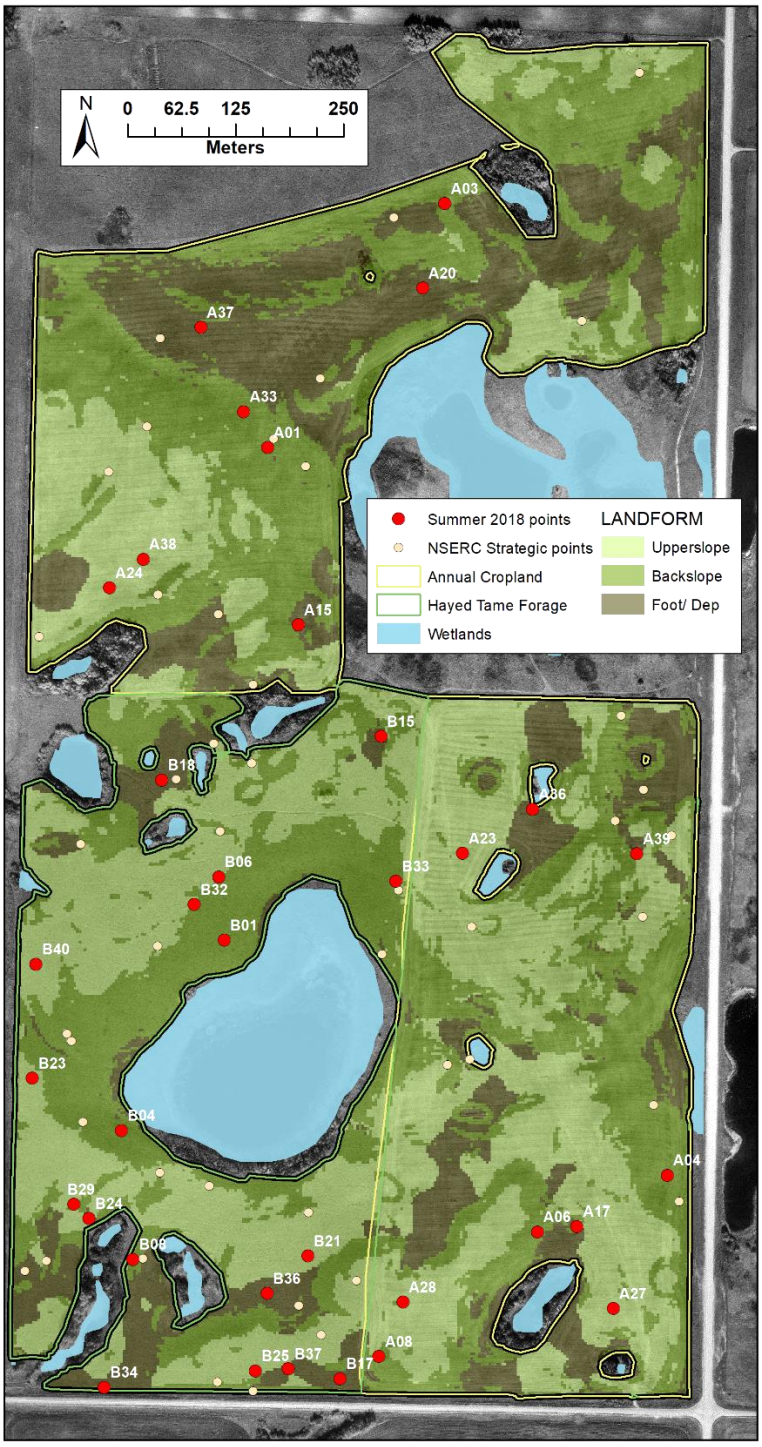


Fig. 3.1. Map of the St. Denis National Wildlife Area west block. Sample points for this study are highlighted in red, selected from a larger set of sampling points, shown in yellow.

3.1.2 Conservation Learning Centre

The second site was located at the Conservation Learning Centre (CLC) located approximately 126.5 km from Saskatoon SK, (Latitude: 53.032431, Longitude: -105.774186), along the northern limit of the Aspen Parkland Ecoregion. This region, located mostly within the Black Soil Zone, is characterized by Orthic Black Chernozems, with Orthic Dark Brown Chernozems, Orthic Gleysols, Orthic Luvisols, and Orthic Regosols being found in relation to landscape variation (Acton & Ellis, 1978). The topography at CLC is rolling hills with more, though smaller, ephemeral and permanent wetlands across the site than SDNWA. The climate at CLC is cooler and wetter than at SDNWA which coincides with its location in the Black Soil Zone. The site was chosen, as with the SDNWA, because it contained adjacent quarter sections of land that supported both annual and perennial cropping systems (Figure 3.2). The annual cropping system hosts a crop rotation of oil seed-grain-oil seed with canola being harvested in 2017 and wheat harvested in 2018. Unlike SDNWA the producer at CLC follows a more typical prairie crop rotation that includes canola. The perennial crop, like the SDNWA is a mixture of non-native and native forage species, with greater abundance and diversity of native species at CLC, particularly in the wetter areas and along the edge between annual and perennial crop. This regrowth is assumed to be natural as no known human reintroduction of native species has taken place.

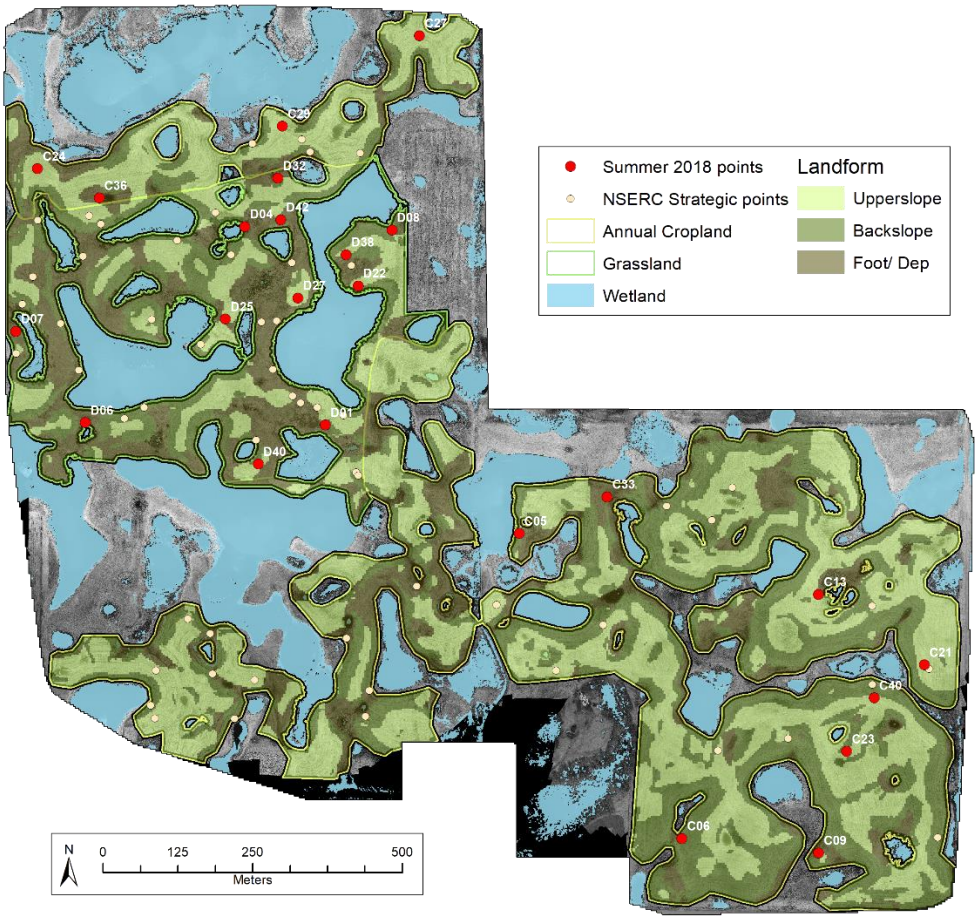


Fig. 3.2. Map of Conservation Learning Centre site. Sampling points for this study are highlighted in red, selected from the larger collection of sampling points, shown in yellow. Higher occurrences of wetlands on this site meant the need for more land area.

3.2 Field sampling methodology

A stratified random sampling design was established on both sites in 2018 as part of a broader sampling campaign for an NSERC Strategic Project led by Dr. Angela Bedard-Haughn (see all points on Figures 3.1 and 3.2). At each site the sampling design is distributed across two land uses: annual and perennial crop. Points within each land use were further split among landscape positions which were chosen to capture landscape variability across the entire site. The landscape positions include upper slopes, backslopes and footslopes at SDNWA and only upper

slopes and footslopes at CLC. An additional landscape position was included to capture the greater variability in the landscape at SDNWA, which was characterized by steeper slopes being more common within the study site, whereas it was decided that two landscape positions were sufficient to capture the landscape variability at CLC, based on the less severe topographic variation there. In total, 18 sampling points in each land use area at SDNWA and 12 points at CLC were selected, with more points at SDNWA to account for the third landscape position; six sampling points in each landscape position in each land use at SDNWA and six per landscape position in each land use at CLC. Soils from each sampling point were collected at SDNWA and CLC respectively, at depth increments of 0-15, 15-30 and 30-45 cm using 5 cm diameter sledge cores on July 9 and July 16, 2018. Surface soils (0-15 cm) were also collected on June 14 and 18, 2018 and August 13 and 16, 2018 using 2 cm diameter back saver probes. All samples were transported on ice to the laboratory and stored at 4°C overnight. Samples were sieved (2 mm) within 24 h of field collection and stored at -20°C until further analysis. Prior to analysis, sieved soils were subsampled for both enzyme assays and PLFA. For enzyme analysis soils were weighed into 1 g subsamples, while for PLFA bulk subsamples were freeze-dried and then subsampled further into 4 g samples.

3.3 Laboratory methodology

3.3.1 Fluorometric enzyme assays

A fluorometric microplate enzyme assay was used to detect potential hydrolytic soil enzyme activity for β - glucosidase, N – acetyl glucosaminidase, and phosphatase in soils at 0-15, 15-30, and 30-45 cm depths . These assays provide simultaneous, sensitive and rapid quantification of the activity of multiple enzymes within the same sample (Dick et al., 2018).

Enzyme activities were assayed using the 4-methylumbelliferone (4-MUB) fluorometric microplate method adapted from Bell et al. (2013). Soils (1 g fresh weight) were mixed with MUB buffer at pH 6.5 in a blender and then transferred to a stir plate. This buffer pH was determined as the optimal pH averaged for all three enzymes. This buffer pH was chosen by assessing enzyme activities in soils from both sites at four different buffer pH's: 5.5, 6.0, 6.5 and 7.5. The pH that produced the highest enzyme activity across all three enzymes collectively was chosen as the optimal buffer pH for use in this study. Substrates used included 25 mM MUB-β-D-glucopyranoside, 25 mM MUB-β-D-cellobioside, and 4-MUB-N-acetyl- β -D-glucosaminide for BG, NAG, and Phos, respectively. The soil-slurry was pipetted into three separate 5 mL centrifuge tubes, one for each enzyme: BG, NAG and PHOS, with 450 μL of substrate added to each vial. Seven vials were used for the standards: 0, 2.5, 5, 10, 25, 50 and 100 μM. The standards were created using a stock concentration that was then diluted, 450 μL of standard were added to each vial specified for that standard. The standards were then used to create a standard curve. The samples were incubated at room temperature for three hours, then centrifuged at 2000 rpm for 5 minutes in a Sorval RC 6+ centerfuge (Thermo Fisher Scientific Inc, Langenselbold, Germany). The supernatant was then pipetted into a microplate and the fluorescence peaks were read and recorded on the Filtermax F5 microplate reader (Molecular Devices LLC, San Jose, CA) at 360 nm excitation and 465 nm emission wavelengths. Enzyme assay data is expressed as μmol g^{-1} soil h^{-1}.

3.3.2 Phospholipid fatty acid extraction

Phospholipid fatty acid extraction was used to quantify soil microbial community structure and viable biomass and followed the method outlined in Helgason et al. (2010). PLFAs were extracted from soils sampled at depth only in July of 2018. July was selected to capture peak

productivity within plant communities. PLFAs were extracted from 4 g of freeze-dried soil using a phosphate buffer solution. In the organic phase phospholipids, neutral lipids and glycolipids were separated from the solution using solid phase extraction columns (0.50 g Si; Varian Inc. Mississauga, ON). Once separated, the phospholipids were methylated through alkaline methanolysis which produces fatty acid methyl esters (FAMEs) that were analyzed using a GC-FID (Scion 436-GC, Scion Instruments, Livingston, WL). Chromatograph peaks were identified by comparing existing Kovat indices with retention times that were normalized to fatty acid standards. These were quantified based on the addition of the internal standard methyl nonadecanoate (19:0). Microbial biomass was assessed using a total of 131 biomarkers. Of those samples, bacteria was split up into gram + : 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, 18:0 iso, gram - : 15:1 w6c, 16:1 w9c, 16:1 w7c, 17:0 cyclo w7c, 18:1 w9c, 18:1 w7c, 18:1 w5c, 19:0 cyclo w9c, 19:0 cyclo w7c, 19:0 cyclo w6c, actinobacteria: 16:0 10-methyl, 18:0 10-methyl, and general bacteria 17:0 10-methyl. Fungal groups were split into general fungi: 18:2 w6c, and AMF: 16:1 w5c.

3.4 Statistical analyses

All statistical analyses were completed using R v3.6.0 (R Core Team, 2019). For both enzyme activity and microbial abundance, general linear mixed effects models were used to test for differences. Main effects included, land use, landscape position, depth for PLFA's and land use landscape position, depth, and seasonality for enzymes. Comparisons of individual group means were then tested using Tukey's HSD test. Where necessary, data was log transformed to achieve a normal distribution. Main effects and interactions were declared significant at $p < 0.05$.

Non-metric multidimensional scaling (NMDS) was used on log(x+1) transformed PLFA (mol%) data to visualize microbial community structure and those factors which influenced variation in structure. Analysis of similarity (ANOSIM) and permutational multivariate analysis of variance (PERMANOVA) was used to test differences within and between main factors respectively, with PERMANOVA also being used to test significance interactions of main effects. All main effects and interactions were declared significant at $p < 0.05$.

4. RESULTS

4.1 Inherent soil properties at SDNWA and CLC

4.1.1 General Findings

Soil chemical and physical properties for NSERC Strategic project were first reported in Smith, 2020. To summarize, soils at SDNWA were clay dominant with secondary texture being primarily sand, while silt was found in equal parts to sand depending on landscape position. There was a high occurrence of coarse fragments throughout the soil profile, reflecting the till deposit that underlays the soils at the site. At CLC the soils were once again clay dominant however sand and silt were found to exist in more equal parts across the site regardless of topography. There were little to no coarse fragments found in the soil profile at CLC due to the superglacial lacustrine deposit overlaying till at this location. Overall nutrient availability and C concentrations were similar between the two sites, but appeared to be slightly higher at CLC than SDNWA. There was a difference in the pH between sites, with soils at SDNWA being more alkaline with an average pH of around 7.5 and soils at CLC being more acidic with an average pH of 6.5.

4.1.2 Ancillary soil property trends

To support the interpretation of the biological data collected for this study, SOC (%), organic N (mg/g) and available P (µg/g) are presented in Figs. 4.1- 4.3; a fuller suite of soil chemical and physical properties are presented in the Appendix. The choice of SOC, organic N and available P is due to their direct relation to enzyme activity and microbial biomass, the properties that were used in this study. For SOC at SDNWA, it follows the known trend of decreasing with depth through the soil profile, a trend that was consistent for both land uses and was seen in organic N and available P concentrations in the soils. In the annual land use, it appears that backslopes had the lowest concentrations of SOC, organic N and available P, a trend which

was not shared by the perennial system (Fig 4.1-4.3). At CLC, SOC, organic N and available P concentrations all followed the trend of decreasing with depth, with a notable deviation from this trend occurring in the 30-45 cm depth of the depressions in the annual system (Fig 4.1), this deviation was not exhibited by organic N or available P. Overall, depressions had higher levels of nutrient and C than upper slopes (Fig 4.1-4.3).

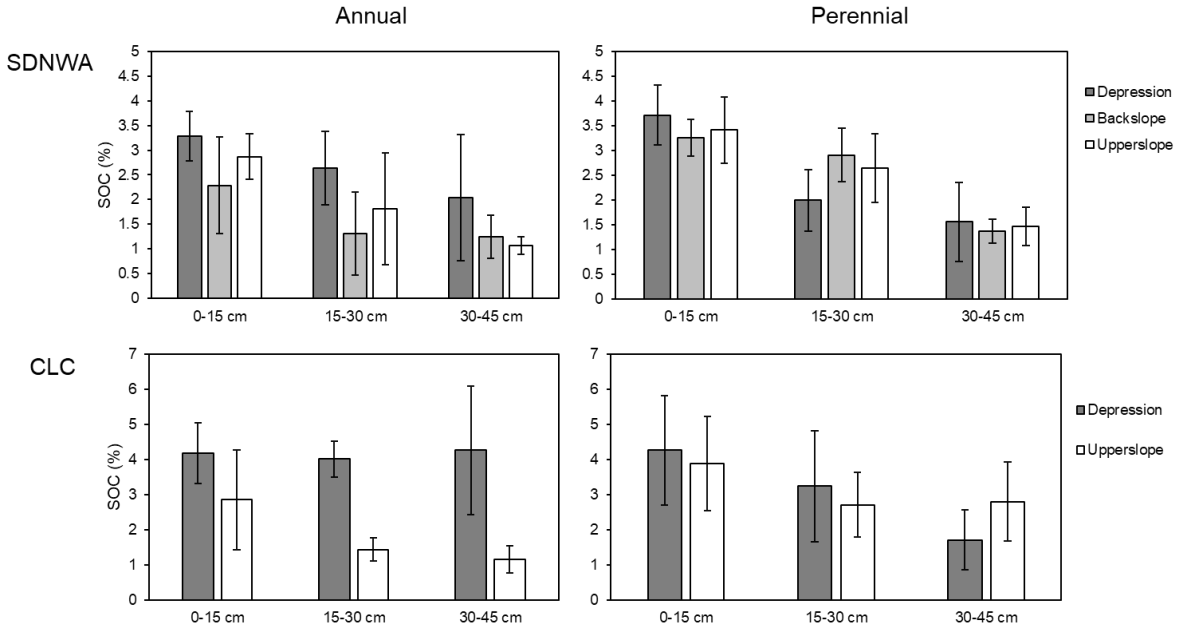


Fig. 4.1. Mean ± standard deviation SOC (%) at three depths for three (SDNWA) and two (CLC) landscape positions in perennial and annual land uses at SDNWA (n=18) and CLC (n=12).

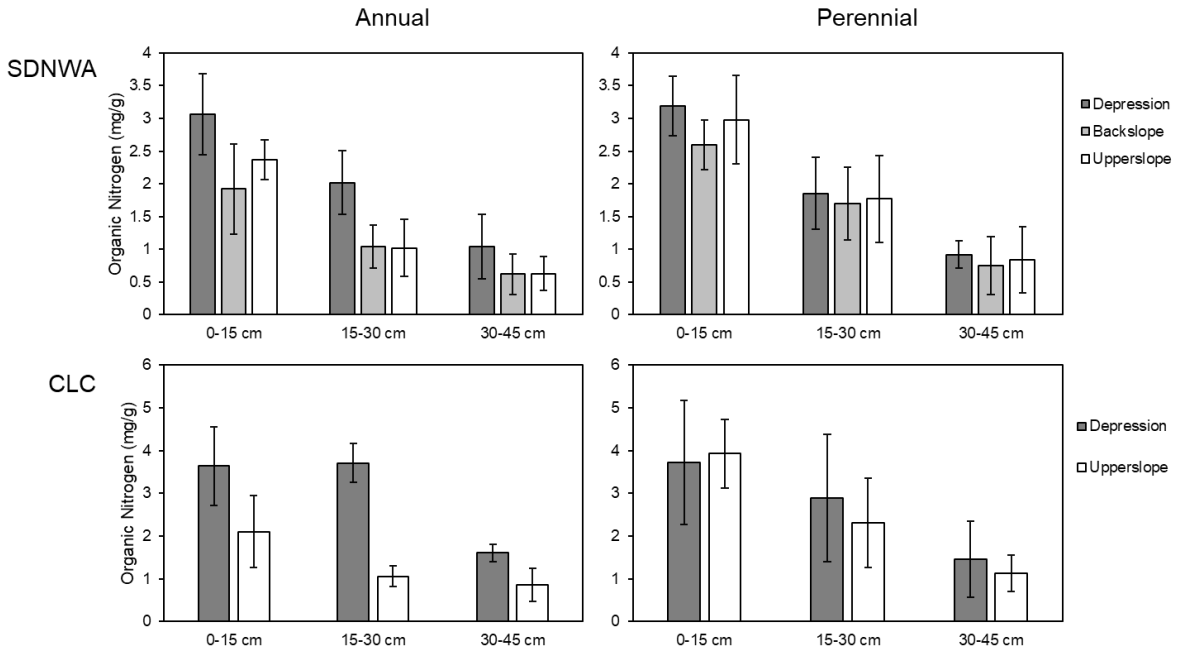


Fig. 4.2. Mean ± standard deviation soil organic nitrogen (mg/g) at three depths for three (SDNWA) and two (CLC) landscape positions in perennial and annual land uses at SDNWA (n=18) and CLC (n=12).

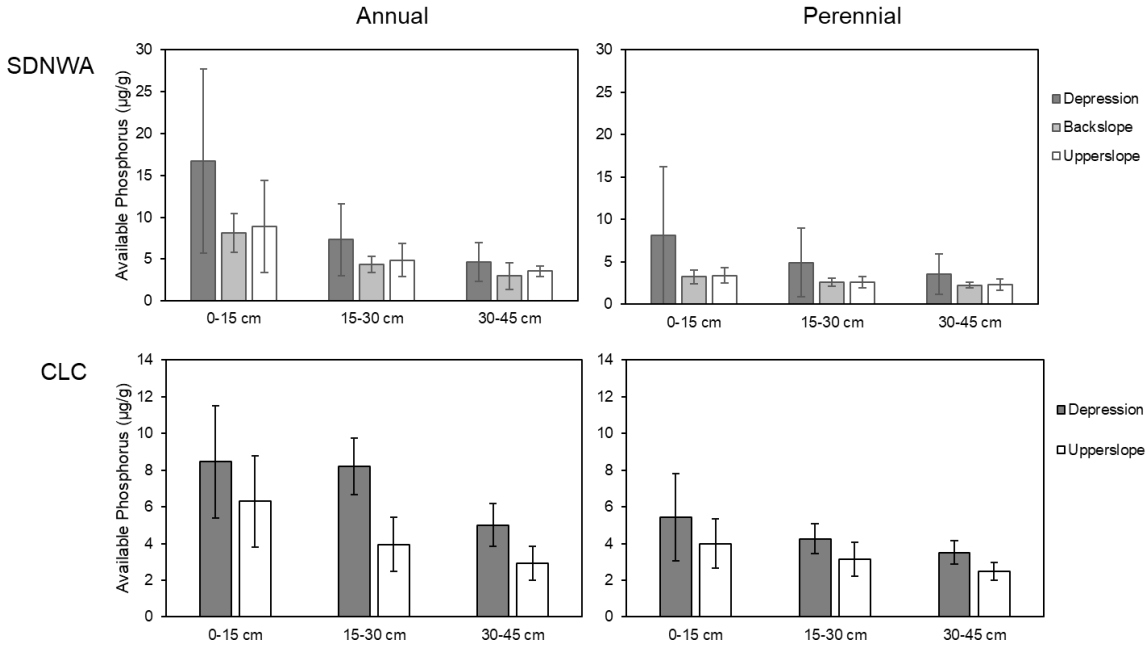


Fig. 4.3. Mean ± standard deviation soil available phosphorus (µg/g) at three depths for three (SDNWA) and two (CLC) landscape positions in perennial and annual land uses at SDNWA (n=18) and CLC (n=12).

4.2 Extracellular enzyme activity

4.2.1 St. Denis National Wildlife Area

4.2.1.1 β-glucosidase activity

A two-way interaction between land use and seasonality ($p < 0.0001$; Table 4.1) at SDNWA revealed that BG activity was 98% higher in the annual compared to the perennial system in surface soils collected in June ($p < 0.0001$). However, this land use difference did not persist through July and August (Fig. 4.1; $p > 0.05$). Soils sampled at depth in July revealed that depth significantly affected BG activity (Table 4.2), with decreased activity with each depth increment through the soil profile (Fig. 4.2).

4.2.1.2 N-acetyl glucosaminidase activity

In surface soils at SDNWA, NAG enzyme activity was affected by a two-way interaction between land use and seasonality ($p<0.0001$; Table 4.1). This interaction revealed significantly higher NAG activity in the annual compared to the perennial system in June ($p<0.0001$; Fig. 4.1). This pattern did not continue through the growing season, however, as NAG activity was higher in the perennial system in July and no difference in August.

Unlike BG, there was also a two-way interaction between land use and landscape position in surface soils ($p = 0.0102$; Table 4.1) that indicated NAG activity tended to be higher in the perennial system only in the upper slope positions of the fields ($p=0.0635$; Fig. 4.3). In July when samples were taken at multiple depths, this same two-way interaction between land use and landscape position was detected ($p = 0.0157$; Table 4.2). With data pooled from the three soil depth increments in July, the interaction was more pronounced; NAG activity was 31% lower in the annual compared to the perennial system at the upper slope position, with no differences in activity in the depression or backslope positions (Fig. 4.3) Like BG, depth had an expected significant

effect on NAG activity (Table 4.2) with activity decreasing with each depth increment (Figure 4.2).

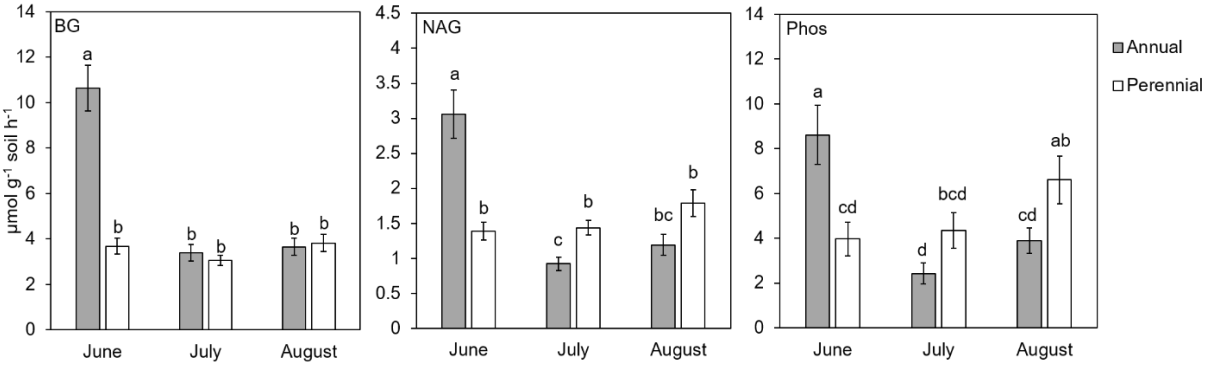
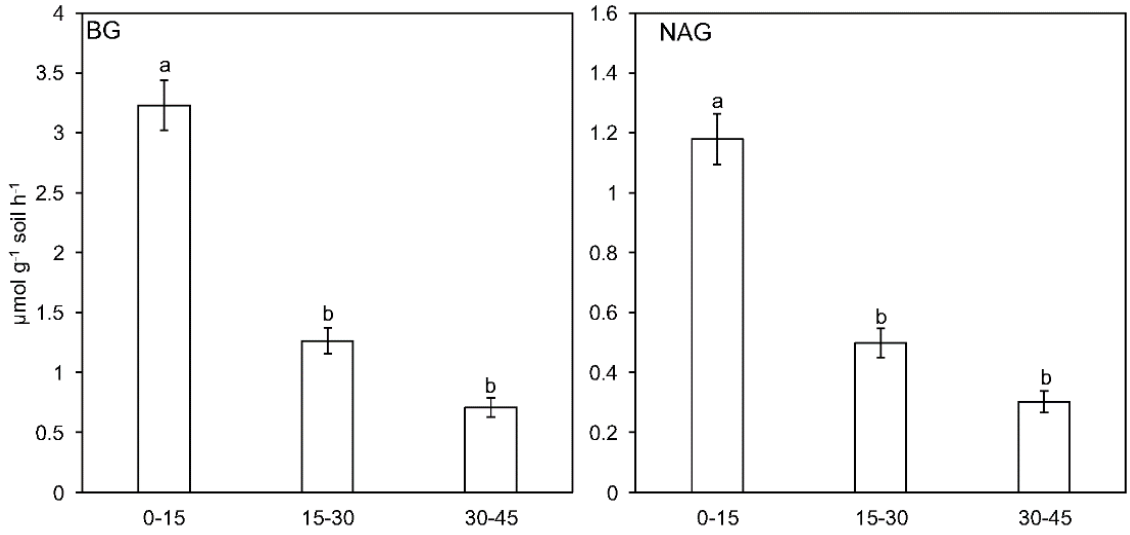


Fig. 4.4. Activity of β-glucosidase (BG), N-acetyl glucosaminidase (NAG), and phosphatase (Phos) in surface soils (0-15 cm) across two land uses over the growing season at St. Denis National Wildlife Area (SDNWA). Bars are means ± standard errors (n=17).



1Fig. 4.5. Activity of β-glucosidase (BG) and N-acetyl glucosaminidase (NAG) in soils sampled in July from 0-15, 15-30, and 30-45 cm depth increments at St. Denis National Wildlife Area (SDNWA). Bars are means ± standard errors (n=34).

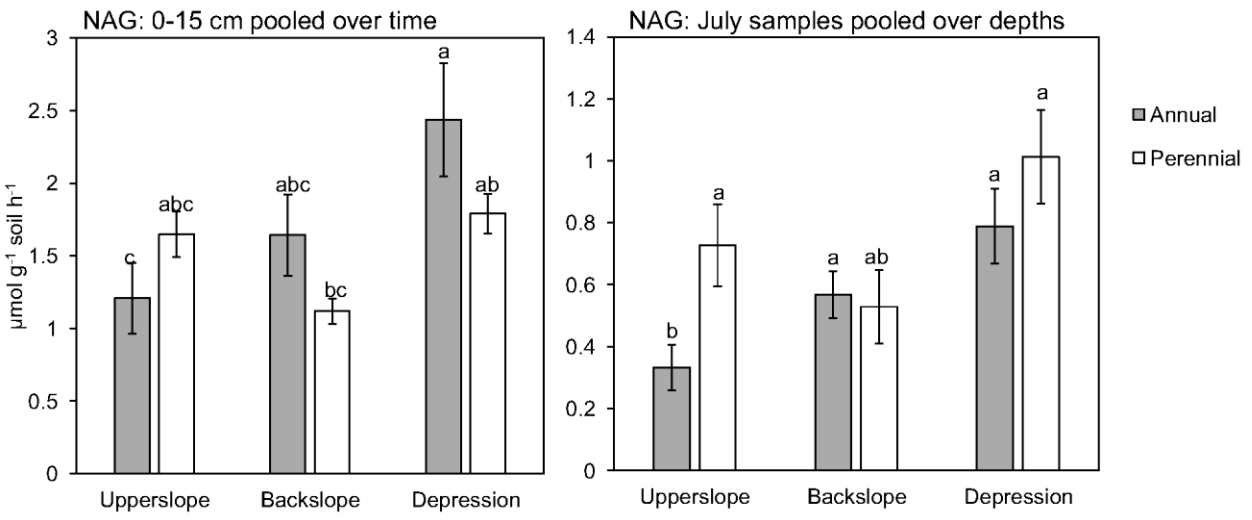


Fig. 4.6. Activity of N-acetyl glucosaminidase (NAG) in surface soils (0-15 cm) pooled over the three sampling times (left panel) and for samples collected in July pooled over three depth increments (right panel) for two land uses in upper slope, backslope, and depression landscape positions at St. Denis National Wildlife Area (SDNWA). Bars are means ± standard errors (n=18).

4.2.1.3 Phosphatase activity

Phosphatase activity in surface soils over the growing season was significantly affected by a two-way interaction between land use and sampling date (p=0.0001; Table 4.1). There was higher Phos activity in June in the annual system, followed by higher Phos activity in the perennial system in July and August (Fig. 4.1). There was generally more variability in Phos enzyme activity compared to BG and NAG enzymes.

In SDNWA, soils sampled in July at depth within the soil profile, there was a two-way interaction between land use and depth (Table 4.2), with higher Phos activity in the surface soils (0-15 cm) of the perennial compared to annual system; however, the difference did not continue through the soil profile (Fig. 4.4). A two-way interaction between landscape position and depth was observed (p=0.0273; Table 4.2). Phos activity was significantly higher in 15-30 and 30-45 cm

in depressions than in backslopes or upper slopes while in surface soils (0-15 cm) Phos activity was significantly lower only in the backslopes ($p<0.05$; Fig. 4.4).

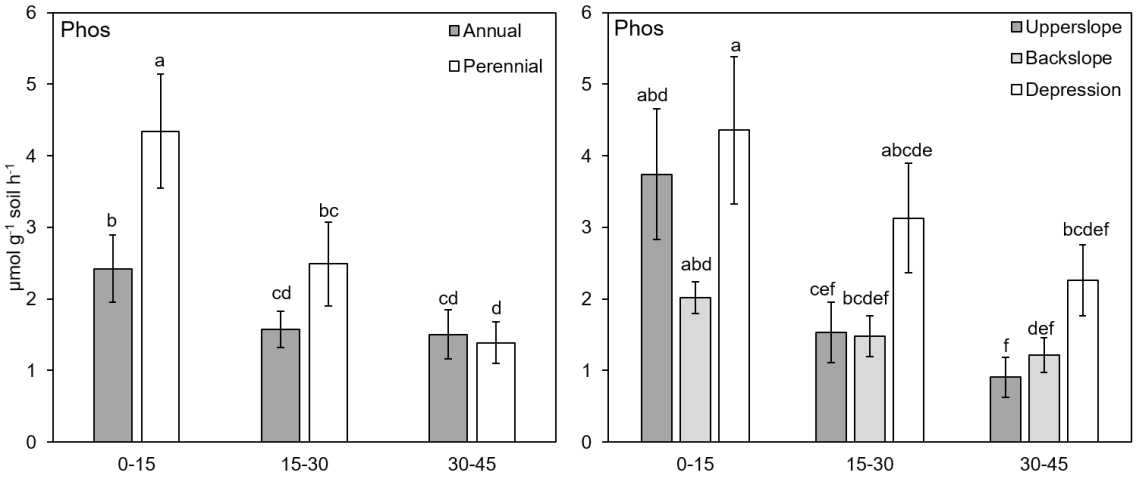


Fig. 4.7. Activity of phosphatase (Phos) for samples collected in July from three depth increments for two land uses (left panel, n=17) and for samples collected in July from three depth increments in upper slope, backslope, and depression landscape positions (right panel, n=12) at St. Denis National Wildlife Area (SDNWA). Bars are ± standard errors.

Table 4.1: Analysis of variance of enzyme activities, B-glucosidase (BG), N-acetyl glucosaminidase (NAG), and phosphatase (Phos) in surface (0-15 cm) soils over the growing season at the St. Denis National Wildlife Area (SDNWA) and the Conservation Learning Centre (CLC).

	SDNWA			CLC		
	BG	NAG	Phos	BG	NAG	Phos
Land use (LU)	0.0009**	0.5437	0.6887	0.0014**	<0.0001***	0.0347*
Landscape position (LP)	0.1182	0.0012**	0.0855	0.0852	0.0080**	0.3139
Seasonality	<0.0001***	<0.0001***	<0.0001***	0.0246*	0.0032**	0.0024**
LU x LP	0.1706	0.0102*	0.3237	0.0776	0.2571	0.0075**
LU x Seasonality	<0.0001***	<0.0001***	<0.0001***	0.0431*	0.0047**	0.6614
LP x Seasonality	0.7922	0.9155	0.5153	0.9559	0.1923	0.9661
LU x LP x Seasonality	0.7429	0.8896	0.7062	0.9679	0.8463	0.8867

*p=0.05-0.01, **p=0.01-0.0001,***p<0.0001

Table 4.2: Analysis of variance of enzyme activities, β-Glucosidase (BG), N-acetyl glucosaminidase (NAG), and phosphatase (Phos) in soil samples collected from three depth increments (0-15, 15-30, 30-45 cm) in July at the Conservation Learning Centre (CLC) and St. Denis National Wildlife Area (SDNWA).

	SDNWA			CLC		
	BG	NAG	Phos	BG	NAG	Phos
Land use (LU)	0.4319	0.0777	0.2695	0.4477	0.0231*	0.3113
Landscape position (LP)	0.0765	0.0008**	0.0690	0.0411*	0.0322*	0.0672
Depth	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
LU x LP	0.1385	0.0157*	0.1065	0.0044**	0.0055**	0.0001**
LU x Depth	0.6517	0.0507	0.0087**	0.0186*	0.0700	0.1163
LP x Depth	0.6186	0.2301	0.0273*	0.2321	0.5452	0.0255*
LU x LP x Depth	0.7139	0.6145	0.2400	0.0105*	0.0258*	0.1199

*p=0.05-0.01 **p=0.01-0.0001, ***p<0.00

4.2.2 Conservation Learning Centre

4.2.2.1 β-glucosidase activity

In surface soils sampled across the growing season at CLC, BG activity was affected by a two-way interaction between land use and seasonality (p=0.0431; Table 4.1). While BG activity was elevated in the perennial compared to annual system in June and July, by August activity was significantly higher with the perennial system having approximately 2.5 times higher BG and NAG activity than the annual system (Fig. 4.5). In CLC soils sampled in July at three depths, a three-way interaction between land use, landscape position, and depth was found (p=0.0105; Table 4.2). There was consistently higher BG activity in perennial compared to annual upper slopes for all soil depth increments (p<0.05), while BG activity was higher in annual compared to perennial depressions at the 30-45 cm depth only (p=0.0022; Figure 4.6).

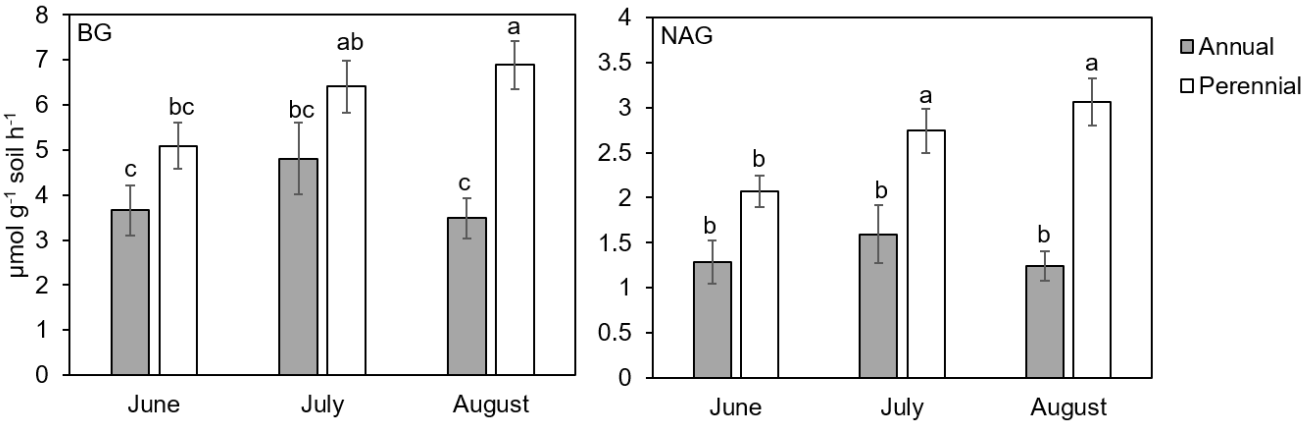


Fig. 4.8. Activity of β-glucosidase (BG) and N – Acetyl glucosaminidase (NAG) for samples collected over the growing season in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=12).

4.2.2.2 N-acetyl glucosaminidase activity

Like with the BG enzyme, there was a two-way interaction between seasonality and land use for NAG activity in surface soils at CLC ($p=0.0047$). This interaction was evidenced by enzyme activity that was increasingly higher in the perennial system than the annual system over the course of the growing season (June, July, August = $p<0.05$; Fig. 4.5). There was also a significant effect of landscape position, with NAG activity being highest in upper slope positions ($p=0.0080$).

In soils sampled in July, a three-way interaction between land use, landscape position, and depth ($p=0.0258$; Table 4.2) was found. Following the same pattern as BG activity, this interaction illustrated significantly higher NAG activity in the perennial compared to the annual system at all depths (0-15, 15-30, and 30-45 cm; $p<0.05$) in the upper slope position. However, in depressions, NAG activity was 75% higher in the annual system in the 30-45cm depth, significantly higher than other landscape positions (Fig. 4.6).

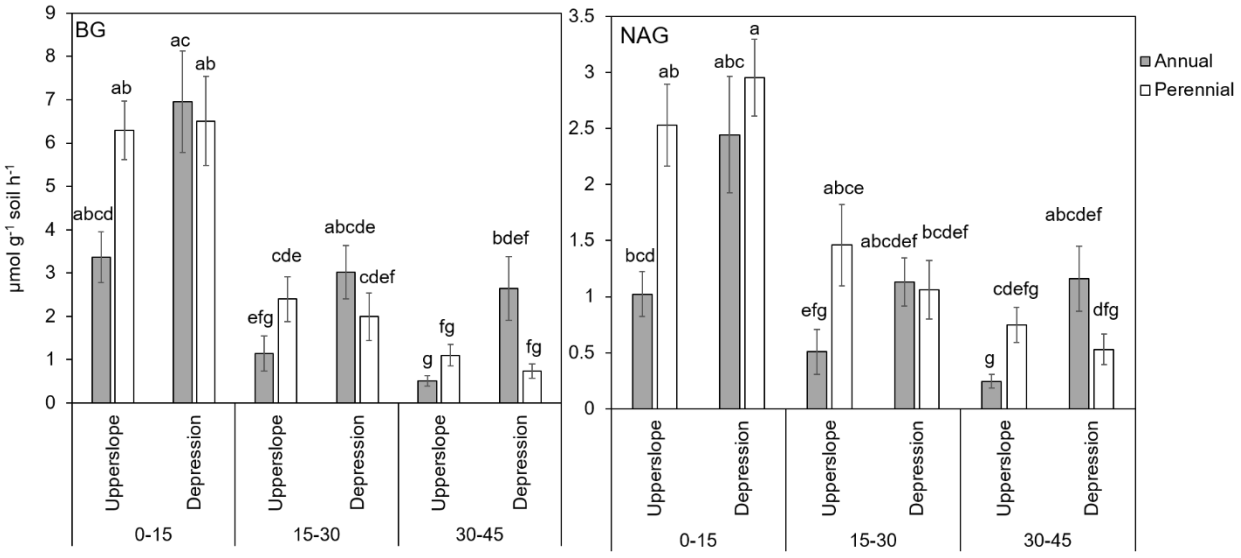


Fig. 4.9. Activity of β-glucosidase (BG) and N – Acetyl glucosaminidase (NAG) for samples collected at three depth increments 0-15, 15-30 and 30-45 cm, in two slope positions, upper slopes and depressions, in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=6).

4.2.2.3 Phosphatase activity

Phosphatase activity in surface soils varied over the growing season, with lower activities occurring in June compared to July and August ($p < 0.05$). In surface soils at CLC, a two-way interaction between land use and landscape position was found ($p=0.0075$; Table 4.1). Phosphatase activity was higher in perennial compared to annual upper slopes, but there was no difference between land uses in the depression (Figure 4.7). When data was analyzed for soil samples collected at three depths in July, the same two-way interaction between land use and landscape position ($p=0.0016$; Table 4.2) was found. Similarly, Phos was higher in perennial compared to annual soils in the upper slopes; across soil depths, Phos was higher in the perennial system (Fig. 4.7). Phosphatase activity was also affected by a two-way interaction between landscape position and depth (0.0255; Table 4.2). NAG activity tended to be higher in depressions throughout the

profile; however, this difference was significant only at the deepest 30-45 cm sampling increment (Fig. 4.8).

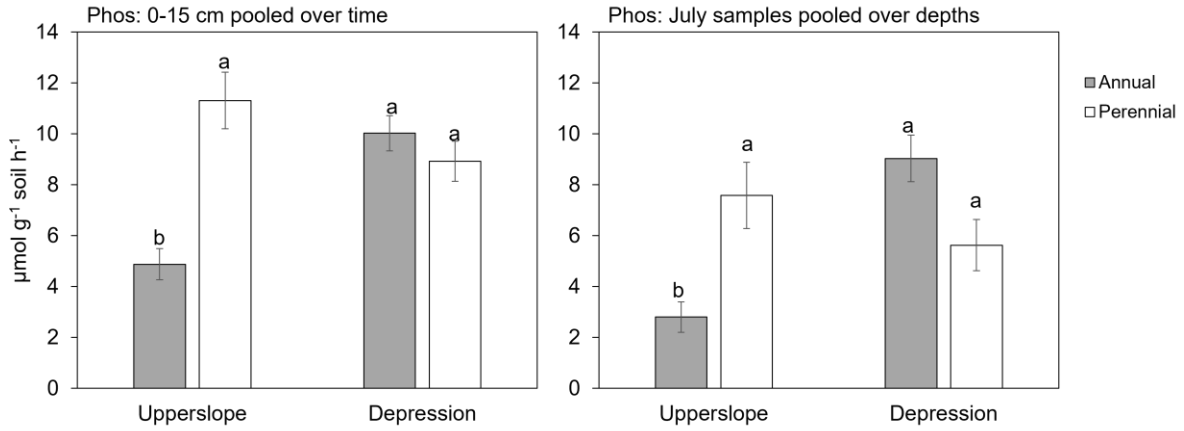


Fig. 4.10. Activity of phosphatase (Phos) for surface soils (0-15cm) pooled over time (left panel) and samples pooled over depth increments 0-15, 15-30 and 30-45 cm (right panel), in two slope positions, across two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=18).

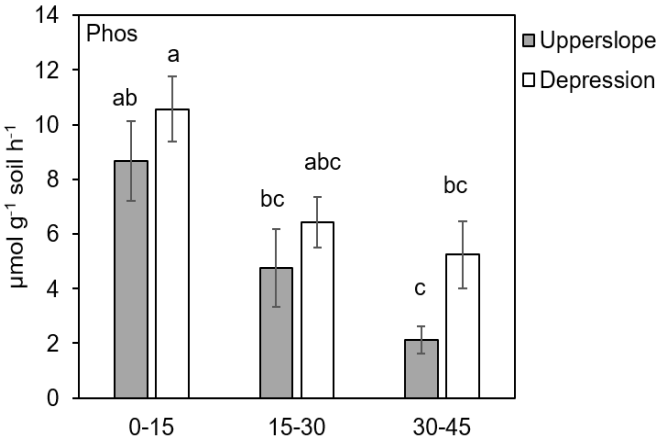


Fig. 4.11. Activity of phosphatase (Phos) for samples collected at three depth increments 0-15, 15-30 and 30-45 cm, in two slope positions, at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=12).

4.3 Phospholipid fatty acid abundance

4.3.1 St. Denis National Wildlife Area

Phospholipid fatty acids were extracted from soil samples collected at depth within the soil profile (0-15, 15-30, 30-45 cm increments) in July only. At SDNWA, total PLFA, and the abundance of all bacterial groups responded to main effects only (Table 4.3) and followed similar patterns (Table 4.4). Depth had a significant effect ($p<0.0001$; Table 4.3) with PLFA abundance being highest in the surface soils ($p<0.05$) and decreasing with soil depth (Table 4.4). Higher PLFA abundance was found in the perennial cropping system than in the annual cropping system for total PLFA and all bacterial groups, except for actinobacteria where the difference was not significant (Table 4.3). Finally, landscape position affected abundance of total PLFA and all bacterial groups ($p<0.05$), where abundance was greater in depressions than in upper slopes (Table 4.3).

For both general fungal and AMF biomarkers, there was a two-way interaction between land use and landscape position (Table 4.4). General fungal abundance was higher in perennial than annual upper slopes, but not at positions downslope (Fig. 4.9). Conversely, AMF abundance was higher in perennial compared to annual upper slopes and backslopes but there was no significant difference in AMF concentrations between annual and perennial depressions (Fig. 4.9). Similar to total PLFA and bacterial groups, depth also affected general fungi and AMF (Table 4.3),

and in a similar pattern with abundance declining with depth (data not shown). Bacterial to fungal (B:F) ratios only changed when associated with depth alone ($p<0.0001$, Table 4.3).

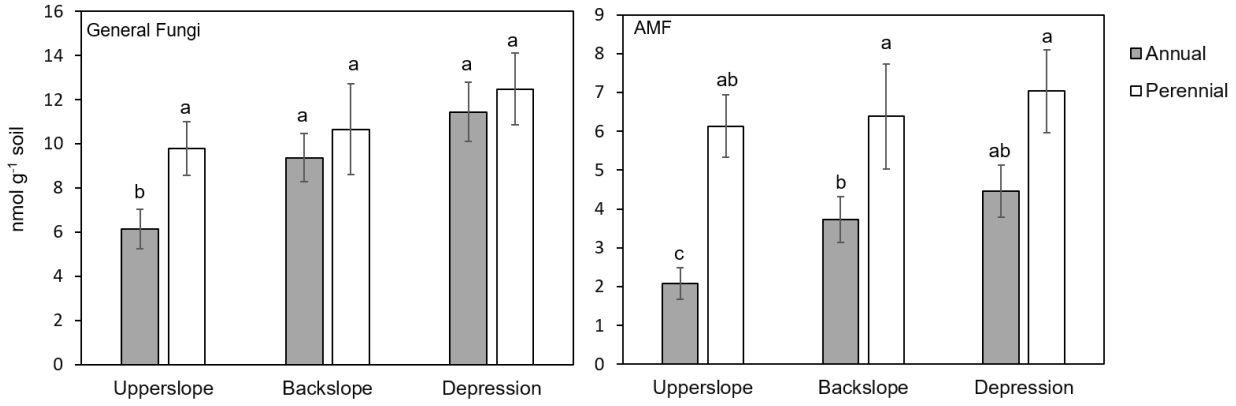


Fig. 4.12. Abundance of general fungi (GF) and arbusuclar mycorrhizal fungi (AMF) for samples collected, in three slope positions, upper slopes, backslopes and depressions, in two land uses at St. Denis National Wildlife Area (SDNWA). Bars are means ± standard errors (n=34).

Table 4.3: Analysis of variance of phospholipid fatty acid (PLFA) abundance for total PLFA, microbial groups, and bacterial to fungal (B:F) ratios for soils sampled under different land use (LU), landscape position (LP), and soil depth (D) in July 2018 at St. Denis National Wildlife Area (SDNWA).

	Total	General Bacteria	Gram +	Gram -	Actinobacteria	General Fungi	AMF	B:F
Depth	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
(LU)	0.0075**	0.0415**	0.0376*	0.0489*	0.1174	0.0266*	<0.0001***	0.9254
Landscape Position (LP)	0.0002**	0.0002**	0.0002**	0.0001***	0.0033**	0.0002**	0.0016**	0.0962
D:LU	0.4604	0.4482	0.3140	0.5971	0.4068	0.2863	0.7259	0.6000
D:LP	0.7947	0.7363	0.8595	0.6445	0.8818	0.8128	0.8356	0.5968
LU:LP	0.0516	0.0737	0.0780	0.0700	0.1080	0.0498*	0.0031**	0.3724
D:LU:LP	0.3351	0.3560	0.3811	0.3643	0.4828	0.0.3539	0.4133	0.6052

4.3.2 Conservation Learning Centre

In contrast to SDNWA, the effects of land use and sampling location (topography and depth) on PLFA abundance were more complex at CLC. A three-way interaction between land use, landscape position, and depth existed for total PLFA abundance and all microbial groups, except B:F ratios (Table 4.4). This interaction revealed that the perennial system had significantly higher PLFA abundances than the annual system in all three depth increments in the upperslope positions, while PLFA abundance was higher in the perennial compared to the annual system in the depressions, but only in the surface soils (Figures 4.10-4.12). A two-way interaction between land use and landscape position ($p=0.0027$; Table 4.5) existed for B:F ratios, revealing lower B:F ratios in perennial *vs*. annual upperslope positions (Fig 4.10). Depth also affected B:F ratios ($p=0.0012$; Table 4.4) with a significant increase in B:F ratio with depth through the soil profile.

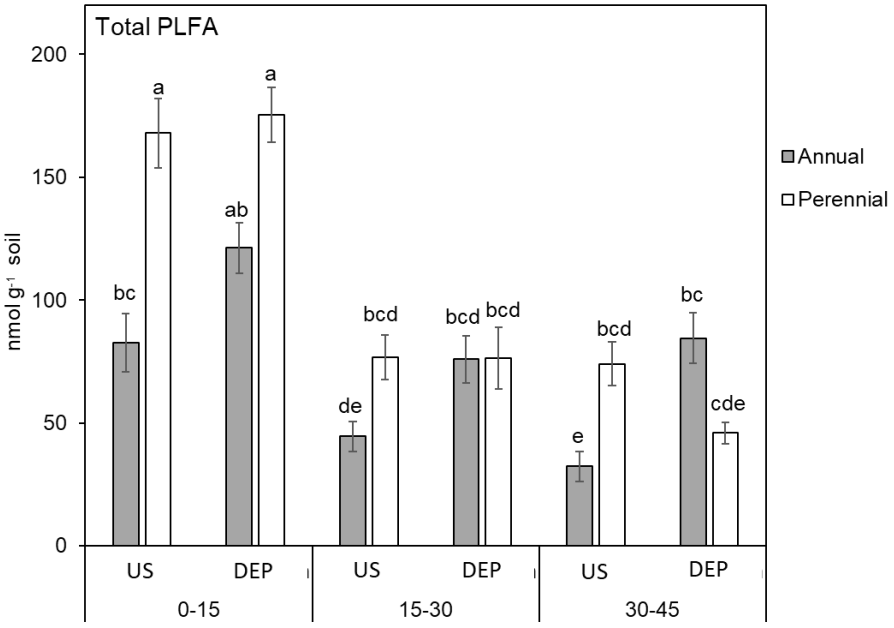


Fig. 4.13. Abundance of total PLFA for samples collected in July at three depth increments 0-15, 15-30 and 30-45 cm, in two slope positions, upper slopes and depressions, in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=6)

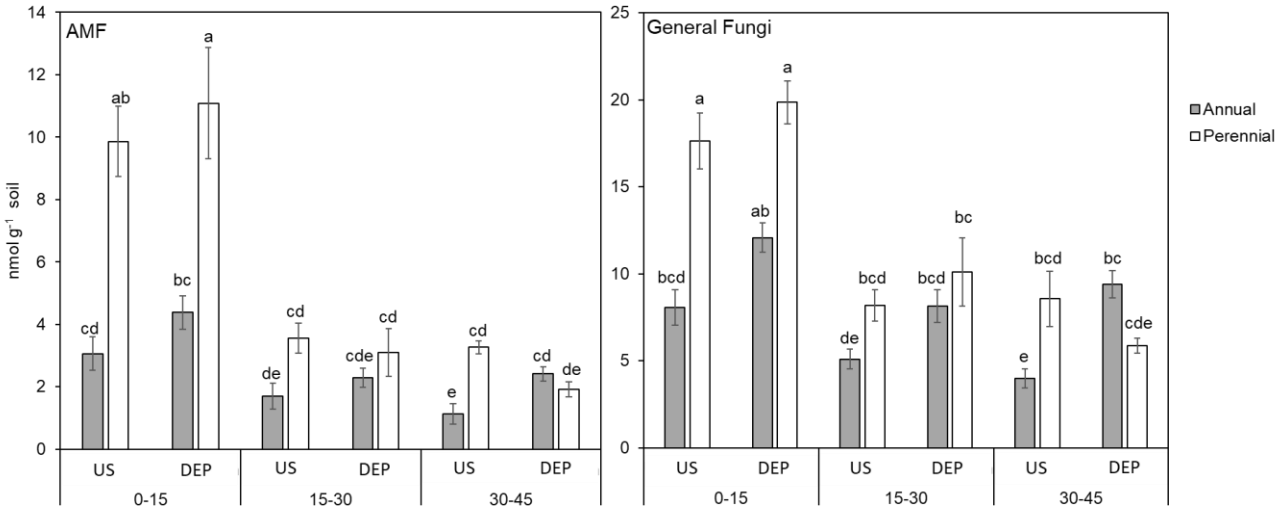


Fig. 4.14. Abundance of arbuscular mycorrhizal fungi (AMF) and general fungi (GF) for samples collected in July at three depth increments 0-15, 15-30 and 30-45 cm, in two slope positions, upper slopes and depressions, in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=6).

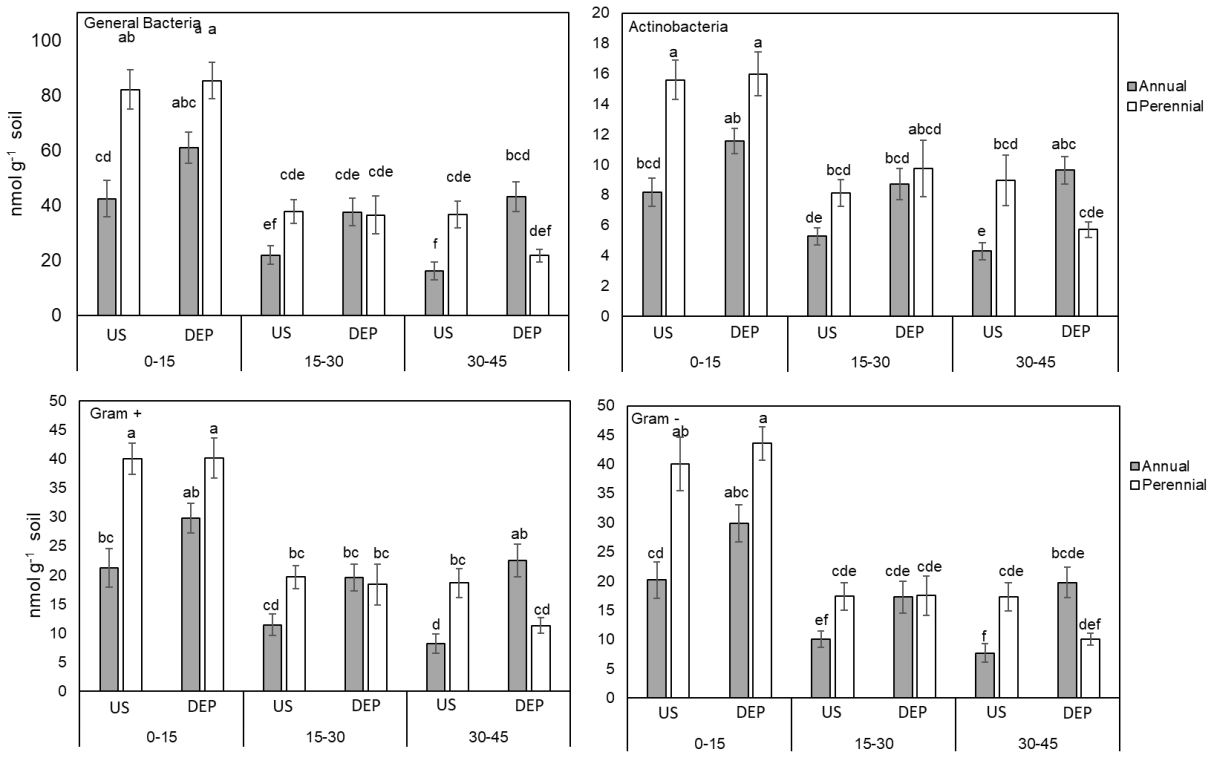


Fig. 4.15. Abundance of general bacteria (GB), actinobacteria (AB), Gram + (G+) and Gram – (G-) bacteria for samples collected in July at three depth increments 0-15, 15-30 and 30-45 cm, in two slope positions, upper slopes and depressions, in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=6).

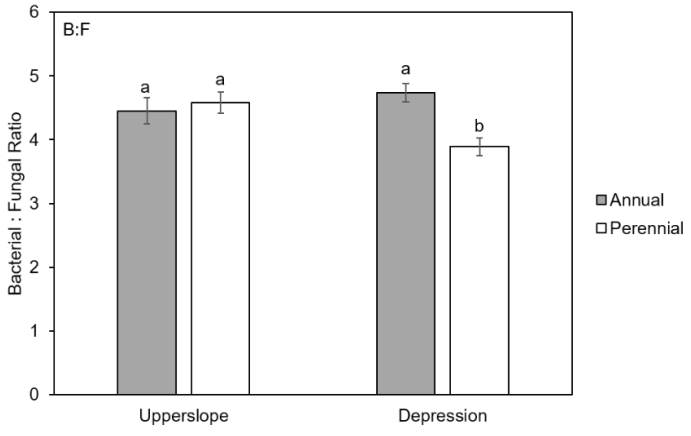


Fig. 4.16. Difference in bacterial: fungal ratios (B:F) for samples collected in July, in two slope positions, upper slopes and depressions, in two land uses at the Conservation Learning Centre (CLC). Bars are means ± standard errors (n=12).

Table 4.4: Analysis of Variance for phospholipid fatty acid (PLFA) Signatures in July sampled soils in 3 depths at the Conservation Learning Centre.

	Total	General Bacteria	Gram +	Gram -	Actinobacteria	General Fungi	AMF	B:F
Depth	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	0.0012**
Land Use (LU)	<0.0001***	0.0003**	0.0006**	0.0002**	0.0001**	<0.0001***	<0.0001***	0.0216*
Landscape Position (LP)	0.0012**	0.0040**	0.0050**	0.0038**	0.0009**	0.0001***	0.00640	0.1829
D:LU	0.0770	0.1128	0.1504	0.1037	0.0697	0.0100*	0.0270*	0.4178
D:LP	0.9639	0.9700	0.8640	0.9917	0.6885	0.9611	0.7618	0.7765
LU:LP	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001**	<0.0001***	0.0017*	0.0027**
D:LU:LP	0.0066**	0.0085**	0.0097**	0.0091**	0.0113*	0.0078**	0.0329*	0.1963

4.4 Microbial community composition

4.4.1 St. Denis National Wildlife Area

At SDNWA, microbial community composition based on the relative abundance of PLFA biomarkers grouped strongly by depth with the tightest groupings forming among surface soils (0-15 cm; Fig 4.14) and a less distinct gradient between 15-30 cm and 30-45 cm points. Of the 0-15 cm soils, upper slopes and depressions grouped more closely together than backslopes (Fig 4.14). There was also a notable separation between perennial and annual points with perennial points grouping in the upper portion of the NMDS ordination and annual points grouping in the lower portion. ANOSIM within-group variation revealed that each main effect was significantly different from the others (LU: R = 0.2352, p = 0.001; LP: R 0.06425, p= 0.002; D: R = 0.1952, p= 0.001), while results from between groups found through PERMANOVA showed that 2 and 3-way interactions between main effects were also significant (LU:LP = 0.001, LP:D = 0.001, LU:D = 0.001, LU:LP:D = 0.001). Second NMDS solution for SDNWA can be found in Appendix 2 (Fig 8.2)

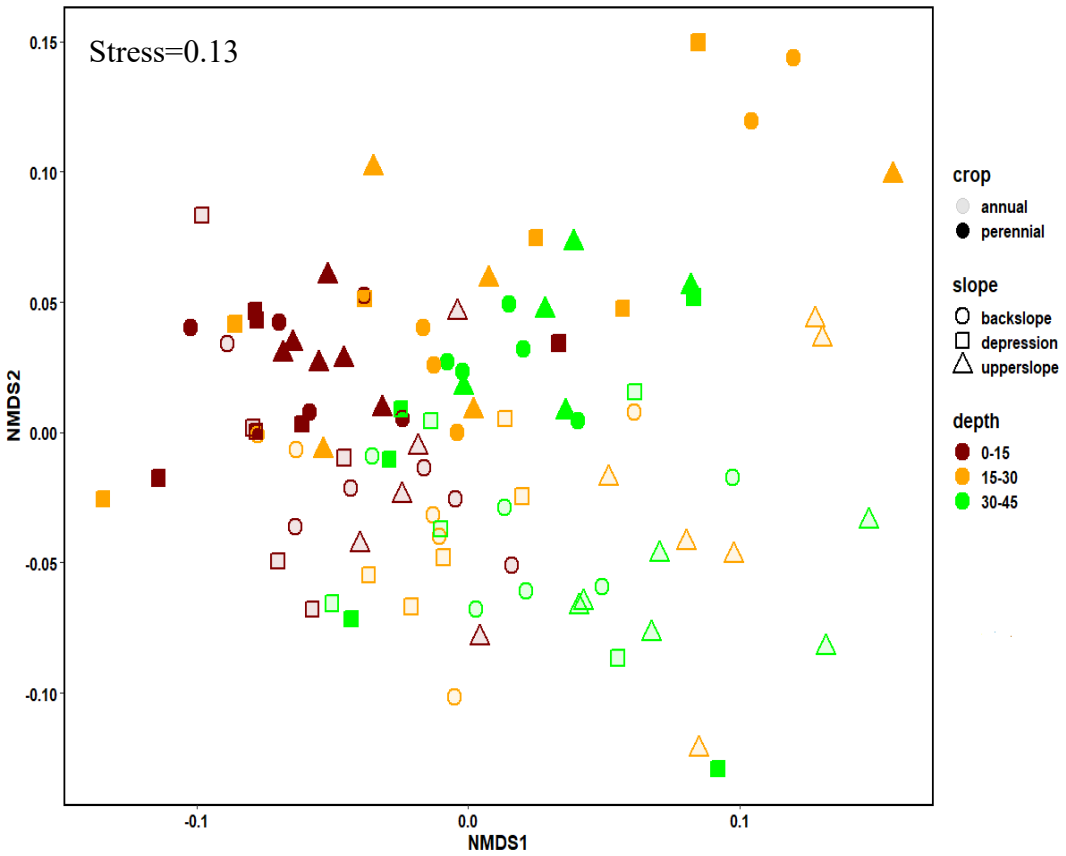


Fig. 4.17. NMDS plots for 2018 soil community composition at St Denis National Wildlife Area. NMDS depicts results from three depth increments, three slope positions and two land uses, 3D solution.

4.4.2 Conservation Learning Centre

Unlike SDNWA, the community at CLC had more distinct separation between treatment groups within the 0-15 cm depth increment, as well as more variation amongst treatment groups in general (Fig 4.15). Results from the accompanying ANOSIM showed that each main effect were significantly different (LU: R = 0.1197, p = 0.001; LP: R = 0.1109, p = 0.001; D: R = 0.2921, p = 0.001). Results between groups measured by the PERMANOVA showed that interactions between main effects were also significant (LU:LP = 0.001, LP:D = 0.001, LU:D = 0.001, LU:LP:D = 0.001). The second NMDS solution for CLC can be found in Appendix 2 (Fig 8.2)

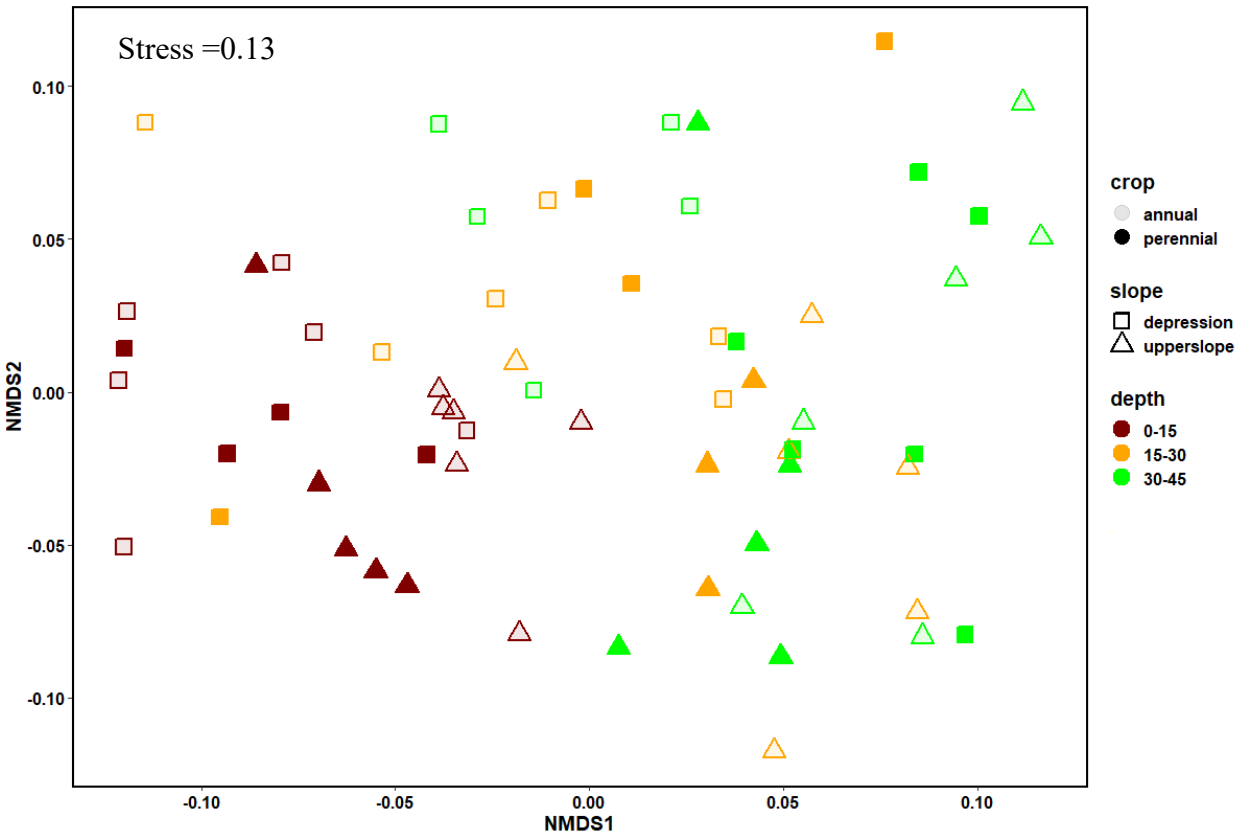


Fig. 4.18. NMDS for 2018 soil community composition at the Conservation Learning Centre. NMDS depicts result for points from three depth increments, two slope positions and two land uses, 3D solution.

5. DISCUSSION

The purpose of this study was to evaluate the effects of annual and perennial cropping systems on soil biological properties in topographically variable landscapes. I evaluated two novel biological soil health indicators, extracellular enzyme activities, and viable microbial biomass and community composition based on PLFA extraction, both of which are not typically used in soil health tests despite their role in supporting many soil ecosystem services including nutrient cycling and soil organic matter formation (Bünemann et al., 2018). I found that while these biological properties often responded to differences in land use, the effects were sometimes dependent on location of sampling within the landscape and soil profile, as well as time of sampling within the growing season. The results from my study indicate that there are other factors, in addition to management history and current land use, that need to be taken into consideration when evaluating soil biological response in a topographically variable landscape.

This study evaluated soil biological properties at two sites; thus, throughout the discussion I will compare results between the sites. In the section below I will discuss: 1) how the effects of land use on soil enzyme activity were moderated by time within the growing season; 2) how topographic effects were less severe under perennial cover compared to annual crops; 3) the importance of soil depth in the distribution of soil microbial properties; and 4) the more complex relationships among soil resources, the physical landscape, and the soil microbial community seen at CLC but not at SDNWA.

5.1. Seasonal variation moderated land use effects on extracellular enzyme activity

Seasonality—reflecting changes in weather, plant growth rates and productivity, and management interventions—had a strong effect on the soil extracellular enzyme activities over the course of the growing season. However, seasonal effects differed between land uses—and these

land use differences were not the same at each site. For instance, at SDNWA, enzyme activity (BG, NAG, and Phos) was especially high in the annual compared to the perennial system in June, which was the earliest sampling period. High enzyme activity earlier in the growing season in the annual system likely coincided with the decomposition of the above and belowground plant residues from the previous season. At SDNWA, the previous crop residues were from the flax crop harvested in 2017. Flax fibre is a low quality organic input comprised of complex organic compounds that are not readily available for microbial decomposition (Fanin, Alavoine, & Bertrand, 2020). This requires production of more enzymes by the soil microbial community, leading to higher extracellular enzyme activity. Further, elevated soil moisture early in the growing season because of spring meltwater may also be driving higher enzyme activity levels. A study by Kotroczó et al. (2014) found that high soil moisture coupled with the detrital inputs from the previous season resulted in significantly higher enzyme activity in the spring than at any other point in the growing season. Granular fertilizer application at the time of seeding in the annual system may have caused an increase in activity particularly of the BG and NAG enzymes (Burns, 1982). Typically, Phos activity tends to decrease in areas of synthetic fertilizer use (Nash et al., 2014); however, like BG and NAG, Phos was highest in June in the annual system at SDNWA as well (Fig. 4.1). Another study indicated that enzyme activity levels showed significant correlations with organic amendments rather than synthetic fertilizers (Chang, Chung, & Tsai , 2010), suggesting that crop residue decomposition coupled with higher moisture levels early in the growing season may override the varying effects of fertilizer application on specific enzyme activities.

In contrast, in the annual system at CLC, BG and NAG enzyme activities were rather stable over the course of the growing season and the initially high activities observed in June at SDNWA

did not occur at CLC. At SDNWA, BG and NAG activities were 2.9 and 2.4 times higher respectively than at CLC in June. Differences in enzyme activities between the two sites in July and August were not as severe. Also, in contrast to SDNWA, the crop residues were more decomposable at CLC, as the crop from the previous year was canola instead of flax. A study by Stevenson (1962) found that amino acid oxidation rates in soils supplemented with flax residues showed the largest increase in bacterial biomass and activity in the first 10 days of decomposition despite flax residue being more persistent in the soil than alfalfa or wheat straw residues. This further highlights the role of plant residue inputs and the timing of decomposition on microbial activity.

Seasonal variation can affect the activity enzymes such as BG, NAG, and Phos (Carpenter-Boggs et al., 2003; Kotroczó et al., 2014). At SDNWA, the initial spike in activity in the annual system early in the growing season subsided in July and August, while in the perennial system significantly higher activity for Phos and a tendency for higher activity for NAG occurred during July and August. This same trend was observed at CLC for BG and NAG, with the difference in enzyme activity increasing between the perennial and annual land uses with each month of the growing season. This “slow burn” effect in activity later in the season may be a response to the diversity and late-season productivity of the plant community in the perennial agroecosystem. In the annual system however annual crop species used on the prairies tend to hit peak productivity in mid to late July for the plants to have time to die back and the seed to cure prior to harvest, and so plant productivity is often more concentrated early in the season. Older and more established plants in perennial systems contribute a greater volume and variety of detrital and root exudate inputs to the soil later in the growing season (Tarafdar & Jungk, 1987). The peak productivity of plants also changes across the growing season, and in grasslands late season productivity is

common for some species used for forage and hay production (Knowles, 1987; Hamel et al, Hamel, Iwaasa, & Schellenberg, 2008).

5.2 Perennial cover buffers topographic effects on soil biological properties

The effects of land use on soil biological properties can both exacerbate and buffer topographic variation. At SDNWA, land use moderated the difference in enzyme activity and microbial abundance between the landscape positions, especially for metrics related to soil fungi. The annual cropping system supported lower NAG activity and general fungal abundance in the upper slopes, and lower AMF abundance in both the upper slopes and backslopes compared to the perennial system. In annual systems the effects of landscape position are often more severe in upper slopes than in the depressions, where soil development is usually slower with thinner surface horizons (Block and Van Rees, 2006), and can also exacerbate the effects of management such as tillage erosion (Pennock et al., 1994; Wickings et al., 2015). Conversely, permanent cover under a diverse plant community has been shown to increase SOM, organic C, soil aggregation, water percolation, and hosts a denser root zone which acts as a buffer to topographic effects such as erosion and excess runoff experienced by soils in annual cropped upper slopes (Block & Van Rees, 2006; DuPont et al., 2010). Increased plant diversity also supports higher fungal abundance, while providing higher concentrations of varied organic inputs to the soil (van der Heijden et al., 2006), which can increase microbial activity and biomass (Hedlund, 2002). Evidence of the buffering quality of perennial cover on topographic variation was observed in a study by Culman et al. (2010) who found that SOC, total N, and water stable aggregates were greater in perennial grasslands than annual croplands. In this study at SDNWA, soil total N, total C, and organic C were all highest, and bulk density lower, in the depressions of the perennial system than in the annual system. These same soil resources that provide buffering qualities within the perennial system are also conducive

to supporting the higher fungal biomass and NAG activity—through increased substrate availability (i.e., chitin found in fungal cell walls).

Further, reduced disturbance of surface soils by heavy farm equipment, which is known to disrupt fungal hyphae and promote bacterial dominance in surface soils, may also play a role in the mitigating effect of perennial land cover on topographic variation in biological properties (Miller, Acton, & St. Arnaud, 1985; Bardgett & McAlister, 1999; Bünemann et al., 2018). Indeed, AMF abundance was 32% higher in the perennial at SDNWA and 38% higher in the perennial system at CLC compared to the annual system (Fig. 4.9). This is supported by the findings of Hedlund. (2002) who found that AMF abundance increased in agricultural plots where perennial cover was allowed to establish over time, in comparison to actively cultivated plots. Abundance of AMF is likely related to the buffering of the perennial system due to the consistent presence of a living root system, and lack of disturbance, enabling the symbiotic relationship that exists between AMF and the plant community to remain undisturbed.

Due to more complex three-way interactions at CLC for most soil biological properties (discussed below), only Phos and B:F ratios were affected by a two-way interaction between land use and landscape position. Bacterial to fungal ratios were lower in perennial compared to annual depressions with no difference in upper slopes, likely resulting from the effects of perennial cover. In the perennial depressions thicker soil horizons and more diverse plant communities are conducive to higher fungal biomass (Bardgett & McAlister, 1999; Schloter et al., 2003; Allison et al., 2005). However, site-specific landscape characteristics may also be affecting how the soil microbial community responds to land use and topography at CLC. A study by Lauber et al. (2008) found that increases in bacterial biomass and/or changes in community composition were correlated with heavier soil textures (i.e., silts and clays); soil texture at CLC was classified as clay

loam, while the texture at SDNWA was primarily sandy clay loam. Soils in the perennial depressions were also saturated at many of the sampling points in July of 2018 at CLC (but not at SDNWA). Many sampling points were located at or close to the edge of wetlands, creating anaerobic conditions for the microbial community for parts of the year. It is possible that these periods of stagnant saturation affected the microbial community structure enough to sufficiently modify the bacterial to fungal ratios. There is evidence of this occurring in a study by Unger, Kennedy, & Muzika (2009), who observed decreased ratios of bacteria to fungi in the microbial community of in-field stagnant flood treatments.

At CLC, the perennial system supported higher Phos activity in upper slopes, but not in depressions (Fig. 4.7) The high Phos activity in the perennial system may be caused by the occurrence of plant-produced phosphatases associated with a more diverse plant community in the perennial cropping system, rather than any increase of labile organic P substrate in the soil (Eivazi & Tabatabai, 1977; Joner & Jakobsen, 1995; DuPont et al., 2010). Soil available P at CLC was higher in the annual system than the perennial system (in both upper slopes and depressions), which could supress Phos activity if the soil microbial community was not P limited in the annual soils.

5.3 The importance of depth to microbial distribution

Depth plays a significant role in the abundance, structure, and function of the soil microbial community. At SDNWA, all PLFA groups as well as BG and NAG activity were affected by a main effect of depth, with activity and abundance decreasing significantly through the soil profile. With increasing depth, soils often see a decrease in available resources such as SOM, SOC, total N, and P. This is typically associated with fewer organic inputs at depth as root density decreases, and fewer inorganic inputs as weathering of mineral material lessens (Block and Van Rees, 2006;

Pennock, D., A. Bedard-Haughn, 2011; Zilverberg et al., 2018). Soil resource availability at SDNWA followed this trend at each landscape position, within each land use, with total C, SOC, total N, SON, and P all decreasing with depth through the soil profile (Appendix 8.1).

Land use and landscape position moderated the effect of depth, particularly on Phos activity at SDNWA. In surface soils sampled in July, the perennial system supported higher Phos activity than the annual system, but this difference did not persist with depth. Phosphatase targets labile organic P, and perennial systems are known to have higher concentrations of organic P associated with increased organic matter and root density present particularly in surface horizons (Eivazi & Tabatabai, 1977; Tarafdar & Jungk, 1987). While phosphatase activity can increase with available substrate, it can also decrease with available P. As a result, Phos activity has been shown to decline with the application of synthetic fertilizers (Nash et al., 2014) . With so much readily available P in the surface soils, microorganisms are unlikely to be P limited, and are less likely to invest in enzyme production in fertilized annual cropping systems (Carpenter-Boggs et al., 2003).

Topography can also influence the effects of depth on soil biological properties. At SDNWA, a landscape position by depth interaction occurred where Phos activity was highest in depressions compared to positions upslope in the 15-30 and 30-45 depths, but in surface soils, activity was just as high in upper slopes as in depressions. Higher Phos activity in the depressions is likely a result of the translocation of material from upper to lower slopes and the increased soil moisture and nutrient concentrations in depressions (Taylor et al., 2002; Wickings et al., 2015). Depressions often experience more soil formation because of this deposition as well as accelerated plant growth (Miller et al., 1985; Pennock et al., 1994; Block & Van Rees, 2006). However, Phos activity was also high in the surface soils of the upper slopes at both SDNWA and CLC

In this study, higher Phos activity in deep soils in the depressions may simply indicate that the sampled soils may be from only one or two upper horizons and not into the less biologically active parent material as we would expect from farther upslope. Both areas of elevated Phos activity, be it upper slopes or depressions at either site, are likely due to pre-existing P concentrations within soils at each site; however, higher P concentrations in surface soils may be linked to higher plant derived inputs and detrital turnover. Phosphorus concentrations at both sites decreased with depth; however, concentrations of P were highest in the depressions at both sites while upper slopes saw higher P concentrations than backslopes at all depths (see Appendix 8.1). Relatively low P concentrations and corresponding activity in backslope positions is not unexpected, as backslopes are often the slope position in a landscape that experiences the most erosion over time, particularly in annual systems (Pennock et al., 1994). Erosional history as a result of previous cultivation in the perennial system – the perennial system having been converted from annual agriculture in the early 2000's - seems to have long term effects on P concentrations at these sites as the trend was consistent in both annual and perennial systems.

5.4 Land use effects moderated by topography and depth at CLC

The most complex soil biological response to land use occurred at CLC and was dependent on both depth and landscape position. Like SDNWA, perennial land use at CLC generally supported higher enzyme activity and PLFA abundance, with differences becoming less severe in the depressions compared to upper slopes, likely due to greater soil redistribution downslope in the annual system. Further, these biological properties tended to be highest, and differences between land uses most stark, in surface soils, especially in upper slope positions where erosion and poorer plant productivity in annual upper slopes likely explain these trends (Pennock et al., 2017; Zilverberg et al., 2018). However, unlike at SDNWA, enzyme activity (BG and NAG) and

PLFA abundance experienced a shift in trend in the 30-45 cm depth in the depressions; values in the depression at depth were more elevated under the annual cropping system. This trend was also seen in SOC concentrations at this depth and location, but was not present for organic N or available P (Fig 4.1-4.3). Where soil biological properties declined expectedly with each depth increment in the perennial system, there was not a similar decline between the 15-30 cm and 30-45 cm depth in the annual system (Figs. 4.6, 4.10-4.12). Community structure within the annual depressions at 30-45 cm was also different from perennial depressions as seen by a slight grouping of the annual depression points in the 30-45 cm depth increment on the NMDS (Fig. 4.14) and noted by the PERMANOVA results for the LU:LP:D interaction at CLC ($p=0.001$).

Higher soil biological property values and microbial biomass and activity at depth in depressions of annual cropping systems is sometimes indicative of buried A horizons (Helgason et al., 2014). Buried horizons are commonly a by-product of erosion from previous land management such as tillage. Soil migrates downslope as a result of the erosive events, covering the pre-existing surface soils in lower slope positions with layers of less developed soil which originated from the upper slopes (Pennock et al., 1994). As a result, buried surface horizons are typically found in footslopes and depressions (Papiernik et al., 2009). Although both CLC and SDNWA are currently managed using no-till methods, prior to the 1980's at both sites tillage was an annual occurrence, and the legacy of tillage may still be present. Both sites have been employed under agricultural management for more than 75 years, in the case of SDNWA more than 100, and so long-term effects of that management could be expected. Buried A horizons were found in a study by Helgason et al. (2014), there distinguished as "inverted soil profiles" in multiple locations at SDNWA; however, my study did not find the same elevated microbial biomass, enzyme activities, and SOC (%) occurring at SDNWA. In contrast, at CLC it appears that a stronger effect

of past erosion events has led to two possibilities, the first being the findings indicate the possible presence of a buried A horizon and the second being the presence of thick A or B horizons in the depressions in the annual system that extend beyond the sampling depth (45 cm) of this study. However, due to similar microbial activity levels, microbial abundance, and SOC (%) in the 15-30 cm depth increment as in the 30-45 cm depth increment (Fig 4.1) it is most likely that soil development in the depressions of the annual land use at CLC have resulted in a horizon whose bottom boundary extends beyond the sampling depth.

It is common in topographically variable sites like SDNWA and CLC for translocation of material and nutrients to occur through both physical displacement and water solubility (Wickings et al., 2015). However, there was not as much variation in soil C or N across landscape positions at SDNWA than what was expected given the steeper slopes found at SDNWA compared to CLC, and previous reports of buried A horizons in SDNWA depressions, than those at CLC. Instead, concentrations of both C and N remained relatively uniform across the site, at odds with the topographic variation that exists there. In contrast, more obvious variation in existing soil resources across the landscape was found at CLC instead. The CLC location's topography is rolling rather than hummocky and as such the contrast in soil resource distribution between landscape positions was unexpected. The more limited variability in topography is the reason that backslope positions at CLC were not sampled in this study, but this may have resulted in the statistical model detecting differences for CLC where there was only two landscape positions compared to SDNWA where there were three landscape positions. However, different management history between the two sites may also explain why there appears to be more sustained soil microbial parameters at depth in the annual depressions at CLC.

6. CONCLUSION

6.1 Summary of key findings

The purpose of this study was to evaluate the response of two novel soil biological properties, enzyme activity and PLFA signatures (viable biomass and community structure), to the effects of land use in topographically variable landscapes over the growing season. I hypothesized that these properties would differ under different land uses and that effects of land use would also be affected by landscape position, depth, and timing within the growing season (seasonality).

A key thread throughout all the trends observed for microbial activity and PLFA functional groups lies in the differences between perennial and annual land use systems. Characteristics such as differences in plant density, diversity and cover type, root density, and total C and N concentrations were some of the driving forces behind differences in activity, biomass concentration and community structure. However, it is important to note that though land use differences were a key driver, they often interacted with season, depth, and landscape position in ways that made it clear that land use should not be evaluated alone within landscapes such as those at SDNWA and CLC.

As demands for methods of soil health testing increase from academia, producers, and the public, soil properties of various kinds are being evaluated for their suitability to be used as indicators within soil health tests. Of the tests available, soil biological properties are often absent from the suites of indicators used. As a result, more research is being done to try and quantify their value as potential soil health indicators, and studies such as this one aim to add to the growing pool of knowledge on how these properties respond to varied stimuli within agroecosystems, particularly stimuli associated with management decisions related to land use. The biological properties evaluated in this study, enzyme activity and PLFA abundance, have the potential to be

used as proxy measurements of changes to soil resources such as SOC and SOM, particularly over short time frames such as a growing season, allowing for quantification of management changes across landscapes faster than relying on soil chemical and physical properties alone. This was supported by the findings in this study, as enzyme activity in particular was sensitive to seasonal variability. In addition to proxy measurements, the value of using soil microbial function itself as a measure of soil health cannot be understated. This is due to the integral role that microbes play in the C and nutrient cycles that are part of a soil's basic function, and that supply the building blocks needed to support other organisms within the ecosystems that operate in tandem across the landscape. An active and diverse microbial community could in itself be indicative of factors within sites that affect to soil health.

6.2 Future research

In seeking to answer the question of how microbial activity and community responds to land use differences, there were several questions that arose during this study that can guide future research. The plant communities on both sites in both land uses drew questions on how microbial response might respond to vegetation specifically. Enzymes such as Phos are produced by the plant community in addition to soil microbes, and studying how plant community diversity and density in surface soils affects enzyme activity and community abundance would be valuable in understanding why perennial agroecosystems create such a buffer to other factor effects, such as topography. An extension of this could be evaluating how much of an effect late season perennials – plants whose peak productivity is relatively late in the growing season – have on microbial activity in perennial systems.

With respect to topography, both sites hosted microtopography within the larger topographic variation across the site, such microtopography occurred more frequently at SDNWA

because of greater in-situ topographic variation. This meant that some depressions were sampled that were higher within the landscape than some upper slopes. Questions arose as to whether the soil microbial community in landscape positions associated with the microtopography responded the same way as the microbial community within the larger scale topography of the site. In a similar vein, many low-lying sampling areas were saturated for at least part of the growing season, and it could be valuable to explore the effects of prolonged saturation on soil microbial response since wetlands are so prevalent in variable landscapes in Saskatchewan.

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8. APPENDICES

8.1 Appendix 1: Soil property tables

Table 8.1: Soil Properties (mean ± standard deviation for three soil depths at three landscape positions in two land uses at SDNWA.

		SDNWA - Chemical and Physical Properties					
		Annual			Perennial		
		Depression	Backslope	Upperslope	Depression	Backslope	Upperslope
		Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev
0-15 cm	Total N (mg/g)	3.08 ± 0.62	1.94 ± 0.69	2.39 ± 0.30	3.20 ± 0.45	2.61 ± 0.38	2.99 ± 0.68
	NH4+ (µg/g)	2.60 ± 0.82	2.02 ± 0.83	2.56 ± 1.70	5.20 ± 1.43	3.48 ± 0.65	5.49 ± 2.57
	NO3- (µg/g)	14.89 ± 7.63	16.56 ± 7.75	14.40 ± 6.39	2.94 ± 1.05	5.44 ± 3.13	5.99 ± 2.27
	Organic N (mg/g)	3.06 ± 0.62	1.92 ± 0.69	2.37 ± 0.30	3.19 ± 0.45	2.60 ± 0.38	2.98 ± 0.68
	EC (µS/cm)	0.49 ± 0.31	0.17 ± 0.05	0.17 ± 0.38	0.21 ± 0.14	0.22 ± 0.04	0.16 ± 0.04
	P (µg/g)	16.72 ± 10.97	8.10 ± 2.32	8.91 ± 5.53	8.13 ± 8.08	3.24 ± 0.82	3.38 ± 0.89
15-30 cm	Total N (mg/g)	2.04 ± 0.49	1.05 ± 0.33	1.03 ± 0.44	1.86 ± 0.55	1.70 ± 0.56	1.78 ± 0.66
	NH4+ (µg/g)	2.26 ± 1.22	1.64 ± 0.80	1.96 ± 0.82	1.95 ± 0.90	1.46 ± 0.77	3.03 ± 1.50
	NO3- (µg/g)	11.95 ± 6.76	11.45 ± 5.64	10.10 ± 5.49	2.18 ± 0.79	4.62 ± 3.40	3.74 ± 1.80
	Organic N (mg/g)	2.02 ± 0.49	1.04 ± 0.33	1.02 ± 0.44	1.85 ± 0.55	1.70 ± 0.56	1.77 ± 0.66
	EC (µS/cm)	0.5 ± 0.4	0.14 ± 0.05	0.14 ± 0.06	0.19 ± 0.17	0.22 ± 0.07	0.13 ± 0.05
	P (µg/g)	7.32 ± 4.28	4.36 ± 1.01	4.88 ± 1.94	4.93 ± 4.09	2.59 ± 0.47	2.60 ± 0.65
30-45 cm	Total N (mg/g)	1.05 ± 0.49	0.63 ± 0.32	0.64 ± 0.26	0.92 ± 0.21	0.75 ± 0.44	0.85 ± 0.51
	NH4+ (µg/g)	2.47 ± 1.55	1.58 ± 0.85	1.56 ± 1.03	2.34 ± 1.32	0.66 ± 0.54	2.55 ± 1.71
	NO3- (µg/g)	6.97 ± 4.41	14.11 ± 16.36	10.40 ± 4.83	1.3 ± 0.57	2.23 ± 1.3	2.71 ± 4.34
	Organic N (mg/g)	1.04 ± 0.49	0.62 ± 0.31	0.63 ± 0.26	0.92 ± 0.21	0.75 ± 0. 44	0.84 ± 0.51
	EC (µS/cm)	0.39 ± 0.32	0.15 ± 0.09	0.39 ± 0.34	0.32 ± 0.22	0.42 ± 0.28	0.22 ± 0.11
	P (µg/g)	4.67 ± 2.32	2.98 ± 1.57	3.54 ± 0.60	3.54 ± 2.39	2.23 ± 0.33	2.28 ± 0.67

Table 8.2: Soil properties (mean ± standard deviation) for three soil depths, at three landscape positions, and two land uses at SDNWA.

		Annual			Perennial		
		Depression	Backslope	Upperslope	Depression	Backslope	Upperslope
		Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev
0-15 cm	Org C (%)	3.28 ± 0.50	2.29 ± 0.98	2.87 ± 0.46	3.71 ± 0.60	3.25 ± 0.37	3.41 ± 0.67
	Total C (%)	3.48 ± 0.38	2.42 ± 1.11	2.98 ± 0.44	3.71 ± 0.69	3.43 ± 0.45	3.48 ± 0.62
	pH	7.73 ± 0.16	7.65 ± 0.54	7.53 ± 0.38	7.57 ± 0.30	7.93 ± 0.20	7.74 ± 0.22
	Sand (%)	35 ± 12	53 ± 14	58 ± 3	43 ± 17	52 ± 13	42 ± 22
	Silt (%)	24 ± 8	16 ± 5	13 ± 3	20 ± 8	20 ± 8	26 ± 16
	Clay (%)	43 ± 8	33 ± 12	35 ± 11	38 ± 12	28 ± 5	33 ± 10
15-30 cm	Org C (%)	2.64 ± 0.74	1.31 ± 0.84	1.82 ± 1.13	1.99 ± 0.62	2.90 ± 0.54	2.64 ± 0.70
	Total C (%)	2.82 ± 0.98	1.35 ± 0.92	1.85 ± 1.29	2.16 ± 0.70	3.28 ± 0.55	2.96 ± 0.91
	pH	7.55 ± 0.44	7.27 ± 0.61	7.55 ± 0.41	7.41 ± 0.63	7.92 ± 0.13	7.64 ± 0.58
	Sand (%)	46 ± 15	44 ± 18	35 ± 27	33 ± 12	55 ± 10	43 ± 22
	Silt (%)	18 ± 9	21 ± 7	28 ± 18	24 ± 7	17 ± 7	27 ± 14
	Clay (%)	40 ± 10	35 ± 12	39 ± 11	44 ± 7	30 ± 8	33 ± 13
30-45 cm	Org C (%)	2.04 ± 1.27	1.25 ± 0.44	1.07 ± 0.18	1.55 ± 0.80	1.37 ± 0.24	1.46 ± 0.39
	Total C (%)	2.56 ± 1.27	1.72 ± 0.96	2.41 ± 0.90	1.61 ± 0.91	3.41 ± 0.56	2.62 ± 1.49
	pH	7.24 ± 3.13	6.50 ± 2.93	8.32 ± 0.83	7.09 ± 0.81	7.90 ± 0.13	7.57 ± 0.48
	Sand (%)	15 ± 16	19 ± 19	10 ± 9	32 ± 6	48 ± 10	35 ± 4
	Silt (%)	43 ± 24	28 ± 21	55 ± 32	28 ± 3	23 ± 7	30 ± 10
	Clay (%)	42 ± 21	29 ± 20	35 ± 27	40 ± 9	30 ± 8	35 ± 8

Table 8.3: Soil properties (mean ± standard deviation) for three depths at two landscape positions in two land uses at CLC.

		CLC - Chemical and Physical Properties			
		Annual		Perennial	
		Depression	Upperslope	Depression	Upperslope
		Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev
0-15 cm	Total N (mg/g)	3.66 ± 0.92	2.12 ± 0.84	3.74 ± 1.45	3.94 ± 0.80
	NH4+ (µg/g)	6.44 ± 6.00	2.97 ± 1.52	6.06 ± 3.48	4.43 ± 0.72
	NO3- (µg/g)	10.80 ± 3.49	14.50 ± 8.22	2.49 ± 1.50	1.84 ± 2.09
	Organic N (mg/g)	3.64 ± 0.92	2.10 ± 0.84	3.73 ± 1.45	3.93 ± 0.80
	EC (µS/cm)	0.38 ± 0.29	0.13 ± 0.03	0.93 ± 1.03	0.88 ± 0.77
	P (µg/g)	8.45 ± 3.07	6.30 ± 2.48	5.43 ± 2.37	4.00 ± 1.36
15-30 cm	Total N (mg/g)	3.72 ± 0.45	1.08 ± 0.25	2.90 ± 1.49	2.31 ± 1.04
	NH4+ (µg/g)	7.46 ± 7.11	2.29 ± 1.17	3.65 ± 2.37	2.85 ± 0.93
	NO3- (µg/g)	11.20 ± 4.78	11.09 ± 9.24	3.70 ± 4.20	1.08 ± 0.63
	Organic N (mg/g)	3.71 ± 0.45	1.06 ± 0.24	2.89 ± 1.48	2.31 ± 1.04
	EC (µS/cm)	0.33 ± 0.31	0.12 ± 0.04	0.90 ± 0.95	1.19 ± 0.86
	P (µg/g)	8.20 ± 1.54	3.95 ± 1.48	4.25 ± 0.82	3.14 ± 0.92
30-45 cm	Total N (mg/g)	1.62 ± 0.20	0.87 ± 0.38	1.46 ± 0.89	1.14 ± 0.42
	NH4+ (µg/g)	3.27 ± 0.83	2.42 ± 0.44	4.64 ± 6.59	2.50 ± 1.95
	NO3- (µg/g)	6.21 ± 1.56	3.93 ± 2.06	2.46 ± 2.03	0.91 ± 0.53
	Organic N (mg/g)	1.61 ± 0.20	0.86 ± 0.38	1.46 ± 0.89	1.13 ± 0.42
	EC (µS/cm)	0.24 ± 0.18	0.11 ± 0.06	0.43 ± 0.37	0.78 ± 0.41
	P (µg/g)	5.00 ± 1.16	2.94 ± 0.92	3.50 ± 0.64	2.48 ± 0.49

Table 8.4: Soil properties (mean ± standard deviation) for three depths at two slope positions in two land uses at CLC.

		Annual		Perennial	
		Depression	Upperslope	Depression	Upperslope
		Mean ± StDev	Mean ± StDev	Mean ± StDev	Mean ± StDev
0-15 cm	Org C (%)	4.18 ± 0.86	2.85 ± 1.43	4.26 ± 1.56	3.89 ± 1.34
	Total C (%)	4.10 ± 0.89	2.36 ± 0.82	4.28 ± 1.56	4.49 ± 0.95
	pH	6.11 ± 0.86	6.19 ± 1.30	6.89 ± 0.96	6.47 ± 0.98
	Sand (%)	35.00 ± 17.32	50.83 ± 19.08	39.17 ± 14.97	25.00 ± 10.95
	Silt (%)	20.00 ± 8.66	16.67 ± 8.76	22.50 ± 8.22	30.83 ± 9.70
	Clay (%)	48.33 ± 2.89	37.50 ± 16.66	40.00 ± 10.00	44.17 ± 7.36
15-30 cm	Org C (%)	4.01 ± 0.51	1.43 ± 0.33	3.24 ± 1.58	2.71 ± 0.92
	Total C (%)	4.15 ± 0.54	1.55 ± 0.47	3.28 ± 1.53	3.00 ± 1.14
	pH	6.18 ± 0.72	6.45 ± 1.14	6.87 ± 1.03	7.02 ± 0.66
	Sand (%)	25 ± 0	50 ± 28	37 ± 18	25 ± 19
	Silt (%)	25 ± 0	20 ±17	22 ± 5	32 ± 16
	Clay (%)	50 ± 0	33 ± 16	42 ± 13	45 ± 5
30-45 cm	Org C (%)	4.26 ± 1.83	1.16 ± 0.39	1.71 ± 0.86	2.8 ± 1.13
	Total C (%)	4.00 ± 1.72	1.76 ± 0.51	1.90 ± 0.93	2.76 ± 0.88
	pH	6.03 ± 1.01	7.44 ± 0.33	6.68 ± 0.98	7.76 ± 0.31
	Sand (%)	37 ± 20	60 ± 5	60 ± 0	28 ± 6
	Silt (%)	22 ± 6	17 ± 8	15 ± 0	27 ± 3
	Clay (%)	42 ± 14	27 ± 18	25 ± 0	45 ± 9

8.2 Appendix 2 Second axis NMDS ordinations

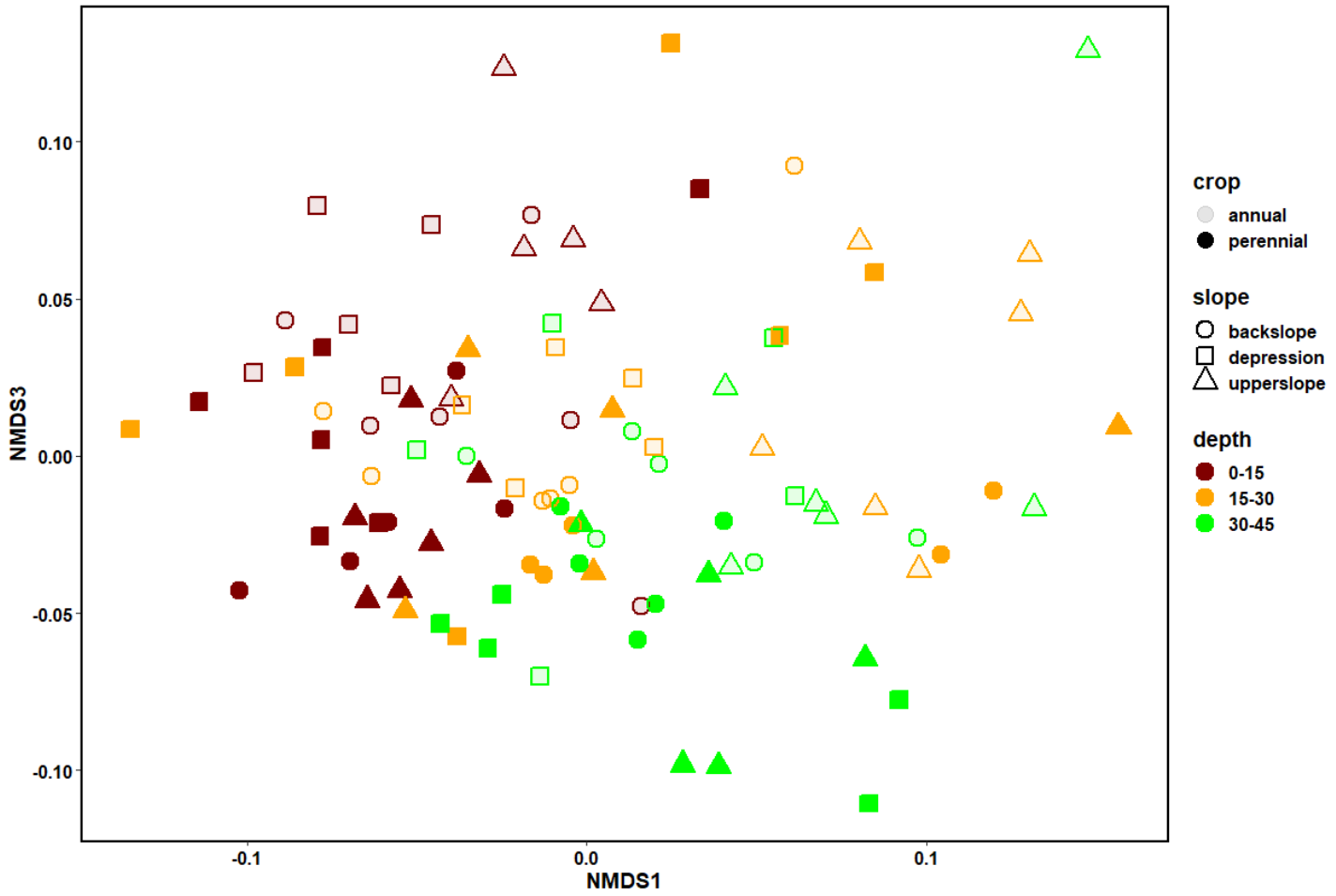

Fig. 8.1. NMDS second axis NMDS1xNMDS3 SDNWA. NMDS depicts 2018 soil community composition for SDNWA, 3D solution.

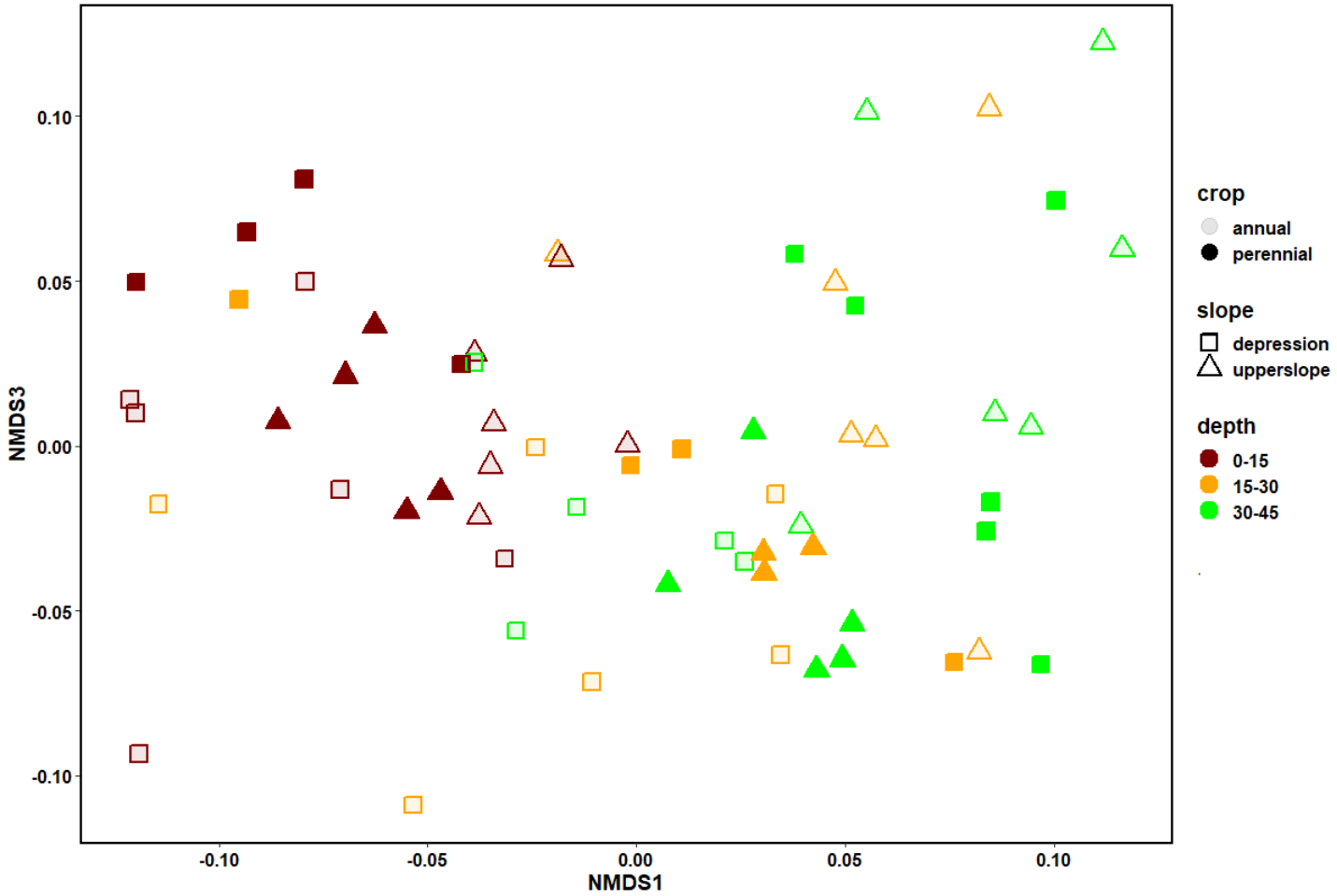

Fig.8.2. NMDS second axis NMDS1xNMDS3 CLC. The NMDS depicts the 2018 soil community composition for CLC, 3D solution.