Simulated Livestock Soil Compaction, Plant Defoliation and Litter Removal Effects on Extracellular Enzyme Activity and Vegetation Across a Moisture Gradient in Southern Alberta, Canada

by

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Abstract

Preservation of grasslands is vital for the continuation of the numerous ecosystem goods and services (EG&S) provided by these ecosystems, including forage for livestock, nutrient cycling, carbon sequestration and habitat for flora and fauna. All EG&S in grasslands are supported by microbial biological functions and the vegetation community. Recent attention on adaptive multi-paddock (AMP) grazing, which focuses on effects to the soil and plant community through trampling and compaction of litter into the soil surface, have raised questions about the relative roles of the different mechanisms through which cattle affect grasslands, namely, compaction, defoliation and subsequent depletion of litter.

Few studies have been conducted that isolate the mechanisms through which grazing may alter grasslands, particularly in western Canada. This study investigated relationships of soil biological activity, vegetation production and diversity with individual grazing mechanisms across a moisture gradient. We conducted a plot-level factorial study that simulated cattle trampling and defoliation as well as litter depletion at three locations with different climates and vegetation types in Alberta rangelands.

In the first year of study, litter was removed or retained to examine the importance of litter presence, which generally decreases with heavy grazing. To simulate seasonal defoliation and trampling, plots were clipped and compacted, or not, in the spring or the fall of the first and second year of study. Data were collected in the second and third year of study. Extracellular enzyme activity (EEA) is a measure of enzyme availability and used in this study as a metric of microbial community function at each site. Enzymes are used to degrade specific substances by plant and soil microbes, and were measured in this study in both soil and litter.

Our study determined that litter manipulation had a strong influence on EEA in both soil and litter, as litter presence affects moisture, though patterns fluctuated between sites. Response of both soil and litter EEA to defoliation and compaction treatments did not present consistent patterns.

Production and structure of the vegetation community are known to be key influences on biodiversity; our study determined that defoliation, particularly in the fall, had a greater influence on vegetation production and diversity than trampling in either season. Response of species with different grazing tolerance indicates variation in the effects of grazing mechanisms on species with varied grazing tolerances.

The results of this study demonstrate the key role that litter presence plays in biological function and influence of defoliation on the vegetation community in grasslands. From this study, the implications for grassland preservation and management are on the importance of litter management for decomposition and nutrient cycling. This study highlights the importance of monitoring effects of seasonal grazing in grasslands, as production and structure of the vegetation community are affected variably at different locations.

Preface

The research conducted for this thesis is part of a research collaboration between the University of Alberta and Agriculture and Agri-Food Canada (AAFC); the lead collaborator at the University of Alberta is Dr. Cameron Carlyle, with Dr. Xiying Hao being the lead collaborator at AAFC in this study.

The design for this project was determined by Dr. Cameron Carlyle, Dr. Xiying Hao and Dr. Don Thompson of Agriculture and Agri-Food Canada. Field site selection and initial setup and treatment of sites was conducted in 2016 by Dr. Don Thompson and Ryan Beck. Most laboratory procedures were conducted at the Lethbridge Research and Development Center (AAFC) unless otherwise mentioned.

Data analysis, interpretation and conclusions in Chapter 2 and 3 are my original work, as well as the literature review in Chapter 1 and conclusions in Chapter 4. The laboratory work, data collection, data analysis and the manuscript composition were my responsibility. Dr. Cameron Carlyle and Dr. Xiying Hao were the supervisory authors; Dr. Carlyle was involved with data analysis and manuscript composition, and Dr. Hao assisted with the data collection and contributed to manuscript edits.

No part of this thesis has been previously published.

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1.1 Introduction

1.1.1 Overview

Worldwide, grasslands are being degraded swiftly through crop production, land development and the effects of climate change. These diverse landscapes provide many ecosystem goods and services (EG&S) necessary for the support of life, such as air and water purification, carbon sequestration, habitat for flora and fauna, and nutrient cycling. Cattle can affect soil properties and ecosystem processes in grasslands through mechanical breakdown of litter, compaction of litter and soils and removal of vegetation, which reduces biomass inputs and alters plant community composition. Recent focus on these particular actions, or mechanisms, of grazing through systems such as adaptive multipaddock (AMP) grazing (Savory 1983) raises questions of ecosystem response to individual grazing mechanisms. Adaptive multipaddock grazing theory attributes success in a grassland system to trampling, defoliation, and plant litter cycling, though this is contested in the literature (Briske et al. 2008, Teague 2013). To examine the effect of these grazing mechanisms on soil and vegetation in western Canadian prairies, we conducted a factorial experiment at three climatically different sites in southern Alberta. In this experiment, litter removal, defoliation (clipping) and trampling (compaction) were simulated to gain an understanding of how these specific mechanisms affect sensitive grassland environments.

1.1.2 Grasslands and Rangelands

Grasslands are herbaceously dominated landscapes where rainfall is sparse enough that growth of trees is not supported, covering approximately 30-40% of the world's landmass

(Wisley 2018). These ecosystems have been utilized as rangelands; areas grazed by livestock or wildlife, primarily dominated by grasses, forbs or shrubs. Grazing in grasslands is an important source of revenue and a way of life for many within the ranching industry. The effect of grazing on grassland can be variable depending on the season of grazing as well as intensity of grazing applied, with higher grazing intensities generally having a greater effect on EG&S (Milchunas et al. 1988, Tang et al. 2019).

1.1.3 Grasslands in Canada

Canadian prairie grasslands span southern parts of Alberta, Saskatchewan and Manitoba (Bailey et al. 2012). Five prairie ecosystems exist in the Canadian prairies – the Foothills Fescue, Tall Grass Prairie, Parkland Northern Fescue, Mixed Grass and Dry Mixed Grass, though classifications may differ slightly between provinces (Bailey et al. 2012). Climate on the Canadian prairies is continental and is sheltered from northwestern weather systems by the western Rocky Mountains (Bailey et al. 2012).

Before settlement of the Canadian prairies, grassland vegetation communities adapted to natural disturbances such as fire and grazing animals. Early bison populations of North American rangeland have been estimated to be over 10 million individuals (Shaw 1995); due to their immense population size, grazing, herd behavior and migration habits, bison shaped and maintained the vitality of the grassland landscape. European colonization and hunting efforts drove the bison population to near extinction in the late 19th century (Sanderson et al. 2008). Efforts to populate the Canadian prairies resulted in the majority of native grassland being converted to cropland (Bailey et al. 2012). The loss of native vegetation, which was spurred on by poor management practices and drought conditions contributed to the ‘Dust Bowl’ era of the 1930s, when severe wind erosion and dust storms were seen across the North American prairies

(Schubert et al. 2004). Due to cultivation, colonization efforts and poor management practices, the Canadian prairies, which originally spanned approximately 61 million hectares, now contain only a fraction of these native landscapes (Bailey et al. 2012).

Current data from a 2006 census by Statistics Canada reports that there are approximately 11.4 million hectares of natural grassland remaining in Canada (Bailey et al. 2012, Statistics Canada 2006), which is mostly used for grazing of domesticated livestock (Wang et al. 2014). A 2017 review of Canada's agriculture and agri-food industry determined that in 2016 6.7% of Canada's GDP was generated by this sector, and approximately 12.5% of Canadians were employed by this sector, which depends heavily on the Canadian grasslands for grazing land (Agriculture and Agri-Food Canada 2017).

1.1.4 Grasslands in Alberta

The province of Alberta is known to have diverse topography, biodiversity and climate. Known as part of the North American Great Plains, grasslands cover 14.4% of Alberta, and are characterized by a warm, dry climate (Downing and Pettapiece 2006). Alberta grasslands are known for Chernozemic soil, undulating plains, and vegetation communities generally dominated by grasses (in drier landscapes) or shrubs (in more moist landscapes) (Downing and Pettapiece 2006). Natural subregions within the Grasslands region of Alberta include Northern Fescue, Foothills Fescue, Mixed Grass and Dry Mixed Grass, largely separated based on climate, vegetation and soil (Downing and Pettapiece 2006). Due to both the climatic and biological diversity of Albertan grasslands, subregions with different conditions must be considered when evaluating ecological and management effects across the province to gain an accurate representation of Alberta's grassland diversity. By understanding grazing responses in different areas of the province, the Alberta livestock industry, which are supported by grasslands, can

continue to prosper. The agriculture industry employed 15.8 thousand Albertans in 2017 (Government of Alberta 2017), making this a key part of the Albertan economy.

1.1.5 Dry Mixed Grass

The Dry Mixed Grass (DMG) subregion is located in the southeast corner of Alberta, covering area from the Northern Fescue subregion to the Alberta-Montana border. The DMG covers 49% of the Grasslands natural region, making it the largest grassland subregion in Alberta (Downing and Pettapiece 2006). Species are generally drought-tolerant in this subregion due to dry conditions. The DMG is characterized by rolling plains, with coulees, badlands and valleys dispersed throughout the landscape (Downing and Pettapiece 2006). This region has a mean annual temperature (MAT) of 4.2 °C and mean annual precipitation (MAP) of 333.3 mm, making the DMG the driest subregion in the province (Downing and Pettapiece 2006). Soils that typically occupy this subregion are Orthic Brown Chernozems, with Solonetzic soils occurring in areas of glacial till (Downing and Pettapiece 2006). Communities such as *Stipa-Bouteloua-Agropyron* or *Stipa-Bouteloua* are common in the DMG (Adams et al. 2013, Smoliak et al. 1972). As grazing pressures increase, shorter grasses become more abundant, but midgrasses are plentiful under proper range management (Adams et al. 2013, Smoliak et al. 1972).

1.1.6 Mixed Grass

The Mixed Grass (MG) subregion covers approximately 21% of the Grasslands natural subregion and is intensively cultivated (Downing and Pettapiece 2006), with 31% of the native grassland remaining (Adams et al. 2004). The MG subregion spreads north of the Alberta-Montana border, extending to the Northern Fescue subregion, with the Foothills Fescue subregion to the west and the Dry Mixed Grass subregion to the east. Soils in this subregion are typically Dark Brown Chernozems, with Rego Chernozems and Regosols often occurring in

eroded or sandier areas (Downing and Pettapiece 2006). The MG subregion has a MAT of 4.4 °C, and MAP of 394.1 mm. Common species in the MG subregion include *Heterostipa comata*, *Pascopyrum smithii*, *Bouteloua gracilis* and *Agropyron cristatum* (Downing and Pettapiece). Vegetation communities on productive, medium textured dark brown soil are typically *Stipa-Agropyron*, while on drier, exposed slopes (such as south-facing slopes), *Heterostipa-Bouteloua-Agropyron* communities are common (Adams et al. 2004, Coupland 1961). Similar responses to grazing are seen in the DMG and the MG prairies – shallow-rooted grass species replace deep-rooted species when grazing intensity increases (Smoliak et al. 1972). In these relatively drier subregions, soil deteriorates at a slower rate in response to grazing than the Foothills Fescue subregion, because the soil does not compact as much as it does in a wetter climate, and because lower ground cover protects soil from erosion and exposure (Adams et al. 2004).

1.1.7 Foothills Fescue

The Foothills Fescue (FF) subregion in Alberta is characterized by its cooler summers, warmer winters and high amounts of precipitation compared to other Grassland subregions. Located just southeast of the Rocky Moutains, the climate of the FF ecoregion is influenced by its proximity to the mountains, along with Chinooks that occur (Downing and Pettapiece 2006). Native FF grassland once occupied approximately 1.5 million ha and due to cultivation only 16.8% of this area remains as native grassland (Adams et al. 2003, Downing and Pettapiece 2006). The FF is dominated by Black Orthic Chernozemic soil, although Dark Brown Chernozems can occur on southern slopes or due to wind erosion (Downing and Pettapiece 2006). The MAT of the FF subregion is 3.9 °C, and the MAP is 469.6 mm (Downing and Pettapiece 2006). On loamy, well-drained Black Chernozemic soils, *Danthonia parryi*, *Festuca campestris* and *Pseudoroegneria spicata* are all species ordinarily found in this subregion, with

the *Festuca campestris-Danthonia parryi* community being common (Downing and Pettapiece 2006). Light grazing on the FF subregion results in little change in the vegetation community, while moderate to heavy grazing has been shown to result in a decline in range conditions and soil quality and major shifts in the vegetation community (Chanasyk and Naeth 1995, Dormaar and Willms 1998, Willms et al. 1985). Shifts in the FF vegetation community in response to grazing are largely due to the high sensitivity of *Festuca campestris* as well as the lesser sensitivity of *Danthonia parryi* to grazing pressure (Willms et al. 1985).

1.2 Grazing Effects and Management

1.2.1 Effects of Cattle on Grasslands

Grazing animals create disturbances on the landscapes they graze through trampling, defoliation, defecation and urination, and grassland plant communities have evolutionarily adapted to these disturbances. While individual plants do not generally benefit from grazing (Ellison 1960), the disturbance that cattle cause while grazing a landscape not only increases spatial heterogeneity and thus biodiversity, but also can cause shifts in vegetative production, soil nutrients and soil physical characteristics (Hobbs 1996).

The action of grazing can impact the plant community, whether by changing community structure or through changes to photosynthetic capability of plants, thereby altering production within the community. Defoliation occurs when animals remove aboveground biomass from the landscape. If defoliation by herbivores is within moderation and occurs at key times of the growing season, depending on the species, plant growth can be stimulated, typically if the plant is in a vegetative growth stage or in a dormant period (Belesky and Fedders 1994, Holechek et al. 2003). This is due to removal of apical dominance when the apical meristem of the grass is removed during defoliation, allowing for the development of lateral branches into buds or tillers;

this is known to increase productivity in grasslands (Murphy and Briske 1992). However, not all species will recover when grazed at any intensity; McNaughton (1983) illustrates in his grazing optimization theory that variety in species necessitates a variety in response to grazing intensity. Optimal timing of grazing on grasses is typically after formation of 3-4 leaves on the individual plant, during the vegetative stage. At this development point, the growing point is elevated for grazing, plant nutrient reserves are still plentiful, and if environmental conditions are generally favourable for regrowth, a grass point can typically recover from grazing (Frank 1996).

Rhizodeposition, which is the release of chemical and root components from the roots of plants, is a response to grazing that aids in grass recovery (Lynch and Whipps 1990). During partial defoliation, rhizodeposition is stimulated, which includes the release of exudates, secretions, lysates and gases, which stimulates the rhizospheric microbial community (Bardgett et al. 1998). As rhizodeposition increases and supplies the microbial community with nutrients, N cycling also increases, as microbes limited by C are supplied with necessary nutrients to mineralize N (Hamilton et al. 2008). Nitrogen is then in an available form to the plant, which subsequently may increase growth (Hamilton et al. 2008).

Grazing at light or moderate intensity can have benefits such as increased photosynthesis, tillering, and reduction in shading (Holechek 1981, McNaughton 1983), though this depends on the status of a particular landscape (Thompson and Uttley 1982). Overgrazing can put nutrient stresses on a plant that can cause stoppage of root growth and eventually a reduction in the health of the plant (Johnston 1961). Briske (1991) emphasizes that regrowth after defoliation must be interpreted with caution – grazed plants might experience compensatory growth after defoliation, but they likely will not experience more growth than ungrazed plants.

Litter production and decomposition is an important part of nutrient cycling in rangelands and grasslands, and grazing animals play a part in the life and death of aboveground plant matter. A large portion of aboveground plant matter becomes plant detritus and enters the C and N cycle within the soil; thus litter is regarded as a key component of nutrient cycling in grasslands (Wang et al. 2018). Litter production is influenced by grazing over time, as overgrazing changes the amount of biomass produced within the system, thereby altering the litter produced (Christie 1979, Mapfumo 2002, Naeth et al. 1991a). Additionally, plant matter such as litter is often trampled into the ground during grazing; this is the beginning of the physical breakdown and decomposition process. Due to lower biomass production as well as increased decomposition and physical breakdown, litter production and accumulation is often lower on overgrazed landscapes (Naeth et al. 1991a). This is often problematic for landscapes that are moisture stressed, as litter protects soil from exposure, aiding in the prevention of temperature increases, moisture loss, and ultimately soil degradation and erosion (Naeth et al. 1991a). Litter decomposition has been found to be indirectly affected by grazing mechanisms – when soil compaction occurs by grazing animals, soil moisture often decreases in heavily grazed sites (Naeth et al. 1991b), decreasing litter decomposition rates and affecting soil nutrient cycling (Wang et al. 2018). Grazing can also alter litter quality, through nutrient additions by livestock/wildlife by urination and defecation (Holland et al. 1992, Penner and Frank 2019).

Due to the impact of the hooves of livestock treading over the soil, soil and vegetation properties will often change due to grazing. Grazing has been associated with higher bulk density, lower water infiltration and lower soil moisture during grazing, as soil is compacted and thus water penetration ability decreases (Greenwood and Mackenzie 2001, Naeth et al. 1991b). Compaction of soil by grazing depends heavily on the soil moisture, with high moisture levels

increasing the possibility of soil compaction (Gifford et al. 1977). As soil compaction decreases root growth (Christie 1979, Unger and Kaspar 1994), vegetation yield and litter cover may decrease on compacted soil (Naeth et al. 1991a, Naeth et al. 1991b), thus leading to increased risk of soil degradation and erosion due to soil exposure. Additionally, soil microbial communities are influenced by trampling; decreased abundance of microbial communities has been found when trampling occurs in sub-arctic grassland (Sorensen et al. 2009). Grazing later in the growing season will lessen trampling effects, due to lower soil moisture levels (Naeth et al. 1991b). While higher amounts of vegetation in ungrazed sites use more soil water than grazed vegetation, ungrazed sites have been found to retain more soil moisture than grazed sites, due to lower soil bulk density and infiltration rates (Naeth et al. 1991b).

1.2.2 Grazing Management Practices

The health of grassland and rangeland ecosystems depends on the disturbance that correctly prescribed grazing provides. Plant communities within grasslands have historically adapted to grazing (Milchunas et al. 1988), and livestock movement can mimic migratory patterns of large, grazing ungulates to the benefit of the grassland ecosystem (Teague et al. 2011). Stocking rate, as well as the timing of grazing, is acknowledged as one of the most important determinants of range response to grazing (Holechek et al. 2003). Stocking rate determines the intensity and duration of grazing in an area, and if overgrazed, can lead to ecosystem stress and shifts to lower seral plant communities and soil health degradation (Manley et al. 1997, Dormaar and Willms 1998).

A variety of grazing systems exist, and grazing managers must choose a system that suits the landscape they care for. Grazing systems often differ in how long a herd is kept within a pasture, as well as movement of the herd between pastures.

The continuous system grazes a single herd on one pasture for the duration of the season; this system has benefits, in that management is limited, and if stocking rate is properly decided on for the area, livestock have access to vegetation at the best points in the growing season, and are therefore able to access as many nutrients as possible (Smoliak 1960). However, continuous grazing can create patches of heavily grazed areas, due to plant preference by the herd (Teague et al. 2003).

Rotational grazing systems allow regular rest of pastures in an attempt to prevent overgrazing or selective grazing by animals, promoting homogenous grazing of a landscape (Holechek et al. 2003); one such system being adaptive multi-paddock (AMP) grazing, a type of short duration grazing system. AMP grazing involves dividing a pasture into multiple small paddocks, generally centralized around a water source, where livestock are frequently moved through paddocks at a high density (Savory 1983). This design allows for long rest periods for each paddock between grazing periods, in contrast to other rotational systems with fewer paddocks (Savory 1983). Adaptive multipaddock grazing theory has claimed to increase soil water infiltration, allowing for grazing homogeneity, increased productivity and animal gain as well as allowing higher stocking rates, among other benefits (Savory 1983, Teague et al. 2011). However, there remains debate in the scientific research as to the validity of these claims, with research often finding little difference in results between continuous and short duration grazing systems (Briske et al. 2008). This uncertainty within the scientific community indicates the necessity for further investigation to determine if the system is beneficial for range in various climates.

1.3 Nutrient Cycling in Grasslands

1.3.1 Controls on Nutrient Cycling in Grasslands

Nutrient cycling in grasslands is an interaction between the biotic and abiotic factors of the environment. Biotic factors such as soil and plant communities interact with the abiotic factors such as climatic variables like precipitation and temperature. To understand grassland nutrient cycles, it is key to understand the flux between soil nutrient acquisition and plant growth and decomposition, both above and belowground.

Native grasslands contribute in a beneficial manner to atmospheric C conversion, in that native forage sequesters and stores more atmospheric C than cultivated crops do (Conant et al. 2001). Grassland management is an important contributor to grassland soil and vegetation vitality; conversion to croplands contributes to C loss from soil, as intensive disturbance that is required for conversion from grasslands to croplands, as well as the maintenance of croplands, leads to disturbance of aggregates. These disturbance events decrease the protection that soil aggregates provide to soil organic matter (SOM) from soil microorganisms, leading to SOM loss from soil (Conant et al. 2001, Zheng et al. 2018). Other systems of grassland management, such as liming, grazing or nutrient fertilization, have been found to significantly affect C to nutrient (mainly N and P) ratios within plants (Heyburn et al. 2017), which reflects variation in soil nutrient availability. Plant-use efficiency tends to be higher in grasslands with lower levels of management, as species adapted to high levels of disturbance tend to have lower C:nutrient ratios (Heyburn et al. 2017). This contrast in plants in areas with different levels of disturbance is reflected in the difference in soil C accumulation between managed and unmanaged areas (Heyburn et al. 2017).

1.3.2 Grazing and Nutrient Cycling

The response of a grassland to grazing practices is complex, being influenced by factors such as management choices and climatic conditions (McSherry and Ritchie 2013). Disturbance models highlight stress such as grazing and environmental factors as necessary for species diversity and ecosystem heterogeneity (Grime 1973, Milchunas et al. 1988), though it is acknowledged that this changes with climatic variation as well as evolutionary history (Conant et al. 2001). Grazing has been shown to shift soil organic carbon (SOC) content; Hewins et al. (2018) found that grazing increases SOC concentration in upper soil layers, and McSherry and Ritchie (2013) report that SOC fluctuations differ in response to grazing depending on the dominant plant community of the site. The latter finding may be due to differences in nutrient inputs as plant communities shift within a site due to disturbance and adaptability (Conant et al. 2001, McSherry and Ritchie 2013).

Change of grazing intensity cause both short and long-term responses within a grassland ecosystem. Immediate changes to vegetation production and the plant community have been observed with an increase in grazing intensity, which is speculated to alter the soil microbial community and thus soil nutrient stocks (Klumpp et al. 2009). Changes to the plant community ultimately alter nutrient input into the ecosystem (Chuan et al. 2018); litter input from the plant community is a crucial component of SOC (Kogel-Knabner 2002, Tian and Shi 2014). The disturbance that grazing creates is related to various actions or mechanisms that occur during grazing, such as plant defoliation, trampling through movement of hooved animals, and nutrient addition to the soil surface through animal waste or dropped food. Grazing has the potential to enhance ecosystem health through use of a proper stocking rate (Dormaar and Willms 1998,

Hewins et al. 2018, McSherry and Ritchie 2013). Through grazing-induced disturbances, a diverse plant community can provide nutrients necessary for soil function and ecosystem vitality.

1.4 Extracellular Enzyme Activity

It is well known that the activity of enzymes secreted by soil microbiota, plants and other organisms plays a key role in the decomposition and organic matter accumulation process (Dick 1994, Gianfreda and Ruggiero 2006). Extracellular enzymes (EE) are protein complexes often secreted for the purpose of the breakdown of complex polymers into simpler compounds that can be used by the organism (Gianfreda and Ruggiero 2006, Sinsabaugh et al. 2002). Extracellular enzymes are a vital component in organic matter accumulation and organic matter breakdown; this activity determines soil stability, plant nutrient availability, soil moisture availability, among other characteristics relating to soil fertility and nutrient availability (Burns et al. 2013). Measurement of soil extracellular enzyme activity (EEA) is a method of estimating potential biological activity within soil, as ideal conditions used in a laboratory enzyme assay do not fully reflect field conditions or substrate availability (German et al. 2011). Due to the diversity of EEs within the soil, careful selection of EEs to be measured is necessary to gain an accurate picture of nutrient cycling (Gianfreda and Ruggiero 2006, Nannipieri et al. 2002).

1.4.1 Important Extracellular Enzymes in Terrestrial Systems

Extracellular enzyme activity is a key driver of soil nutrient cycling in grassland ecosystems. This is due to extracellular enzyme involvement in catalysis of decomposition reactions (Jing et al. 2018, Sinsabaugh et al. 2008). Due to the high specificity and thus variability of EE, the response of EE to environmental variables is dependent on the nature of the specific enzyme (Jing et al. 2018). Variation in EEA has been found to be linked to environmental variables, such as SOM concentration, soil pH, soil moisture content as well as

substrate availability (Jing et al. 2018, Sinsabaugh et al. 2008). For example, decreases in precipitation can decrease EEA depending on the properties of the soil studied (Allison and Treseder 2008, Jing et al. 2018). Additionally, the tendency of soil microbes to be C limited is a driver of soil EE production (Allison et al. 2007).

Due to the importance of plant decomposition within the nutrient cycle, the enzymes involved in the breakdown of lignin and cellulose are of particular interest to those who study terrestrial ecosystems (Sinsabaugh et al. 2002, Sinsabaugh et al. 2008). Within soil enzymology, enzymes that contribute to the production of humus, as well as those that mineralize N and P are of particular importance (Allison et al. 2007, Sinsabaugh et al. 2002). Sinsabaugh et al. (2002) state that within litter decomposition studies, the most commonly examined classes of enzymes are "cellulases, hemicellulases, pectinases, phenol oxidases, peroxidases, chitinases, peptidases, ureases and phosphatases." The broad suite of enzymes that are present in the soil are key for breaking down complex polymers at different stages of degradation (Sinsabaugh et al. 1991, Sinsabaugh et al. 2002). The complexity or simplicity of the substrate present determines the nature of the microbial community that will be able to utilize, and thus produce EE, for the degradation of the substrate (Sinsabaugh et al. 2002). Due to the relative ease of assaying and the availability of substrates, exohydrolytic enzymes are typically studied (Allison et al. 2007), thus the scientific literature on EEA is not exhaustive or completely representative of what is occurring in the soil (Sinsabaugh et al. 1991). When considering a grassland ecosystem, enzymes that decompose plant fiber compounds, as well as those active in the N and P cycle, as these nutrients are not plentiful in plant fibers, are key to nutrient function (Sinsabaugh et al. 2002); these EEs will be the focus of the following paragraphs.

Many of the enzymes examined in grassland studies are involved in cellulose degradation; cellulose is a crystaline polysaccharide that composes plant cell walls, and is the most abundant biopolymer present on earth (Garcia-Garrido et al. 2002, Klemm et al. 2005). Cellulases, enzymes that degrade cellulose, are regarded as one of the most important classes of enzymes in organic matter accumulation (Schimel and Weintraub 2003). β-D-Cellobiohydrolase acts as an exohydrolase in cellulase complexes, acting on the exposed ends of β-1,4-glucan chains of cellulose to release cellobiose (Garcia-Garrido et al. 2002). β-1-4-glucosidase is an important cellulose-digesting enzyme, often focused on because of its production of glucose as an end product to its hydrolytic reaction (Eivazi and Tabatabai 1990, Sinsabaugh et al. 1991), as well as its sensitivity to disturbance, including SOM, vegetation and microbial community fluctuations (Caldwell et al. 1999, Sinsabaugh et al. 2002). In soil and plant ecosystems, glucose is the most common source of energy for organisms, as it is a readily available product of cellulose degradation and a simple monosaccharide (Derrien et al. 2006, Mganga and Kuzyakov 2014). This emphasizes the importance of β-1,4-glucosidase, which catalyzes the final step of cellulose hydrolysis to release glucose (Singh et al. 2016).

Hemicelluloses are polysaccharides also present in plant cell walls, though are simpler than cellulose (Garcia-Garrido et al. 2002). C-acquiring enzymes such as endoxylanase, exoylanase and β-1,4-xylosidase are involved in the breakdown of hemicellulose compounds (Sinsabaugh et al. 1991).

Due to the limited availability of nitrogen in plant litter (Sinsabaugh et al. 2002), this necessitates examination of N-cycling in a grassland ecosystem, and further demands an understanding of the function of the N cycle in response to disturbance events. Nitrogen is a common limiting resource in plant communities, as plants require large amounts of nitrogen for

the production of nucleotides, nucleic acids, amino acids, proteins and chlorophylls (Gurevitch et al. 2006). Involved in both the N and C cycles, N-acetyl-β-glucosaminidase (NAG) breaks down N-acetyl-β-glucosamine residues from polymers such as chitin, the second most abundant biopolymer on the planet (Parham and Deng 2000).

Phosphorous is a key macronutrient in plant life that is limited in plant litter, a major component of SOM; understanding the mechanisms of P acquisition in soil is important for understanding soil and plant ecosystem nutrient cycling (Sinsabaugh et al. 2002). Grasslands have limited reserves of P contained in the soil, and are thus largely dependent on plant detritus for the supply of soil P (Gurevitch et al. 2006). Phosphatases are enzymes that release phosphate through the hydrolysis of ester-phosphate bonds, commonly studied as acid or alkaline phosphatase (Nannipieri et al. 2011, Sinsabaugh et al. 1991), which release available phosphorus for microbial and plant life.

1.4.2 Controls of Extracellular Enzymes

A variety of factors affect the vitality of EEs. These protein complexes are sensitive to environmental pH, temperature, moisture and substrate availability (German et al. 2011, Gianfreda and Ruggerio 2006, Sinsabaugh 1994). The expression and longevity of soil EEA is dependent on the environment surrounding the enzymes (Gianfreda and Ruggerio 2006). In the interaction between environmental patterns and litter decomposition, emphasis on macro- and microscale is key for an accurate perspective of EEA with spatial variation (Sinsabaugh et al. 1991). Therefore, climatic conditions, substrate presence and management decisions are all key factors in the viability of EEA within rangeland soil.

1.4.3 Grazing and Extracellular Enzymes

Recent studies have found response of enzyme activity to grazing. Hewins et al. (2015) examined the effects of grazing on soil nutrient cycling enzymes, and found that grazing had a weak but negative effect on β-D-Cellobiosidase, N-acetyl-β-glucosaminidase and Phosphatase. A study conducted by Xu et al. (2017) to determine microbial response to increasing land use intensity saw an increase in the activity of β-1,4-glucosidase, which is commonly linked with increasing SOC concentrations. These and other studies show variation in response of soil enzyme activity to grazing, dependent on climatic conditions and enzymes studied. Within different ecoregions, soil EEA has been examined to determine response to certain grazing mechanisms. In both agricultural and forest ecosystems, soil EEA was found to decrease with compaction (Siczek and Frac 2011, Tan et al. 2008), though it should be noted that soil from agricultural fields showed an increase in EEA when soil was only moderately, not heavily, compacted (Siczek and Frac 2011). Compaction effects in a study by Dick et al. (1988) attribute lowered EEA to physical factors, such as increased bulk density in compacted soil, and impaired root growth. A defoliation study in grasslands of Alberta, Canada showed that enzyme response to defoliation was largely dependent on environmental conditions (Hewins et al. 2016a). Decreases in plant litter due to heavy grazing results in decreases in soil enzyme activity, likely due to changes in the C:N ratio of litter under the stress of heavy grazing and substrate limitations (Olivera et al. 2014).

1.5 Role of Plant Community Dynamics, Competition and Grazing-Induced Disturbance on Grassland Biodiversity

In evolutionarily adapted grassland ecosystems, grazing often provides a disturbance that is important for maintenance of biodiversity and productivity, two important EG&S of grasslands,

when grazing is utilized at a moderate rate for the climate and evolutionary history of the area being grazed (Milchunas et al. 1988, Milchunas et al. 1993). The linkage of biodiversity and plant productivity (Fraser et al. 2015, Grime 1973) highlight the importance of the maintenance of biodiversity within grasslands, acknowledged as a key driver in ecosystem health and sustainability (Hooper et al. 2012, Lange et al. 2015). The stability of grasslands is driven by biodiversity, in which a higher number of species has been shown to increase resilience to stress (Tilman and Downing 1994).

1.5.1 Plant Competition Theory

When considering the importance of plant biodiversity, an understanding of plant competition dynamics is key to grasping changes in the biodiversity of an ecosystem. Plant competition in grasslands is driven by several factors: environmental conditions, disturbance, and interactions between plants. Resource competition is a driving force in plant competitive interactions (competition for light, nutrients, water, space, etc.) in addition to intraspecific (competition between individuals of the same species) and interspecific (competition between individuals of different species) competition.

Several theories exist on how community biodiversity responds to environmental stress, management intensity, and competition among plants, and which drives ecosystem change. The intermediate disturbance hypothesis (Connell 1978), often termed the "hump-backed model," was first illustrated by Grime (1973), to show that species diversity is highest at an intermediate level of stress, and lowest at low or high stress. Grime's hump-backed model (1973) hypothesizes that a moderate amount of disturbance is key to creating gaps within the ecosystem necessary for new species to proliferate. When considering successional communities within an ecosystem, the state and transition model is currently commonly used in rangeland management.

This theory proposes that vegetation dynamics within a community can take many different steady states, which can have various change thresholds, and transitions to other states can be irreversible (Stringham et al. 2003). State-and-transition models are an important change from traditional climax successional models because they allow for ecosystem response to various combinations of disturbances, and thus many outcomes of change for an ecosystem (Stringham et al. 2003).

The interactions between species for resources is important to consider in the response of a plant community to stress, as these interactions can drive community change. Grubb (1977) argues that the regeneration niche, or the conditions required by a species for germination and establishment, drives community structure and determines if or how two species will coexist. In contrast, the resource-ratio hypothesis (Tilman 1985) hypothesizes that established species are competing for resources, may coexist if they are not limited by the same resource, and one species can become dominant over the other by acquiring the smallest amount of the limiting resource. These two theories lead to different conclusions when considering species response to disturbance; the resource-ratio hypothesis may predict that species will respond differently to disturbance depending on how resource availability is affected for individual species (Tilman 1985), whereas regeneration niche hypothesis may focus on species response to gaps created by disturbances (Grubb 1977).

1.5.2 Response of the Plant Community to Grazing

Grazing is a disturbance that has the ability to shape a plant community, dependent on the intensity of the grazing (Hobbs 1996). Rangeland and grazing theory have long wrestled with this relationship through utilization of the practice of classifying species as “increaser” or “decreaser” based on response in abundance to grazing (Dyksterhuis 1949, Vesk and Westoby

2001). Trampling, the act of compaction of the ground and associated plant material when a grazing animal steps, can have neutral or detrimental effects on plant production or composition, depending on the environmental conditions and intensity (Dunne et al. 2011, Kobayashi et al. 1997). Defoliation or clipping can serve to stimulate production and diversity in grasslands, through gap creation and compensatory growth mechanism of grasses (McNaughton 1983). Litter removal is a known outcome of grazing, and has differing effects based on the climate of the ecosystem in question. Litter removal can allow for gap creation due to the role of litter in soil moisture retention and temperature reduction, allowing for higher production and species richness in mesic systems, or production decline in xeric ecosystems (Deutsch et al. 2010a, Deutsch et al. 2010b, Willms et al. 1986, Willms et al. 1993).

1.6 Thesis Overview

This thesis examines the effects of grazing mechanisms on sensitive grasslands in three different ecoregions in southern Alberta, Canada. Soil and plant community responses to litter removal and seasonal clipping and grazing were studied across a climatic gradient. The main objectives of this study are as follows:

1. Measure the extracellular enzyme activity of five enzymes associated with nutrient cycling in grassland systems and explore if there is a significant effect of litter manipulation, defoliation and trampling on decomposition, as well as an effect of climatic variation on response (Chapter 2).
2. Assess the response of plant production and diversity, including the areal cover of site-specific dominant species, to simulated grazing mechanisms (ie. litter manipulation, defoliation and trampling) from areas within three Alberta natural subregions (Chapter 3).

Chapter 2 examines the response of extracellular enzyme activity in soil and litter in response to treatments, and finds influence of moisture on both soil and litter EEA, particularly within litter manipulation treatments. Chapter 3 examines plant community and production dynamics in response to treatments, and finds a greater decrease in production and diversity with defoliation than trampling, as well as variation in response of species with different grazing tolerances to grazing mechanisms. Grazing mechanisms were found to influence moisture retention in grasslands and is thought to largely drive results seen in enzyme activity and vegetation dynamics. With this work, soil and plant community dynamics in Alberta in response to grazing mechanics will be understood, and furthermore, ecosystem responses to grazing systems in northern temperate grassland can be better predicted and utilized to protect these valuable landscapes.

Chapter 2. Effect of Litter and Moisture on Soil and Litter Enzyme Activity in Grasslands of Alberta, Canada.

2.1 Introduction

The decomposition of plant material is a key ecosystem function that contributes to the provision of important ecosystem goods and services (EG&S) in grasslands, such as carbon sequestration, nutrient cycling and forage production for wildlife and livestock (Bailey et al. 2012). Extracellular enzymes, a key component of decomposition, chemically break down plant material into smaller components that can be incorporated into the soil and consumed by microorganisms and plants (Burns et al. 2013, Gianfreda and Ruggerio 2006). Large grazing mammals may affect extracellular enzyme activity (EEA) indirectly through the consumption of vegetation and trampling of soil. As the vast majority of grasslands in Canada, and globally, are used for livestock production (Bailey et al. 2012, Wisley 2018), understanding how EEA responds to grazing and affects litter and soil is important to ensure livestock management supports this critical ecosystem function.

Through secretion of extracellular enzymes, plants and soil-microbes contribute to organic matter degradation, energy transfer and nutrient availability through polymer breakdown into nutritionally available compounds (German et al. 2011, Gianfreda and Ruggiero 2006). Due to their sensitivity to changes in abiotic factors such as pH, temperature and moisture, EEA can be used as a measure of soil function and microbial response to changes in environmental conditions that land management may cause (Dick and Burns 2011, German et al. 2011, Sinsabaugh 1994). It is possible to gain insight into potential EEA, and thus decomposition, through analysis of field samples under the ideal conditions that a controlled laboratory environment creates (Burns et al. 2013, German et al. 2011, Sinsabaugh 1994)

Recently, interest in adaptive multipaddock (AMP) grazing has placed emphasis on particular aspects of grazing, such as trampling, evenness of grazing and surface litter (Savory 1983, Teague et al. 2011), which could have significant effects on processes regulated by the soil microbial community. The actions of grazing, such as stepping on plants with hooves (trampling) or defoliation of plants, can cause shifts in plant composition and soil physical properties (Greenwood and McKenzie 2001, Milchunas and Lauenroth 1993). These disturbances are known to cause changes in the soil such as pH, soil moisture, total C content and the soil C:N ratio (Smoliak et al. 1972, Willms et al. 1988). Shifts in vegetation productivity and microbial decomposition dynamics due to grazing mechanisms can also cause changes to litter abundance, which is an important regulator of soil water (Chuan et al. 2018, Naeth et al. 1991a, Naeth et al. 1991b, Willms et al. 1993). Changes seen through trampling, defoliation and changes in litter abundance can affect the structure and function of the soil and litter microbial community (Bardgett et al. 1998, Bardgett and Wardle 2003).

Physical and chemical changes due to grazing may have an impact on the EEA of the grassland microbial community, due to the sensitivity of EEA to environmental variables and organic matter availability (Allison and Vitousek 2005, German et al. 2011, Sinsabaugh 1994, Sinsabaugh and Moorhead 1994). Grazing is known to impact EEA through changes in nutrient cycles (Hewins et al. 2016a, Cenini et al. 2016), specific to climate and enzyme composition (Hewins et al. 2015, Xu et al. 2017). Studies have shown varying responses of soil EEA to grazing mechanisms. A reduction in soil EEA was reported by Hewins et al. (2016a) in response to defoliation and water addition. Varying responses of soil EEA to compaction have been reported by Siczek and Frac (2011) and Tan et al. (2008); decreases in soil EEA in response to compaction were found by both studies, though the former also reported increases in soil EEA

under a lower impact treatment. Decreases in litter, due to grazing or experimental manipulation, can decrease soil EEA, likely due to changes in the C:N ratio of litter under the stress of grazing and substrate limitations (Kotroczó et al. 2014, Olivera et al. 2014).

The knowledge of dynamics of EEA activity of soil and litter in grasslands, particularly in respect to grazing, shows certain gaps. Responses to grazing and overall activity vary across classes of enzymes; litter in Canadian grasslands displayed an increase in activity of C cycling enzymes while the activity of N cycling enzymes were reduced (Chuan et al. In press). Another study in the same region found greater activity of C, N and P cycling enzymes in non-grazed soil compared to grazed soil (Hewins et al. 2015). Plant litter EEA has been reported to vary with species, indicating that as species shift due to grazing, nutrient cycling in the ecosystem may also change (Chuan et al. in press, Pei et al. 2017). Though the response of soil EEA to grazing has been widely studied, knowledge is incomplete on the response of litter EEA in grasslands. Furthermore, grazing mechanisms have largely not been individually studied on both soil and litter EEA, both key in grassland nutrient cycling. Contrasting effects of grazing seen in soil EEAs highlights the need for further study into the effect of grazing mechanisms on soil and litter EEAs, as both are key to grassland nutrient cycling.

This study examined the effect of simulated trampling, defoliation and litter removal at three grasslands in southern Alberta, Canada located across a climatic gradient to examine their individual and combined effects on EEA in soils and surface litter. We hypothesize that treatments that reduce moisture in both soil and litter, such as litter removal and defoliation, will reduce EEA. Compaction may have differential effects on moisture availability, increasing it in surface litter but reducing it in soils leading to increased EEA in litter but reductions in soil. We expect these effects to change across study sites with different levels of precipitation; wetter sites

will likely be less affected by these treatments as moisture will remain more constant despite the treatments.

2.2 Materials and Methods

2.2.1 Study Sites

The study was conducted at three different locations within different grassland ecoregions in southern Alberta, Canada. Onefour (49° 07'N, 110° 29'W) is in the Dry Mixedgrass Natural Subregion of Alberta and is characterized by brown Chernozemic soil, has a mean annual precipitation of 333 mm and a mean annual temperature of + 4.2 °C, and is dominated by plant species such as *Hesperostipa comata* (Trin. and Rupr.), *Bouteloua gracilis* (Willd. ex Kunth) and *Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould (Downing and Pettapiece 2006). Lethbridge (49° 43'N, 112° 57'W), in the Mixedgrass Prairie Natural Subregion which is more mesic with a mean annual precipitation of 394 mm and a mean annual temperature of + 4.4 °C, is characterized by dark brown Chernozemic soil and plant communities containing *Pascopyrum smithii* ((Rydb.) Á. Löve, *Koeleria macrantha* (Ledeb.) Schult. and *Nassella viridula* (Trin.) (Downing and Pettapiece 2006). Stavely (50° 12'N, 113° 57'W) is in the Foothills Fescue Natural Subregion, and of the three sites has the highest mean annual precipitation (470 mm), the lowest mean annual temperature (3.9 °C), orthic black Chernozemic soils and its vegetation is dominated by *Festuca campestris* Rydb. and *Danthonia parryi* Scribn. (Downing and Pettapiece 2006).

Weather conditions during set up and sampling years can be seen in Figure 2.1 (A-C). Rainfall events were seen before sampling in June of 2017, though all other sampling periods generally occurred during dry conditions.

2.2.2 Experimental Design

This experiment used a 2 x 7 factorial design with four blocks; treatments included litter removal or retention, as well as 7 different grazing treatments that manipulated clipping and compaction at different times of the year: unclipped – uncompacted (control), and clipped-compacted, clipped – no compaction, or unclipped-compacted which were done in either spring or fall (Figure M1). In total, 14 treatments with 56 plots were monitored at each site. Each plot was 1 m^2 and was separated from adjacent plots by 0.5 m; blocks were at least 1 m apart.

Litter removal was completed in 2016 during initial site setup. Standing dead was removed using a forage harvester to the same height as the clipped treatment at each site and surface litter was removed using rakes, careful to only remove the litter from the LFH horizon. Litter removal occurred before the beginning of the growing season in early spring of 2016. Litter removal was done prior to the initial clipping and compaction treatments, to ensure that litter to be removed was not compacted into the soil.

Clipping and compaction treatments were applied either in the spring or in the fall in both 2016 and 2017. Spring treatments were completed in late June to early July of 2016 and 2017, and fall treatments were completed in mid-September of 2016 and 2017; however, in 2017 the fall treatments at Stavely were pushed to mid-October due to a fire risk. Clipping treatments were done by hand using sheep shears and were intended to simulate moderate levels of grazing by removing 50% of the vegetation. Due to the moisture differences between study sites, Stavely produces more plant biomass and was consequently clipped at a higher level (12.5 cm) than the other two sites (7.5 cm).

Compaction treatments were applied by pulling a cultipacker behind an all-terrain vehicle (ATV). Different cultipackers were used in 2016 and 2017 due to equipment availability. Both

cultipackers were approximately 1 m wide and consisted of a set of independently rotating wheels. The cultipacker used in 2016 weighed 215.5 kg and this weight was increased to 306.2 kg during treatment using sand. Due to equipment availability, the pressure exerted by the cultipacker used in 2016 was not able to be calculated. The approximate pressure of the cultipacker used in 2017 was 142 kPa, which is within range of mechanisms used in other compaction studies (Di et al. 2001). Increases in surface soil compaction are expected with higher ground pressure (Smith and Dickson 1990). During compaction treatment, the weighted cultipacker was pulled using an ATV three times over the treated plots, always moving in the same direction throughout the site for consistency (e.g., plots may have been rolled in an East-West direction during treatment, but not West-East). Because of the need to pull the cultipacker in a straight line, plots were blocked by compaction and litter removal treatments, while clipping treatments were applied randomly within the blocks.

2.2.3 Sample Collection and Processing

Soil and litter samples were collected in June and July of 2017 and 2018 to be used for EEA and chemical analyses. Three 15 cm deep soil cores were taken from each plot and combined at each sampling time. In 2017, a 2-cm diameter JMC Backsaver soil core was used (JMC Soil Samplers, Newton, IA, USA), and in 2018 a 2.5 cm diameter JMC Backsaver soil core (JMC Soil Samplers, Newton, IA, USA) was used. A larger soil core was used in 2018 to obtain more sample for analyses. The soil corer was cleaned with ethanol between the sampling of each plot to prevent cross-contamination. The combined soil samples for each plot were homogenized within collection bags and divided between two separate bags, one for EEA analyses and the other for chemical analyses. Litter was identified as plant material that was senesced, no longer living and on the surface of the soil. This litter was removed from the soil

surface by hand at each location where a soil core was to be taken, from an area 8 cm in diameter. Litter amounts varied between sites and treatments. All samples designated for EEA analyses were stored on ice while in the field and during transport to AAFC Lethbridge where they were promptly stored at -20°C (Hewins et al. 2016b).

2.2.4 Chemical Analyses

Soil samples from each plot designated for chemical analysis were air dried, then coarsely ground to pass through a 2 mm sieve. Soils were analysed for pH, moisture content, non-purgeable (water extractable) C (NPOC), water extractable total N (WETN) and available P (AP).

Soil pH was determined by mixing air-dried soil and deionized water in a 2:1 liquid to solid ratio, and after settling were then measured using a pH meter (used for all litter and 2017 soil samples) (Orion Star A215, Thermo Fisher Scientific, Waltham, MA, USA) or an automatic pH meter (used for 2018 soil samples for efficiency). For use of the automatic pH meter, a 2:1 ratio of deionized water and air-dried soil was mixed, and soil pH was measured using an automated titration analysis system (MT-100, Mantech, Guelph, Ontario, Canada). The system is equipped with a pH electrode. Operation and automation of the system was controlled using PC-Titrate software (version 3). Consistency between pH measurement devices was ensured through calibration and standard soil samples. Litter pH was determined using a protocol from Cornelissen et al. (2006 and 2011), which uses a 1:8 litter to water ratio. pH meters were calibrated using standards of known pH (Thermo Fisher Scientific, Waltham, MA, USA).

Gravimetric soil and litter moisture content were evaluated by determining weight loss after drying field-moist soils and litter at 105^0C for at least 24 hours.

The non-purgeable (water extractable) C and water extractable total N were measured by using a method modified from Chantigny et al. (1999). 15 g of air-dried soil was shaken with 30 mL of ultra-pure water for 0.5 hours, then the solution was syringe-filtered (0.45μm). The levels of NPOC and WETN in the filtrate were measured with TOC-VCSH equipped with a TMN-1 (Shimadzu Corp. Kyoto, Japan).

Available P in soil from each plot was determined using a modified Olsen extractable-P method (Olsen et al. 1954). The soil was extracted (a 1:10 soil/solution ratio) with 0.5 mol L^{-1} $NaHCO_3$, pH 8.5, after which P was measured using an Auto-Analyzer III (Bran and Leubbe, Germany)

2.2.5 Extracellular Enzyme Assays

Litter and soil samples were analysed for activity of β-D-Cellobiosidase (Cello), β-1,4-xylosidase (Xylo), β-1,4-glucosidase (Gluco), N-acetyl-β-glucosaminidase (NAG) and Phosphatase (Phos). β-D-Cellobiosidase and β-1,4-Glucosidase are both cellulase enzymes that have different roles in degrading cellulose, a key component in plant cell walls **(**Eivazi and Tabatabai 1990, Garcia-Garrido et al. 2002, Sinsabaugh et al. 1991**).** β-1,4-Xylosidase is a hemicellulase enzyme that works to degrade hemicelluloses, which are also present, though not as prevalent as cellulose, in plant cell walls (Sinsabaugh et al. 1991). Cello, Gluco and Xylo are thus active in the carbon cycle. N-acetyl-β-Glucosaminidase is a chitinase enzyme active in the nitrogen and carbon cycle; it works to degrade NAG residues from chitin polymers (Parham and Deng 2000). Phosphatase, present in the enzyme class phosphatase, releases inorganic phosphate from phosphate esters (Nannipieri et al. 2011, Sinsabaugh et al. 1991). Samples were analysed for hydrolytic activity using methylumbelliferone (4-MUF)-linked substrates; 4-MUF is a fluorescent commonly used for environmental enzyme assays (Saiya-Cork et al. 2002).

Sample suspensions were prepared using 0.5 g of litter or 1 g of soil in 125 mL of 50 mM acetate buffer made using sodium acetate trihydrate (Thermo Fisher Scientific, Waltham, MA, USA). In order for assay conditions to mimic that of field conditions, buffer was adjusted to be within ± 0.1 of the measured pH of the sample using acetic acid (Thermo Fisher Scientific, Waltham, MA, USA) or sodium bicarbonate (Thermo Fisher Scientific, Waltham, MA, USA).

Hydrolytic enzyme assays were done for both soil and litter samples using a method modified from Saiya-Cork et al. (2002). Standard black Costar (Corning Inc., Corning, NY, USA) or Grenier polysterene (Greiner Bio-One, Kremsmünster, Austria) 96 well-plate were used for this procedure. Using results from an initial substrate optimization test completed by Chuan (2017), ratios of 1.0 g of soil to 200 µM of substrate and 0.5 g of litter to 400µM of substrate (Sigma-Aldrich Corporation, St. Louis, MO, USA**)** were used. Once plated, samples were incubated for 4 hours at room temperature in the dark, and then read using a Gemini XPS Microplate Reader (Lethbridge AAFC) or SpectraMax M3 (University of Alberta) microplate reader (Molecular Devices LLC, San Jose, CA, USA). To account for the differences in microplate readers, standard 4-MUF solution (10 mM 4-methylumbelliferone) was read during each assay to ensure consistency between microplate readers (Figure H1). Microplates were read at 365 nm excitation and 450 nm emission filter setting. Potential enzymatic activity was calculated by using the following equations, with all results reported in nmol/g/h.

$$\text{Hydrolytic activity } \left(\text{nmol g}^{-1}\ \text{h}^{-1}\right) = \left(\left(\frac{\text{NFU}}{Standard\ NFU}\right) * \frac{0.3125}{(DW * hours)}\right) * 1000$$

Expanded version (explains division of 0.3125) below.

$$\text{Hydrolytic activity (nmol } g^{-1}\ h^{-1}) = \frac{\left\{\frac{NFU}{\left[\frac{StandardFU}{\left(10\frac{\mu mol}{L}*0.00005L\right)}\right]}\right\}}{[0.0002L*\left(\frac{DW\ g}{0.125L}\right)*hours} * 1000$$

Where:

$$\text{Net fluorescence units (NFU)} = \left\{\frac{Assay - Sample}{\left[\frac{(Quench\ Control - Sample)}{Standard}\right]}\right\} - Substrate$$

DW = Dry weight of soil/litter sample contained in 125mL of buffer

2.2.6 Statistical analysis

Statistical analyses were conducted using R (R Foundation for Statistical Computing, Vienna, Austria). All EEA data were log transformed before analysis in order to meet the assumptions of homogeneity of variance. Soil and litter characteristics that did not meet normality assumptions (soil available P, non-purgeable organic C (NPOC) and water-extractable total N (WETN)) were log-transformed before analysis. Results and figures are presented as original data. Litter and soil EEAs were analysed separately. All response variables were subsequently analysed using linear mixed models through use of the lmer function in the lme4 R-package (Bates et al. 2015). Fixed effects included month of sampling, litter removal treatment and seasonal clipping/compaction treatment, as well as all interactions. Sites and years were analysed separately due to the variation in weather between sites between years (as seen in Fig. 2.1) and the sensitivity of soil and litter enzymes to environmental variation. Block was used as a random factor. Significance of effects was assessed at $P \leq 0.05$. Post-hoc mean comparisons were conducted using Tukey's test ($\alpha = 5\%$), unless otherwise specified, as biologically interesting results and trends were mentioned when $\alpha = 10\%$ to prevent Type II error.

Levels of both litter and soil EEA were ordinated using non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance metric across all study sites. The relationships between EEAs and soil and litter characteristics (including grazing treatment, soil pH, water-extractable total nitrogen (WETN) and non-purgeable organic carbon (NPOC)) were examined using NMDS. All soil and litter characteristics were fitted in ordination plots as vectors using the envfit function in the R-package vegan (Oksanen et al. 2013). Relationships between treatments within the Bray-Curtis distance matrixes was analysed with a permutational multivariate analysis of variance (perMANOVA), using the adonis function in the R-package vegan (Oksanen et al. 2013).

2.3 Results

2.3.1 Soil Properties

The effects of treatments on soil properties varied by site and year (Table 2.1). With few exceptions, soil properties changed through the growing season likely in response to seasonal change and local weather.

In 2017 in Onefour, the no clip-spring compaction treatment had higher soil pH than other spring treatments, by approximately 0.32 ($p=0.007$, $F_{6,81} = 3.20$, Table D1), as well as the no clip-fall compaction, fall clip – no compaction and control treatment in 2018 ($p= < 0.001$, $F_{6,81}=4.97$, Table D1). Litter removal increased soil pH at Onefour in 2017 and 2018 (2017: litter retained: 6.90 ± 0.05, litter removed: 7.02 ± 0.06, $p=0.022$, $F_{1,81} = 5.45$; 2018: litter retained: 6.93 ± 0.03, litter removed: 7.02 ± 0.03, $p=0.007$, $F_{1,81} = 7.54$). Litter removal also decreased WETN in 2018 (litter retained: 4.17 mg/kg ± 0.17, litter removed: 3.75 mg/kg ± 0.15, $p=0.036$, $F_{1,81}= 4.54$) at Onefour. In 2017, NPOC was greater at Onefour in the litter retained treatment,

but only in July (litter retained: 3.82 mg/kg ± 0.09, litter removed: 3.59 mg/kg ± 0.09, p=0.010, $F_{1,78} = 6.97$).

In Lethbridge, treatments had no effect on soil properties in 2017. In 2018, litter removal decreased soil moisture at Lethbridge (litter retained: 9.69 % ± 0.36, litter removed: 8.21 % ± 0.33, p=< 0.001, $F_{1,3} = 20.02$). Soil pH at Lethbridge showed lower values litter removed treatments in July of 2018 (7.86 ± 0.07) than in both treatments in June (litter retained: 8.00 ± 0.07, litter removed: 8.02 ± 0.07) as well as litter retention in July (8.04 ± 0.01) (p=0.001, $F_{1,81} = 11.94$).

At Stavely, effect of clipping/compaction treatments were found in soil pH in 2017 (p = 0.040, $F_{6, 81} = 2.332$) and 2018 (p=0.032, $F_{6,81} = 2.44$, Table D1). Higher soil pH was found in fall clip – no compact in comparison to spring clip – spring compact treatments in 2017 (Table D1) In 2018, a marginal increase in soil available P was detected in the fall compaction treatment in comparison to the control and spring clip-spring compact treatment (p=0.010, $F_{6,81} = 3.033$, Table D1). Soil NPOC showed interaction between clipping/compaction treatments and month sampled in 2018 (p = 0.041, $F_{6,81} = 2.31$). In 2018, soil nutrients at Stavely decreased in litter removal treatments: soil WETN (litter retained: 15.90 mg/kg ± 0.67, litter removed: 14.08 mg/kg ± 0.67, p=0.011, $F_{1,81} = 6.70$), soil NPOC (litter retained: 184.33 mg/kg ± 8.19, litter removed: 154.67 mg/kg ± 8.59, p = 0.0044, $F_{1,81} = 8.57$) and soil AP (litter retained: 0.46 mg/L ± 0.02, litter removed: 0.39 mg/L ± 0.02, p=0.015, $F_{1,81} = 6.20$). Soil moisture was shown to be significantly higher in June litter retained samples (23.3 % ± 0.72) than July litter retained samples in 2018 (21.3 % ± 0.72) (p=0.020, $F_{1,81} = 5.68$).

2.3.2 Litter Responses

The effects of treatments on litter properties varied by site and year (Table 2.2). Litter moisture content at all sites in both years varied within the growing season (Table 2.2).

Onefour plots treated with spring compaction had greater litter pH than those without in 2017 ($p=< 0.001$, $F_{6,72} = 4.67$, Table E1). Conversely, in July of 2018, clipping treatments in either season, as well as the fall clip – fall compact showed an increase in litter pH in comparison to other treatments ($p=0.048$, $F_{6,78}= 2.24$, Table E1). At Onefour in 2018, litter removal treatment decreased litter pH (litter retained: 5.14 ± 0.03, litter removed: 5.01 ± 0.03, $p= < 0.001$, $F_{1,78}= 17.78$). At Onefour in 2017 litter moisture was higher in litter removed treatments in June (litter retained: 7.55 % ± 0.78, litter removed: 9.24 % ± 0.81, $p = 0.045$, $F_{1,69} = 4.17$). Litter pH was affected temporally at Onefour in 2017 and 2018.

At Lethbridge in 2017 and 2018, litter removal increased litter pH (2017: litter retained: 5.17 ± 0.03, litter removal: 5.23 ± 0.03; $p= 0.049$, $F_{1, 73}= 4.00$; 2018: litter retained: 5.51 ± 0.02, litter removal: 5.62 ± 0.02, $p = 0.001$, $F_{1, 77}=, 12.83$). In 2018, litter removal decreased litter moisture (litter retained: 4.14% ± 0.39, litter removal: 2.81 % ± 0.40, $p=0.001$, $F_{1, 81} = 12.05$) at Lethbridge.

At Stavely, litter pH was affected by the clipping and compaction treatment in 2017 ($p=0.034$, $F_{6,80} = 2.41$, Table E1). Litter moisture was affected by an interaction between grazing treatments (litter manipulation and clipping/compaction) and month sampled in June 2017 samples, with litter removed fall clip – fall compact treated plots having significantly lower moisture than several treatments ($p=0.047$, $F_{6,80} = 2.24$). Litter pH showed significant decrease in the litter removed treatment at Stavely in 2018 (litter retained: 5.80 ± 0.04, litter removed: 5.58 ± 0.03, $p = < 0.001$, $F_{1,81} = 23.93$).

2.3.3 Soil EEA Response

EEA in soil was affected by treatments and time of sampling, but the patterns varied by location, year and enzyme (Table 2.3). Litter removal tended to reduce EEA. At Onefour in 2017, the activity of Cello was reduced (litter: 37.82 nmol g^{-1} h^{-1} ± 2.80, litter removed: 30.30 nmol g^{-1} h^{-1} ± 2.66, $p = 0.025$, $F_{1,81} = 5.22$) and Xylo followed a similar pattern (litter: 25.69 nmol g^{-1} h^{-1} ± 1.63, litter removed: 21.32 nmol g^{-1} h^{-1} ± 1.66, $p=0.029$, $F_{1,81} = 4.97$), as did NAG at Stavely in 2018 (litter: 517.28 nmol g^{-1} h^{-1} ± 54.29, litter removed: 418.87 nmol g^{-1} h^{-1} ± 42.70, $p=0.019$, $F_{1,81} = 5.75$). There were no effects of litter on soil EEA at Lethbridge.

At Stavely in 2017, the activity of Xylo was altered by the grazing treatment ($p = 0.038$, $F_{6, 81.1} = 2.36$) and was greatest under the no clip, fall compaction treatment (Table F1). Similarly, Cello (Figure 2.2A, $p = 0.041$, $F_{6, 81} = 2.32$) and NAG (Figure 2.2B**,** $p = 0.049$, $F_{6, 81} = 2.22$) were affected by a three-way interaction between litter, grazing treatments and month at Onefour in 2017 (Fig. 2.2A and 2.2B). Both NAG and Cello from this interaction show high activity within the June litter removed spring clip – spring compaction treatment. At Lethbridge, there were no treatment effects on EEA in either year.

Our results indicated that month sampled has the potential to have a significant effect on EEA observed. At Stavely and Onefour, soil samples taken in July of 2017 had higher activity than June for various enzymes, whereas all soil EEA from Lethbridge had significantly higher activity in June than July (Table 2.3). In 2018, all soil enzyme activity within all sites were found to be significantly higher in June than July (Table 2.3, $p < 0.001$).

Results from this study have indicated that moisture has an influence on enzyme activity in soil. The activity of Xylo, Gluco and Phos in soil linearly increase with soil moisture content at all sites (Fig. 2.6B, 2.6C, 2.6E, respectively), while the activity of Cello and NAG in soil vary

in their relationship with moisture at sites (Fig. 2.6A, 2.6D, respectively). Differentiation between Cello and NAG activity and moisture at sites is dependent on average moisture at sites. Onefour has a comparatively lower range of soil moisture than a mesic site like Stavely, and thus the influence of moisture on EEA at Onefour is not as strong as Stavely (Fig. 2.6A, 2.6D).

2.3.4 Litter EEA Response

Enzyme response to the litter treatment was dependent on site, year sampled and enzyme (Table 2.4). In 2017, Stavely litter samples showed significantly higher activity in the litter retention treatment, seen in Cello (litter: 1832.44 nmol g^{-1} h^{-1} ± 124.52, litter removed: 1258.54 nmol g^{-1} h^{-1} ± 83.19, p= < 0.001, $F_{1,79}$ = 17.01), NAG (litter: 2358.94 nmol g^{-1} h^{-1} ± 189.64, litter removed: 1807.15 nmol g^{-1} h^{-1} ± 139.98, p=0.011, $F_{1,79}$ = 6.87) and Gluco (litter: 4538.60 nmol g^{-1} h^{-1} ± 269.52, litter removed: 3773.82 nmol g^{-1} h^{-1} ± 271.07, p=0.0094, $F_{1,79}$ = 7.08). An interaction was found between litter manipulation treatment and month in Phos activity in Onefour 2017 samples (June litter: 3248.85 nmol g^{-1} h^{-1} ± 274.25, June litter removed: 2736.97 nmol g^{-1} h^{-1} ± 359.52, July litter: 3496.27 nmol g^{-1} h^{-1} ± 324.03, July litter removed: 4597.57 nmol g^{-1} h^{-1} ± 523.51, p = 0.017, $F_{1,65}$ = 5.95) as well as Gluco in Stavely 2018 samples (June litter: 1558.21 nmol g^{-1} h^{-1} ± 84.05, June litter removed: 1447.54 nmol g^{-1} h^{-1} ± 55.59, July litter: 2326.47 nmol g^{-1} h^{-1} ± 317.43, July litter removed: 2895.33 nmol g^{-1} h^{-1} ± 193.15, p = 0.024, $F_{1,81}$ = 5.26). Both enzymes displayed higher activity in July 2018, as well as higher activity in litter removed treatments in July than in June.

Effect of grazing treatment was seen in Stavely litter samples in 2018; Xylo displayed high activity in spring clipping treatments in comparison to fall clipping or fall compaction treatments (p=0.013, $F_{6,81}$ = 2.88, Table F1). Similarly, Phos activity in Stavely 2018 samples was affected by a two-way interaction between litter manipulation and grazing treatment, where

high activity in plots with litter removed and treated in the spring contrasted with other treatments (Figure 2.3E, p=0.014, $F_{6,81} = 2.87$). Three-way interactions were detected between litter manipulation, clipping and compaction as well as month sampled at both Stavely and Onefour. Samples from Onefour in 2017 displayed interactions in Cello (Figure 2.3A, p=0.0052, $F_{6,64} = 3.44$) and NAG (Figure 2.3B, p = 0.032, $F_{6,64} = 2.48$). NAG generally displayed higher activity in litter removed treatments than litter retained treatments in July. Similarly, Xylo activity in Onefour June 2018 samples, displaying the effects of the same three-way interaction, showed high activity in litter retained fall compaction treatments in comparison to the spring treatments, with low activity in the litter removed fall compaction treatment (Figure 2.3D, p=0.033, $F_{4,65} = 2.79$). The same three-way interaction can be seen in Phos activity in Stavely 2017 samples (Figure 2.3C, p=0.011, $F_{6,65} = 1.23$), where EEA was found to be higher in July samples than June, as well as interaction between litter retained fall clipped plots and other treatments. At Lethbridge, there were no treatment effects on EEA in either year.

ANOVA results from various enzymes at all sites in 2017 indicate that several EEs differed significantly in their activities between months. At Stavely, Cello showed higher activity in June than July, while Gluco and Phos showed higher activity in July than June (Table 2.4). Litter samples from Lethbridge in 2017 showed higher Phos activity in July, and the same pattern was seen in Phos at Onefour. Stavely samples in 2018 show higher activity in July than June for all enzymes but Xylo (Table 2.4). June samples from Lethbridge in 2018 show higher activity than those from July (Table 2.4).

Results from this study indicate that moisture is an influence on enzyme activity in litter. Quadratic relationships were found between the activity of Cello and Gluco and litter moisture content (Fig. 2.7A, 2.7B), while a positive linear relationship was found between NAG activity

in litter and moisture (Fig. 2.7C). Interaction between site, litter manipulation and clipping/compaction treatment was seen in the activity of Xylo in litter (Fig. 2.7D-G, Fig. G1).

2.3.5 NMDS: Environmental Effects on EEAs: Soil

The ordination representing Onefour in 2017 detected association between EEAs, soil pH and soil moisture content (Figure 2.4A, Table A1). Samples showed inverse visual association between the activities of Cello, Xylo, Gluco and NAG and soil moisture content, whereas moisture had a visual association with the activity of Phos in the same samples (Figure 2.4A). The ordination of 2018 samples from Onefour detected association between EEAs, soil pH, available P and NPOC (Table A1). An inverse visual association between NAG and available P was detected. Based on our results, separation of litter manipulation treatments can be seen (Figure 2.4B, Table B1).

The ordination from Lethbridge in 2017 indicated association between EEAs, soil pH and soil moisture content (Figure 2.4C, Table A1). Separation was detected between months likely due to moisture differences (Figure 2.4C), and Phos showed higher activity than other EEs, leading to its separation (Figure 2.4C). The ordination from Lethbridge in 2018 showed visual association between available P, WETN, NPOC, soil pH, soil moisture content and EEAs (Figure 2.4D, Table A1). Positive visual associations were detected from the activities of Cello, Xylo, Gluco and NAG and soil available P, whereas the same enzymes had negative visual associations with soil pH (Figure 2.4D). Phos was detected as having a positive visual association with soil moisture and soil pH, as well as a negative relationship with NPOC and soil available P in Lethbridge samples (Figure 2.4D).

The ordination of Stavely 2017 samples detected association between EEAs and soil pH and available P (Figure 2.4E, Table A1). A positive visual association between Phos and soil pH

was detected (Figure 2.4E); our results detected a negative visual association between pH with Cello, Xylo and Gluco. Stavely samples from 2018 showed a separation between samples from different months, likely due to moisture differences between sampling times (Figure 2.4F).

2.3.6 NMDS: Environmental effects on EEAs: Litter

The NMDS ordination for litter EEA samples from Onefour in 2017 showed mild separation between June and July sampling times, as well as between litter manipulation treatments (Figure 2.5A, Table B1). Cello, Xylo and NAG activity at Onefour in 2018 showed association with litter moisture and litter pH, while Phos displayed an inverse visual association with the same environmental factors (Figure 2.5B, Table A1**)**. Pairwise perMANOVA results examining clipping/compaction treatment interaction at Onefour in 2018 litter (Table C1) show differences between fall treatments (fall clip fall roll – no clip fall roll: p=0.03).

Litter manipulation showed separation between treatments in the ordination of Lethbridge litter EEA samples from 2018 (Figure 2.5D, Table B1). Association was detected between EEA, litter pH, litter moisture content, soil available P, soil NPOC and soil WETN (Figure 2.5D, Table A1). Visual association was detected between litter pH, Cello and Phos at Lethbridge in 2018 – association was positive between litter pH and Cello, while association was negative between litter pH and Phos (Figure 2.5D). In Lethbridge, labile organic matter (NPOC and WETN) shared a positive visual association with NAG, while both shared a negative visual association with Xylo (Figure 2.5D). Additionally, litter moisture content and soil available P are positively associated with Phos at Lethbridge (Figure 2.5D). Differences were detected between months sampled at Lethbridge (Figure 2.5D).

A positive association was detected between the pH of the litter and the activity of NAG in the ordination of Stavely 2017 litter EEA samples (Figure 2.5E, Table A1); a negative visual

association was detected between the litter pH and the activity of Phos at the same site (Figure 2.5E). Pairwise presults examining clipping/compaction treatment interaction at Stavely in 2017 litter (Table C1) show differences between fall treatments and spring treatments (fall clip fall roll – no clip fall roll: p=0.01, fall clip fall roll – spring clip spring roll: p=0.035, no clip fall roll – spring clip no roll: p=0.04). The ordination of litter EEA from Stavely in 2018 shows that Cello is positively associated with litter moisture and litter pH, whereas Phos is negatively associated with the same environmental variables (Figure 2.5F). Pairwise perMANOVA results examining a litter manipulation and clipping/compaction treatment interaction at Stavely in 2018 litter show differences between fall treatments and spring treatments, as well as between spring treatments with different litter manipulation treatments (Table C1).

2.4 Discussion

Extracellular enzyme activity was consistently greater in litter than soils and tended to increase with site moisture levels. EEA in litter is typically greater than in soil, which is likely driven by the greater availability of resources and complexity of chemical compounds (Ge et al. 2017, Papa et al. 2014). Decomposition of litter by extracellular enzymes is a complex community level process, as complex polymers found in plant fibers such as cellulose must be decomposed by a series of enzymes, particularly due to their tendency to link covalently (Papa et al. 2014, Sinsabaugh et al. 1991, Sinsabaugh et al. 1994, Sinsabaugh et al. 2002). As extracellular enzymes are substrate specific, the microbial community responds to the structure of the litter itself and produces the appropriate enzymes for decomposing the substrate present (Ge et al. 2017, Papa et al. 2014, Sinsabaugh et al. 1991, Sinsabaugh et al. 2002). Due to the complex nature of plant fiber polymers and structure, as well as high C/N and C/P ratios found in plant tissues, degradation of litter is a complex process that requires a larger amount of EEA than

soil, which has already been humified (Ge et al. 2017, Papa et al. 2014, Sinsabaugh et al. 1991). The tendency of litter EEA to be more responsive to treatments than in soil is likely the result of greater changes in temperature and moisture within the litter compared to soil. Litter EEA is highly subject to temperature and moisture fluctuations (Ge et al. 2017, Papa et al. 2014), which are sensitive to defoliation effects and above-ground changes (Deutsch et al. 2010a, Deutsch et al. 2010b, Willms et al. 1993, Wisely 2018). Through utilization of the increased activity and reactivity of litter, a clearer image of immediate response to disturbance can be developed.

The removal of litter sometimes led to reduced EEA activity in soil samples. These effects are likely the result of litter removal effects on soil moisture (Deutsch et al. 2010a, Deutsch et al. 2010b, Willms et al. 1993). Links between soil enzyme activity and moisture are well documented across ecosystems, such as forests (Brockett et al. 2012), grazed grasslands in the northern Great Plains (Hewins et al. 2016a) and Patagonian Steppe (Olivera et al. 2014). Only a few other studies have directly examined litter removal effects on enzyme activity. Litter removal decreased soil Phosphatase and B-Glucosidase activity in Central European deciduous forests, which was attributed to changes in the availability of labile carbon (Kotroczó et al. 2014, Veres et al. 2015). Overgrazing is well known to reduce litter in grassland ecosystems (Naeth et al. 1991a), and our study demonstrates that this is likely a key process through which grazing alters nutrient cycling in these systems.

The removal of litter had varying responses through interactions with other factors in litter samples. Our results show that the litter removal treatment often, but not always, resulted in a decrease of litter EEA, which tended to vary between sites. It is known that the removal of litter decreases the amount of available substrate for EEA as well as increases in evapotranspiration due to increased exposure. Due to the complex nature of litter EEA, in which structural

components are degraded by a community of substrate-specific enzymes (Papa et al. 2014, Sinsabaugh et al. 1991, Sinsabaugh et al. 1994, Sinsabaugh et al. 2002), this study hypothesized that as litter availability decreases through litter removal, litter EEA would also decrease due to drier conditions. It is also plausible that litter removal would decrease litter EEA through higher recalcitrance of the remaining litter. Though still in need of further analyses, some studies suggest that solar radiation (termed photodegradation), not moisture, is the greatest influence on litter decomposition in arid environments because it aids in degradation of complex polymers such as lignin, that are often costly and time consuming to break down for microorganisms within litter tissues (Austin et al. 2006, Austin et al. 2016, Gallo et al. 2009, King et al. 2012). This suggests that litter degradation and thus enzyme activity in litter is controlled by different factors in various environments. Increases in litter EEA at a dry site such as Onefour in response to litter removal may therefore be influenced by environmental factors other than moisture. Thus, litter degradation may respond to grazing effects in various ways in different environments, indicating that plant material breakdown and litter EEA may occur in different cycles in different climates.

Litter and soil extracellular enzyme activity varied by site, largely dependent on moisture differences. However, some enzyme activity patterns were different than expected: litter EEA patterns from Onefour, the driest site, as well as Lethbridge, did not respond to treatment as hypothesized. Elevated litter EEA in dry conditions at Onefour suggest that factors other than moisture are controlling the level of enzyme activity at this site. Known drivers of extracellular enzyme activity are moisture, temperature, pH and substrate availability (Allison and Vitousek 2005, German et al. 2011, Gianfreda and Ruggerio 2006, Sinsabaugh 1994, Sinsabaugh and Moorhead 1994). The occurrence of nutrient cycling, particularly enzyme activity, in a dry

ecosystem such as Onefour suggests that even in moisture-limited environments, microorganisms that produce extracellular enzymes are still actively searching for energy sources, though perhaps in patterns that do not mimic that of a moisture-rich site.

Extracellular enzyme activity at the Lethbridge site were not altered by treatments despite treatment effects on pH and moisture in soil and litter. Given the influence of pH and moisture on EEA (Allison and Vitousek 2005, German et al. 2011, Gianfreda and Ruggerio 2006, Sinsabaugh 1994, Sinsabaugh and Moorhead 1994), this result was not expected. A study by Willms et al. (1993) suggests that when moisture is limited in the Alberta Mixedgrass natural subregion, litter reduces evapotranspiration to aid in plant growth; this is due to the positive effect of litter on herbage production in relation to growing conditions. Hewins et al. (2016a) determined that defoliation in the Alberta Mixedgrass natural subregion may interact with water addition to alter soil EEA activity. These findings suggest that moisture plays a large role in the continuation of nutrient cycling in the Alberta Mixedgrass natural subregion. Thus, enzyme activity at the Lethbridge site may not have been responsive to treatment due to the dry conditions in both years of study. However, our finding also suggests the need for further study; perhaps further study of the influence ofvarious environmental variables, or a longer duration of a similar study may find different conclusions.

Soil extracellular enzyme activity is driven by soil moisture (Brockett et al. 2012); however, we observed a few exceptions to this pattern. The activity of two enzymes at Onefour, the dry site, did not increase with soil moisture. This could be due to a higher proportion of sand in the soil at Onefour in comparison to the other two sites. Differences in soil organic carbon (SOC) contents of soil may be another possible influence; Onefour logically has lower soil

organic C than Stavely, for example, which has more organic matter due to higher vegetation production.

Non-linear relationships between the activity of some enzymes and moisture in litter samples may indicate complexities between EEA among sites, or perhaps the greater importance of other influences on litter EEA, perhaps such as litter pH. The relationship between Xylo activity and litter moisture content interacts between litter manipulation, clipping/compaction and site, indicating site differences. Patterns at Stavely and Onefour within this interaction indicate the effect of compaction treatments on litter EEA, though these patterns are inconclusive. The absence of this pattern at Lethbridge echoes the absence of treatment effect found at this site. Both Hewins et al. (2016a) and Willms et al. (1993) highlight the importance of moisture for production and nutrient cycling in the Alberta Mixedgrass natural subregion; in our study, likely due to the dry conditions of both study years, limited response was seen at Lethbridge. Low litter moisture content may have influenced EEA activity in both years at Lethbridge between treatments to see effect.

Litter and soil enzyme activity were not affected by the clipping and compaction treatments in consistent patterns across enzymes and sites; treatments were designed to simulate the physical impact, defoliation and trampling, of grazing livestock. Though pH was affected by clipping and compaction treatments, little treatment effect on soil and litter moisture was likely the reason for inconsistent treatment effect on EEA. Both moisture and pH are known as important factors in the regulation of soil EEA; individual extracellular enzymes are known to function best within a particular range of soil pH and moisture (Gianfreda and Bollag 1996). Treatment effect, though inconsistent, may have been seen due to monthly precipitation fluctuations, substrate differences between treatments and pH fluctuations. Differences in

precipitation between sampling months often contributed to large fluctuations in EEA in both soil and litter. Additionally, infrequent defoliation, as was seen in this study, may allow for root growth increase, thus allowing for root exudates and root decomposition into the soil to increase (Belsky et al. 1986, Hewins et al. 2016a**)**. Furthermore, compaction treatments may have the effect of beginning the physical breakdown process of fresh surface litter, providing the soil with another source of substrate for enzyme activity (Guretzky et al. 2014, Olofsson and Oksanen 2002).While these factors led to response of soil and litter EEA to the clipping and compaction treatments, the absence of treatment effect on soil and litter moisture content was likely the reason for inconsistent EEA response.

2.5 Conclusion

This study shows that soil and litter EEA within southern Alberta grasslands were affected primarily by litter manipulation, likely through the role of litter in modulating moisture. Moisture strongly influenced EEA, although these patterns differed between sites, depending on climatic average moisture. Enzyme activity in both soil and litter did not have consistent responses to compaction trampling and defoliation. The findings of this study demonstrate the role of grazing on nutrient cycling in grasslands, particularly the importance of litter on enzyme activity in a grassland ecosystem, and the varied effects of other grazing mechanisms such as defoliation and compaction. These findings have important implications for nutrient cycling in grasslands where climate change is likely to increase the occurrence of drought which can be exacerbated by grazing. Future analysis of long-term effects of grazing mechanisms on decomposition in grasslands could aid in further understanding response of the microbial community to grazing disturbances.

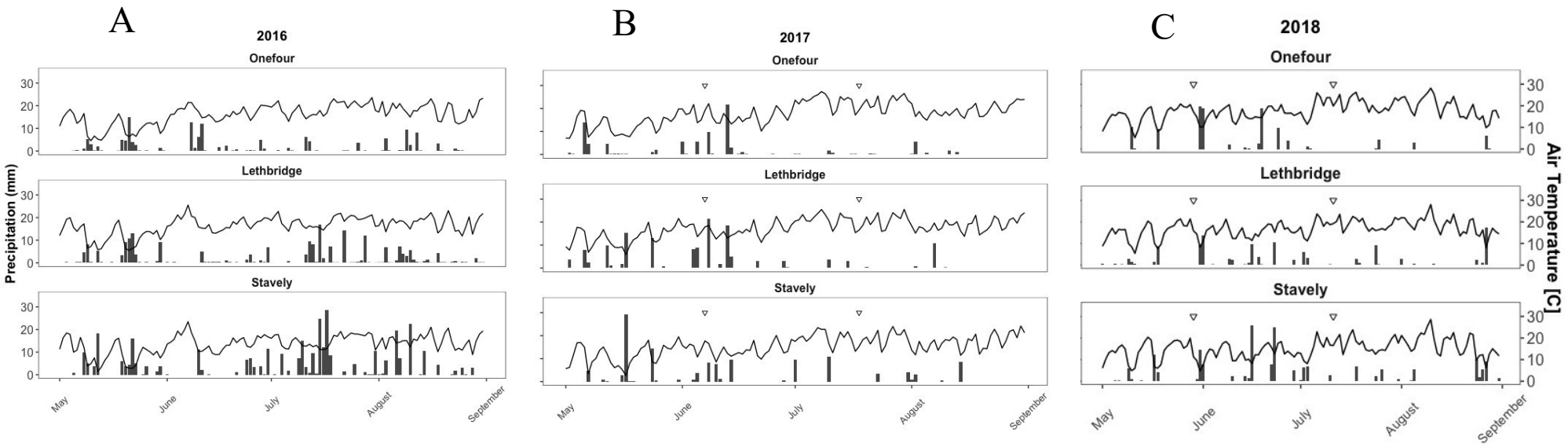

Figure 2.1 (A-C): Precipitation and temperature record for all sites; precipitation events are displayed as bars, daily temperature as a line (Alberta Climate Information Service). Approximate sampling event times are labeled with a downward triangle. A precipitation event of 45.6 mm that occurred at Stavely on June 13, 2017 was removed from this plot to optimize axes.

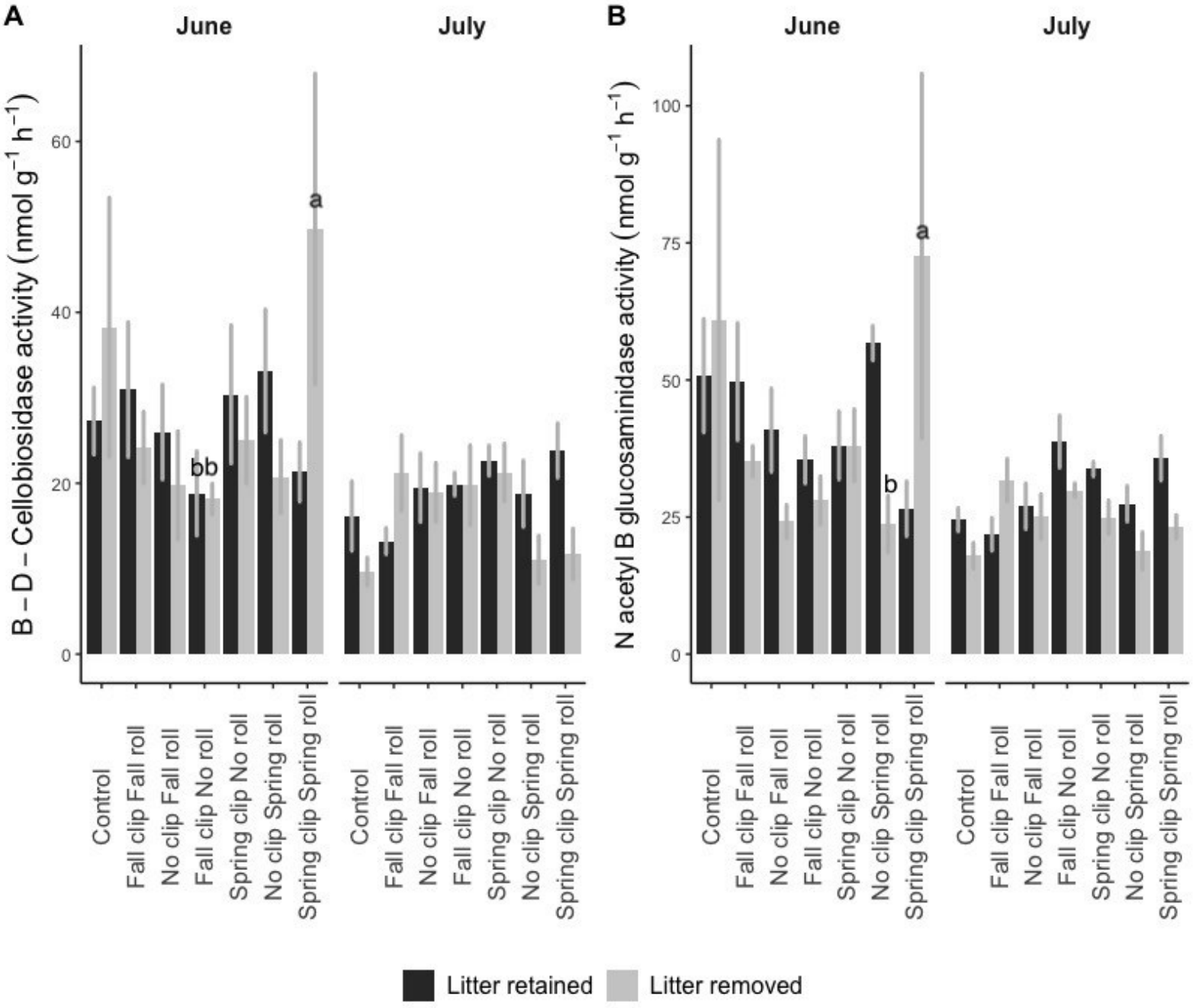

Figure 2.2 (A-B): Mean extracellular enzyme activity in soil at Onefour for (A) β-D-Cellobiosidase and (B) N-acetyl-β-glucosaminidase within litter manipulation treatments, clipping/compaction treatments and months sampled in 2018. Different letters above bars indicate significant differences ($p \leq 0.05$), bars with no letter above them did not differ from any other.

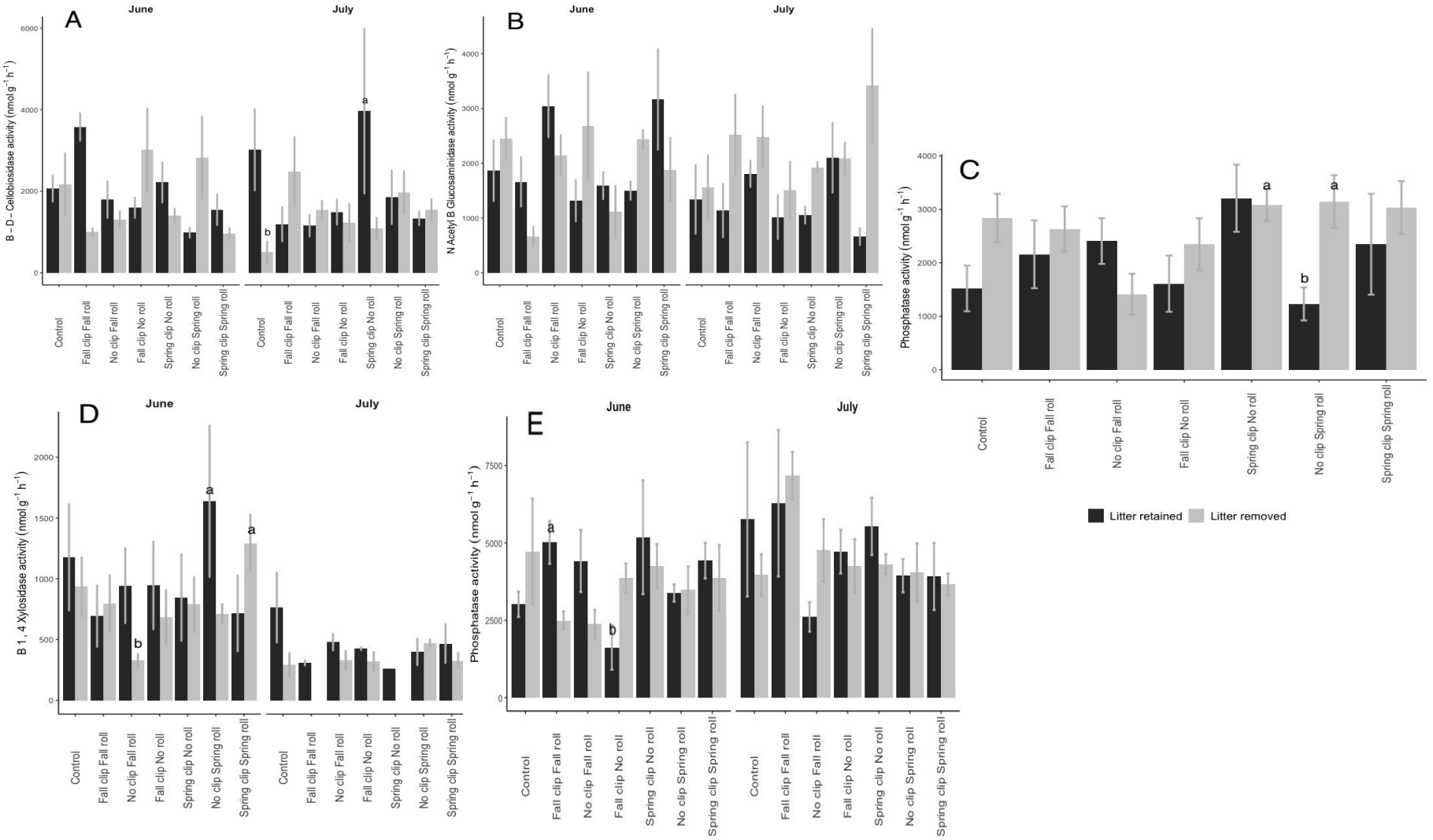

Figure 2.3 (A-E): Mean extracellular enzyme activity in litter for (A) β-D-Cellobiosidase at Onefour in 2017, (B) N-Acetyl-β-Glucosaminidase at Onefour in 2017, (C) Phosphatase at Stavely in 2018, (D) β-1,4-Xylosidase at Onefour in 2018 and (E) Phosphatase at Stavely in 2017 displaying interactions between litter manipulation, clipping/compaction and month sampled (except in the case of C). Significance value adjusted to $\alpha = 10\%$ in E to account for concern of Type II error. Different letters above bars indicate significant differences ($p \leq 0.05$), bars with no letter above them did not differ from any others.

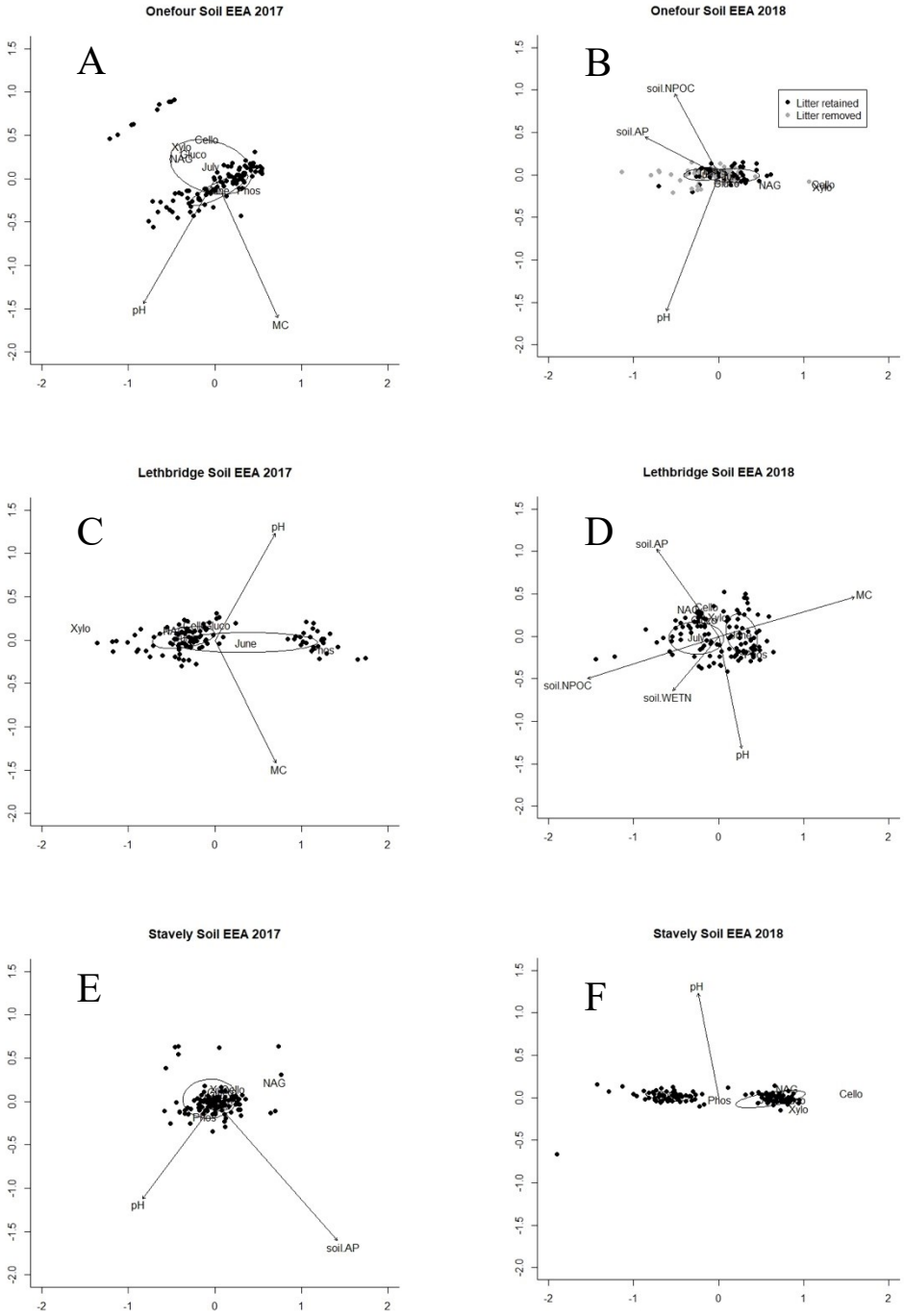

Figure 2.4 (A-F): Non-metric multidimensional scaling ordination of soil extracellular enzyme activity by site and year (Cello - β-D-Cellobiosidase, Xylo - β-1,4-Xylosidase, Gluco - β-1,4-glucosidase, NAG - N-acetyl-β-glucosaminidase, Phos - Phosphatase) in relation to soil characteristics. Significant environmental variables as determined through envfit (soil AP – soil Available Phosphorous, soil. NPOC – soil Non-Purgeable Organic Carbon, soil. WETN – soil Water Extractable Total Nitrogen, MC – Soil Moisture Content) are shown as vectors, and significant treatment factors as determined through perMANOVA (month, litter manipulation or clip/compaction) are shown on legends or ellipses.

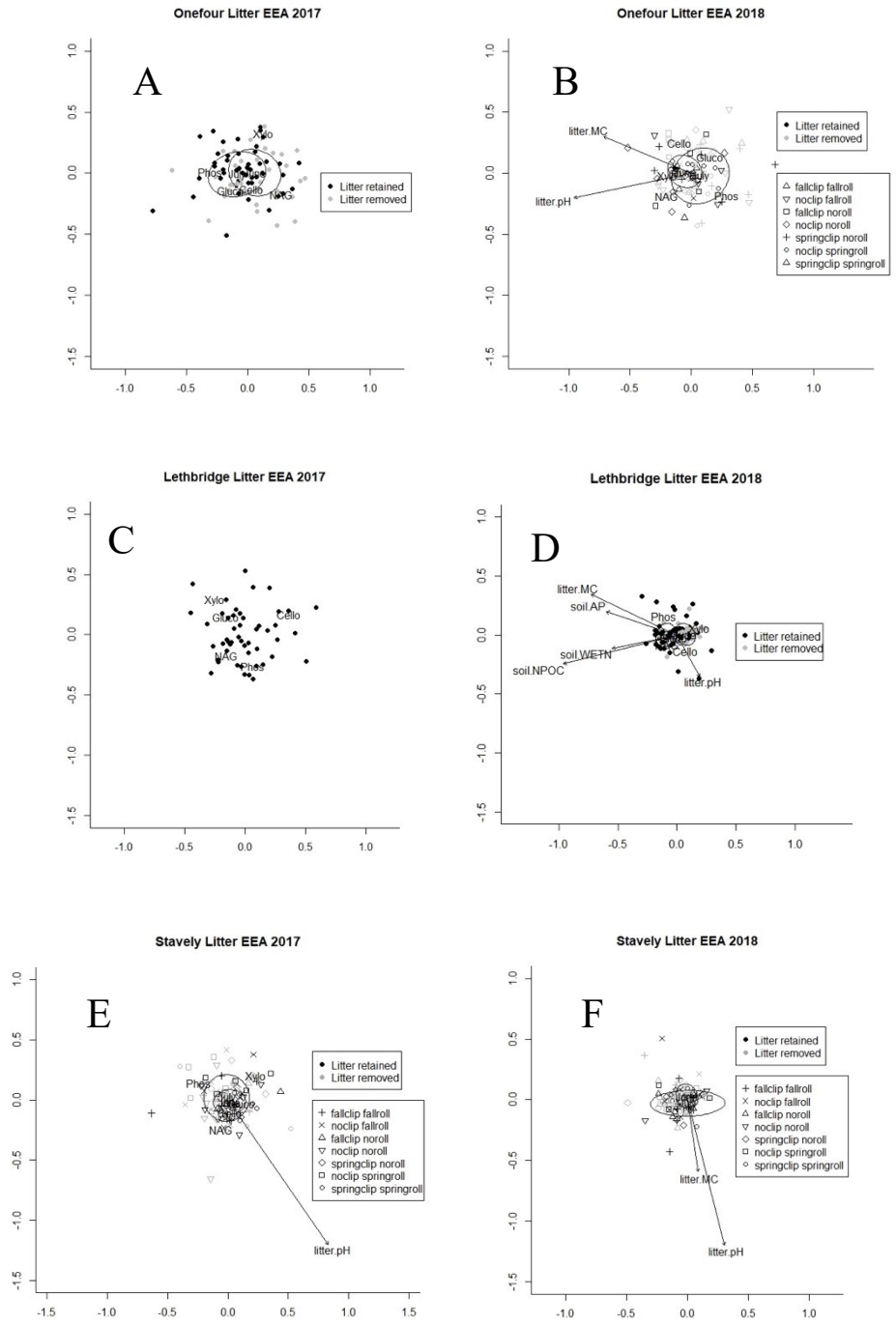

Figure 2.5 (A-F): Non-metric multidimensional scaling ordination of litter extracellular enzyme activity by site and year (Cello - β-D-Cellobiosidase, Xylo - β-1,4-Xylosidase, Gluco - β-1,4-glucosidase, NAG - N-acetyl-β-glucosaminidase, Phos - Phosphatase) in relation to soil and litter characteristics. Significant environmental variables as determined through envfit (soil AP – soil Available Phosphorous, soil.NPOC – soil Non-Purgeable Organic Carbon, soil.WETN – soil Water Extractable Total Nitrogen, litter.MC – Litter Moisture Content) are shown as vectors, and significant treatment factors as determined through perMANOVA (month, litter manipulation or clip/compaction) are shown on legends or ellipses.

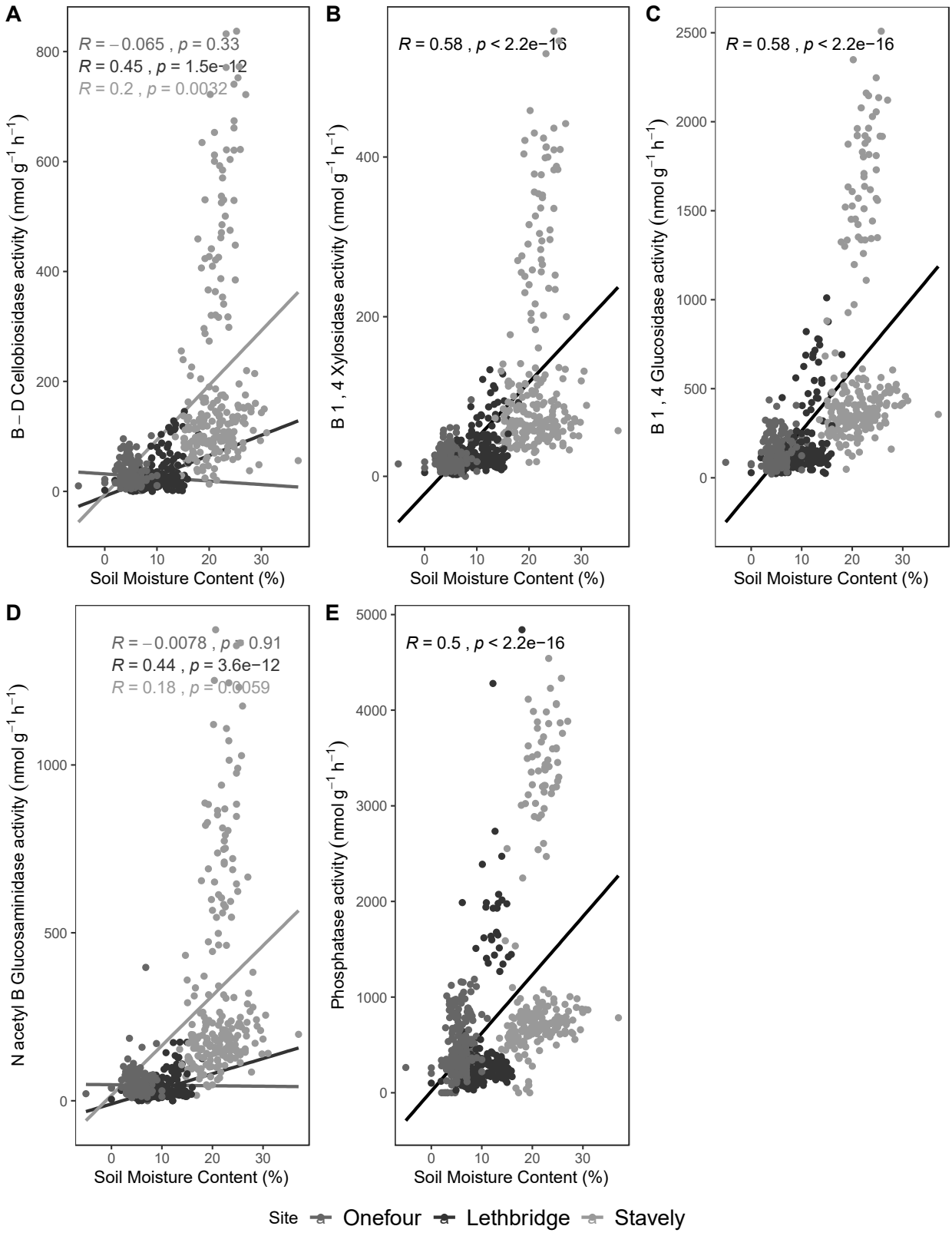

Figure 2.6 (A-E): Linear regressions displaying significant relationships between soil moisture content and soil extracellular enzyme activity, with (A) as β-D-Cellobiosidase, (B) as β-1,4-Xylosidase, (C) as β-1,4-Glucosidase, (D) as N-Acetyl-β-Glucosidase, (E) as Phosphatase.

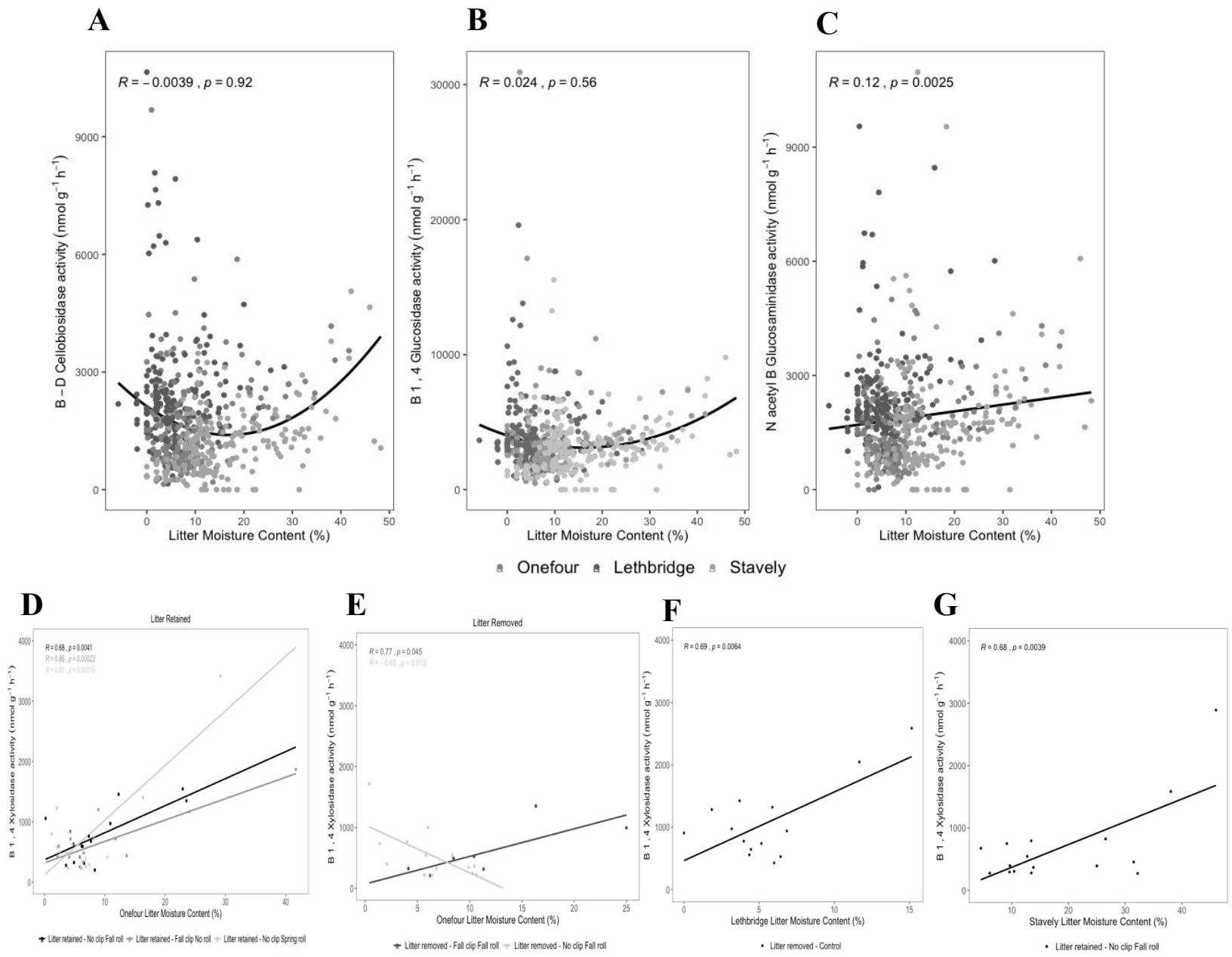

Figure 2.7 (A-G): Linear regressions displaying significant relationships between litter moisture content and litter extracellular enzyme activity, with (A) as β-D-Cellobiosidase, (B) as β-1,4-Glucosidase, (C) as N-Acetyl-β-Glucosidase and β-1,4-Xylosidase at Onefour (D, E, representing litter retained and litter removed treatments, respectively), Lethbridge (F) and Stavely (G), respectively. Note that A-C share a legend. Due to the complexity of the interaction between β-1,4-Xylosidase and litter moisture content, plots D-G are displaying the relationships with $p \leq 0.05$ (regressions and data points). A full display of all regressions and data for D-G can be seen in Fig. G1.

Table 2.1: Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil characteristics at each site and year. Degrees of freedom are the same across soil characteristics, df values are for treatments recorded on the left. Significant values ($P < 0.05$) are in bold.

2017															
		Soil pH			**Soil Moisture Content**			**WETN**			**NPOC**			**AP**		
Treatment	**df**	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
		Onefour														
Litter (L)	1	5.45	81	**0.022**	2.04	81	0.16	0.06	78	0.81	1.30	78	0.26	0.40	81	0.53
Clip / Compact (CC)	6	3.20	81	**0.007**	0.81	81	0.57	1.32	78	0.26	0.54	78	0.78	0.24	81	0.96
Month (M)	1	110.54	81	**< 0.001**	151.83	81	**< 0.001**	17.42	78	**< 0.001**	65.88	78	**< 0.001**	4.06	81	**0.047**
L*CC	6	0.50	81	0.81	0.81	81	0.56	0.84	78	0.54	0.86	78	0.53	0.15	81	0.99
L*M	1	2.70	81	0.10	0.09	81	0.77	0.21	78	0.65	6.97	78	**0.01**	0.01	81	0.93
CC*M	6	0.59	81	0.74	0.56	81	0.76	1.16	78	0.34	0.84	78	0.54	0.30	81	0.94
L*CC*M	6	0.94	81	0.47	0.24	81	0.96	1.08	78	0.38	0.51	78	0.80	0.44	81	0.85
		Lethbridge														
Litter (L)	1	0.38	81	0.54	0.32	81	0.57	0.02	80	0.89	0.0001	80	0.99	0.68	81	0.41
Clip / Compact (CC)	6	0.64	81	0.70	1.03	81	0.41	0.68	80	0.67	0.52	80	0.79	0.16	81	0.99
Month (M)	1	0.90	81	0.34	488.47	81	**< 0.001**	0.57	80	0.45	13.79	80	**< 0.001**	11.06	81	**0.001**
L*CC	6	0.37	81	0.89	1.36	81	0.24	1.14	80	0.35	1.17	80	0.33	0.56	81	0.76
L*M	1	0.01	81	0.93	0.28	81	0.60	0.02	80	0.90	0.06	80	0.81	0.90	81	0.35
CC*M	6	0.43	81	0.85	0.36	81	0.90	0.41	80	0.87	0.48	80	0.82	0.09	81	1.00

L*CC*M	6	1.48	81	0.19	0.94	81	0.47	0.89	80	0.50	0.64	80	0.70	0.41	81	0.87
Stavely																
Litter (L)	1	0.11	81	0.74	0.43	81	0.51	1.08	77	0.30	1.89	77	0.17	0.60	79	0.44
Clip / Compact (CC)	6	2.33	81	**0.040**	1.19	81	0.32	1.59	77	0.16	1.63	77	0.15	2.14	79	0.06
Month (M)	1	0.04	81	0.84	259.33	81	**< 0.001**	24.67	77	**< 0.001**	35.44	77	**< 0.001**	15.44	79	**< 0.001**
L*CC	6	1.43	81	0.21	0.66	81	0.68	0.40	77	0.88	0.48	77	0.82	0.85	79	0.54
L*M	1	0.03	81	0.85	0.01	81	0.93	0.07	77	0.79	0.11	77	0.74	0.04	79	0.84
CC*M	6	0.60	81	0.73	0.73	81	0.63	0.20	77	0.97	0.41	77	0.87	0.19	79	0.98
L*CC*M	6	0.50	81	0.81	1.05	81	0.40	0.63	77	0.70	0.79	77	0.58	0.22	79	0.97

Table 2.1 continued: Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil characteristics at each site and year. Degrees of freedom are the same across soil characteristics, df values are for treatments recorded on the left. Significant values ($P < 0.05$) are in bold.

2018															
		Soil pH			**Soil Moisture Content**			**WETN**			**NPOC**			**AP**		
Treatment	**df**	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
		Onefour														
Litter (L)	1	7.54	81	**0.007**	0.85	81	0.36	4.54	81	**0.036**	2.11	81	0.15	0.40	81	0.53
Clip / Compact (CC)	6	4.97	81	**< 0.001**	0.80	81	0.57	1.20	81	0.31	0.67	81	0.67	0.24	81	0.96
Month (M)	1	1.04	81	0.31	2.44	81	0.12	0.06	81	0.81	116.23	81	**< 0.001**	4.06	81	**0.047**
L*CC	6	1.10	81	0.37	0.88	81	0.52	0.53	81	0.79	1.30	81	0.27	0.15	81	0.99
L*M	1	0.18	81	0.67	0.24	81	0.62	0.02	81	0.90	2.28	81	0.13	0.01	81	0.93
CC*M	6	0.59	81	0.74	0.70	81	0.65	0.27	81	0.95	0.62	81	0.72	0.30	81	0.94
L*CC*M	6	0.49	81	0.81	0.54	81	0.78	0.54	81	0.78	0.70	81	0.65	0.44	81	0.85
		Lethbridge														
Litter (L)	1	8.91	81	**0.004**	20.02	81	**< 0.001**	0.03	81	0.86	1.02	81	0.32	0.12	81	0.73
Clip / Compact (CC)	6	0.94	81	0.47	0.37	81	0.90	0.47	81	0.83	0.16	81	0.99	0.25	81	0.96
Month (M)	1	4.61	81	**0.035**	120.68	81	**< 0.001**	15.62	81	**< 0.001**	224.22	81	**< 0.001**	46.79	81	**< 0.001**
L*CC	6	2.10	81	0.06	0.63	81	0.70	0.12	81	0.99	0.30	81	0.93	0.51	81	0.80
L*M	1	11.94	81	**0.001**	0.71	81	0.40	0.10	81	0.76	1.62	81	0.21	3.08	81	0.08
CC*M	6	0.46	81	0.84	0.28	81	0.94	0.50	81	0.81	0.58	81	0.75	0.76	81	0.61
L*CC*M	6	0.34	81	0.91	0.88	81	0.51	0.21	81	0.97	0.03	81	1.00	0.18	81	0.98

Stavely																
Litter (L)	1	1.32	81	0.25	0.89	81	0.35	6.69	81	**0.011**	8.57	81	**0.004**	6.20	81	**0.015**
Clip / Compact (CC)	6	2.44	81	**0.032**	2.14	81	0.06	1.65	81	0.14	0.99	81	0.44	3.03	81	**0.010**
Month (M)	1	17.29	81	**< 0.001**	2.08	81	0.15	3.73	81	0.06	0.40	81	0.53	2.83	81	0.10
L*CC	6	1.28	81	0.28	0.77	81	0.60	0.23	81	0.96	1.56	81	0.17	0.24	81	0.96
L*M	1	1.38	81	0.24	5.68	81	**0.02**	0.02	81	0.90	0.24	81	0.62	0.95	81	0.33
CC*M	6	0.26	81	0.95	1.29	81	0.27	1.35	81	0.25	2.31	81	**0.041**	0.15	81	0.99
L*CC*M	6	0.21	81	0.97	0.71	81	0.64	0.80	81	0.57	0.51	81	0.80	0.56	81	0.76

Table 2.2 Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on litter characteristics at each site and year. Significant values (P < 0.05) are in bold.

2017								
	Litter Moisture Content				Litter pH			
	F	**df**	**df.res**	***P***	**F**	**df**	**df.res**	***P***
Treatment	**Onefour**							
Litter (L)	3.11	1	69	0.08	0.87	1	73	0.35
Clip / Compact (CC)	0.75	6	69	0.61	4.67	6	72	**< 0.001**
Month (M)	153.88	1	69	**< 0.001**	16.30	1	72	**< 0.001**
L*CC	1.21	6	69	0.31	1.44	6	72	0.21
L*M	4.17	1	69	**0.045**	2.88	1	72	0.09
CC*M	1.31	6	69	0.27	1.59	6	72	0.16
L*CC*M	0.81	6	69	0.56	0.23	6	72	0.96
Lethbridge								
Litter (L)	0.49	1	72	0.49	4.00	1	73	**0.049**
Clip / Compact (CC)	0.72	6	72	0.63	1.66	6	72	0.14
Month (M)	244.12	1	72	**< 0.001**	78.29	1	72	**< 0.001**
L*CC	0.50	6	72	0.81	1.69	6	72	0.14
L*M	3.37	1	72	0.07	0.03	1	72	0.87
CC*M	0.88	6	72	0.51	0.89	6	72	0.51
L*CC*M	0.66	6	72	0.68	0.77	6	72	0.59
Stavely								
Litter (L)	2.28	1	80	0.14	0.38	1	80	0.54

Clip / Compact (CC)	1.12	6	80	0.36	2.41	6	80	**0.034**
Month (M)	245.34	1	80	**< 0.001**	2.75	1	80	0.10
L*CC	2.98	6	80	**0.011**	0.79	6	80	0.58
L*M	5.51	1	80	**0.021**	0.07	1	80	0.79
CC*M	0.79	6	80	0.58	1.36	6	80	0.24
L*CC*M	2.24	6	80	**0.047**	1.20	6	80	0.32

Table 2.2 continued: Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on litter characteristics at each site and year. Significant values (P < 0.05) are in bold.

2018								
	Litter Moisture Content				Litter pH			
	F	**df**	**df.res**	***P***	**F**	**df**	**df.res**	***P***
Treatment	**Onefour**							
Litter (L)	2.11	1	64	0.15	17.78	1	78	**< 0.001**
Clip / Compact (CC)	0.46	6	64	0.84	7.19	6	78	**< 0.001**
Month (M)	7.76	1	64	**0.01**	28.17	1	78	**< 0.001**
L*CC	0.43	6	64	0.86	1.99	6	78	0.08
L*M	0.03	1	64	0.85	0.19	1	78	0.66
CC*M	0.64	6	64	0.70	2.24	6	78	**0.048**
L*CC*M	0.30	4	64	0.88	0.61	6	78	0.72
Lethbridge								
Litter (L)	12.05	1	81	**0.001**	12.83	1	77	**0.001**
Clip / Compact (CC)	1.45	6	81	0.21	0.97	6	77	0.45
Month (M)	42.55	1	81	**< 0.001**	1.80	1	77	0.18
L*CC	2.19	6	81	0.05	2.00	6	77	0.08
L*M	0.004	1	81	0.95	0.08	1	77	0.78
CC*M	1.13	6	81	0.35	0.42	6	77	0.86
L*CC*M	0.58	6	81	0.75	0.59	6	77	0.73
Stavely								
Litter (L)	0.25	1	81	0.62	23.93	1	81	**< 0.001**

Clip / Compact (CC)	0.17	6	81	0.98	1.88	6	81	0.09
Month (M)	39.38	1	81	**< 0.001**	0.70	1	81	0.40
L*CC	0.17	6	81	0.98	0.98	6	81	0.44
L*M	0.16	1	81	0.69	0.01	1	81	0.94
CC*M	0.25	6	81	0.96	0.93	6	81	0.47
L*CC*M	0.21	6	81	0.97	0.75	6	81	0.61

Table 2.3 Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil extracellular enzyme activities at each site and year. Degrees of freedom are the same across soil extracellular enzymes, df values for treatments are recorded on the left. Significant values ($P < 0.05$) are in bold.

Soil EEA 2017															
		Cello			**Xylo**			**Gluco**			**NAG**			**Phos**		
Treatment	**df**	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
		Onefour														
Litter (L)	1	5.22	81	**0.025**	4.97	81	**0.029**	1.00	81	0.32	1.40	81	0.24	3.74	81	0.06
Clip/Roll (CC)	6	1.33	81	0.25	1.07	81	0.39	0.65	81	0.69	1.02	81	0.42	0.75	81	0.61
Month (M)	1	41.47	81	**< 0.001**	45.27	81	**< 0.001**	6.13	81	**0.015**	7.53	81	**0.007**	3.78	81	0.06
L*CC	6	0.31	81	0.93	0.46	81	0.83	0.41	81	0.87	0.84	81	0.54	0.43	81	0.85
L*M	1	0.01	81	0.93	0.02	81	0.88	0.43	81	0.51	3.63	81	0.06	0.01	81	0.93
CC*M	6	0.90	81	0.50	1.10	81	0.37	1.04	81	0.41	0.56	81	0.76	0.99	81	0.44
L*CC*M	6	0.85	81	0.53	0.53	81	0.79	1.05	81	0.40	1.50	81	0.19	0.58	81	0.74
		Lethbridge														
Litter (L)	1	0.87	79	0.35	0.60	79	0.44	0.68	79	0.41	0.16	79	0.69	0.18	79	0.67
Clip/Roll (CC)	6	0.41	79	0.87	0.20	79	0.97	0.15	79	0.99	0.25	79	0.96	0.10	79	1.00
Month (M)	1	52.50	79	**< 0.001**	40.58	79	**< 0.001**	48.34	79	**< 0.001**	43.11	79	**< 0.001**	44.65	79	**< 0.001**
L*CC	6	0.26	79	0.95	0.08	79	1.00	0.10	79	1.00	0.30	79	0.94	0.20	79	0.97
L*M	1	1.76	79	0.19	0.94	79	0.34	0.46	79	0.50	0.02	79	0.89	0.17	79	0.69
CC*M	6	0.39	79	0.88	0.19	79	0.98	0.16	79	0.99	0.15	79	0.99	0.17	79	0.98
L*CC*M	6	0.57	79	0.75	0.25	79	0.96	0.19	79	0.98	0.35	79	0.91	0.14	79	0.99
		Stavely														
Litter (L)	1	0.05	81	0.82	0.53	81	0.47	0.58	81	0.45	0.74	81	0.39	1.58	81	0.21
Clip/Roll (CC)	6	0.82	81	0.55	2.23	81	**0.049**	0.95	81	0.46	0.92	81	0.49	1.02	81	0.42

Month (M)	1	0.23	81	0.63	3.30	81	0.07	0.66	81	0.42	0.01	81	0.93	14.15	81	< **0.001**
L*CC	6	0.26	81	0.95	0.62	81	0.71	0.86	81	0.53	1.02	81	0.42	2.15	81	0.06
L*M	1	2.55	81	0.11	2.62	81	0.11	1.94	81	0.17	2.98	81	0.09	0.86	81	0.36
CC*M	6	0.39	81	0.88	0.76	81	0.60	0.21	81	0.97	0.80	81	0.57	0.71	81	0.64
L*CC*M	6	0.98	81	0.44	1.29	81	0.27	0.67	81	0.67	0.75	81	0.61	1.20	81	0.31

Table 2.3 continued: Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil extracellular enzyme activities at each site and year. Degrees of freedom are the same across soil extracellular enzymes, df values for treatments are recorded on the left. Significant values ($P < 0.05$) are in bold.

Soil EEA 2018															
		Cello			**Xylo**			**Gluco**			**NAG**			**Phos**		
Treatment	**df**	**F Onefour**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
Litter (L)	1	0.17	81	0.68	0.03	81	0.86	1.41	81	0.24	1.22	81	0.27	2.53	81	0.12
Clip/Roll (CC)	6	0.67	81	0.67	0.87	81	0.52	0.83	81	0.55	0.52	81	0.79	1.38	81	0.23
Month (M)	1	17.60	81	**< 0.001**	15.33	81	**< 0.001**	39.20	81	**< 0.001**	15.01	81	**< 0.001**	27.72	81	**< 0.001**
L*CC	6	0.82	81	0.56	1.46	81	0.20	1.75	81	0.12	1.36	81	0.24	0.85	81	0.54
L*M	1	0.74	81	0.39	0.37	81	0.55	0.06	81	0.81	0.14	81	0.71	0.44	81	0.51
CC*M	6	1.54	81	0.18	0.52	81	0.79	0.59	81	0.74	1.44	81	0.21	0.61	81	0.72
L*CC*M	6	2.32	81	**0.04**	1.79	81	0.11	1.94	81	0.08	2.22	81	**0.049**	0.80	81	0.58
Lethbridge																
Litter (L)	1	0.13	81	0.72	0.33	81	0.57	0.33	81	0.57	0.005	81	0.95	3.62	81	0.06
Clip/Roll (CC)	6	1.59	81	0.16	1.02	81	0.42	0.88	81	0.52	1.08	81	0.38	1.41	81	0.22
Month (M)	1	17.34	81	**< 0.001**	34.19	81	**< 0.001**	60.91	81	**< 0.001**	42.31	81	**< 0.001**	93.79	81	**< 0.001**
L*CC	6	0.43	81	0.85	0.42	81	0.86	0.59	81	0.74	0.56	81	0.76	1.73	81	0.12
L*M	1	1.10	81	0.30	1.17	81	0.28	1.88	81	0.17	2.06	81	0.15	0.88	81	0.35
CC*M	6	1.00	81	0.43	1.22	81	0.31	0.47	81	0.83	0.96	81	0.46	0.77	81	0.59
L*CC*M	6	0.37	81	0.90	1.13	81	0.35	0.46	81	0.84	0.95	81	0.47	1.53	81	0.18
Stavely																
Litter (L)	1	0.08	81	0.78	0.91	81	0.34	0.004	81	0.95	5.66	81	**0.020**	0.11	81	0.75
Clip/Roll	6	0.71	81	0.64	1.03	81	0.41	1.01	81	0.42	0.84	81	0.54	0.72	81	0.63

(CC)																
Month (M)	1	268.09	81	< **0.001**	307.75	81	< **0.001**	433.60	81	< **0.001**	279.11	81	< **0.001**	598.11	81	< **0.001**
L*CC	6	0.39	81	0.88	1.14	81	0.35	0.86	81	0.53	0.79	81	0.58	1.19	81	0.32
L*M	1	0.03	81	0.86	0.21	81	0.64	0.00	81	0.96	1.59	81	0.21	0.62	81	0.43
CC*M	6	0.77	81	0.60	0.86	81	0.53	0.94	81	0.47	1.01	81	0.43	0.78	81	0.59
L*CC*M	6	0.60	81	0.73	1.22	81	0.30	0.62	81	0.72	0.25	81	0.96	1.11	81	0.36

Table 2.4 Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on litter extracellular enzyme activities at each site and year. Degrees of freedom are the same across litter extracellular enzymes, df values for treatments are recorded on the left. Significant values ($P < 0.05$) are in bold.

Litter EEA 2017															
		Cello			**Xylo**			**Gluco**			**NAG**			**Phos**		
		F	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
Treatment	**df**	**Onefour**														
Litter (L)	1	1.81	65	0.18	0.66	56	0.42	0.02	55	0.88	4.56	65	**0.037**	0.04	65	0.84
Clip/Roll (CC)	6	0.51	64	0.80	0.80	55	0.57	1.14	54	0.35	2.19	64	0.06	0.33	64	0.92
Month (M)	1	0.77	65	0.38	1.30	57	0.26	0.01	56	0.91	1.08	65	0.30	8.77	65	**0.004**
L*CC	6	2.38	64	**0.038**	0.82	55	0.56	1.15	54	0.35	0.48	64	0.82	0.60	64	0.73
L*M	1	0.00	64	0.98	0.07	57	0.79	4.01	56	0.05	7.80	64	**0.007**	5.95	65	**0.017**
CC*M	6	0.71	64	0.64	1.65	55	0.15	0.60	54	0.73	0.89	64	0.51	0.24	64	0.96
L*CC*M	6	3.44	64	**0.005**	1.04	55	0.41	1.17	54	0.34	2.48	64	**0.032**	1.23	64	0.30
Lethbridge																
Litter (L)	1	0.01	73	0.93	0.14	74	0.70	0.02	73	0.89	0.07	63	0.79	0.56	73	0.46
Clip/Roll (CC)	6	0.54	72	0.78	1.34	72	0.25	0.61	72	0.72	0.62	60	0.72	0.40	72	0.88
Month (M)	1	0.47	72	0.49	0.57	72	0.45	2.11	72	0.15	0.88	61	0.35	16.52	72	**< 0.001**
L*CC	6	1.08	72	0.38	0.21	72	0.97	0.72	72	0.64	0.54	61	0.77	0.52	72	0.79
L*M	1	0.00	72	0.97	3.49	73	0.07	1.48	72	0.23	0.38	60	0.54	0.09	73	0.77
CC*M	6	0.88	72	0.51	0.23	72	0.97	0.80	72	0.57	0.44	61	0.85	0.26	72	0.96
L*CC*M	6	1.44	72	0.21	1.18	72	0.33	0.46	72	0.83	0.11	61	1.00	1.18	72	0.33
Stavely																
Litter (L)	1	17.78	79	**< 0.001**	3.44	79	0.07	6.73	79	**0.011**	6.74	79	**0.011**	0.001	79	0.97
Clip/Roll (CC)	6	0.92	79	0.48	1.13	79	0.35	1.18	79	0.33	0.58	79	0.74	1.84	79	0.10
Month (M)	1	9.98	79	**0.002**	0.04	79	0.84	9.68	79	**0.003**	0.98	79	0.33	7.66	79	**0.007**
L*CC	6	0.82	79	0.56	1.92	79	0.09	0.76	79	0.60	0.44	79	0.85	0.76	79	0.60
L*M	1	2.41	79	0.12	0.01	79	0.92	0.49	79	0.49	0.00	79	0.96	0.19	79	0.67
CC*M	6	0.96	79	0.46	0.92	79	0.48	0.14	79	0.99	0.22	79	0.97	1.33	79	0.26

L*CC*M	6	1.59	79	0.16	0.55	79	0.77	1.56	79	0.17	0.72	79	0.63	2.99	79	**0.011**

Table 2.4 continued: Statistical results of mixed-model ANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on litter extracellular enzyme activities at each site and year. Degrees of freedom are the same across litter extracellular enzymes, df values for treatments are recorded on the left. Significant values ($P < 0.05$) are in bold.

Litter EEA 2018															
		Cello			Xylo			Gluco			NAG			Phos		
		F	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
Treatment	**df**	**Onefour**														
Litter (L)	1	0.00	64	0.96	1.57	64	0.22	0.04	64	0.84	2.53	64	0.12	0.13	64	0.72
Clip/Roll (CC)	6	1.11	64	0.36	1.88	64	0.10	0.82	64	0.56	1.15	64	0.34	0.87	64	0.52
Month (M)	1	1.18	64	0.28	0.41	64	0.52	0.40	64	0.53	3.30	64	0.07	0.01	64	0.91
L*CC	6	1.30	64	0.27	2.23	64	**0.051**	0.97	64	0.45	0.77	64	0.59	0.29	64	0.94
L*M	1	0.66	64	0.42	4.37	64	**0.041**	0.42	64	0.52	1.00	64	0.32	0.91	64	0.34
CC*M	6	0.93	64	0.48	1.63	64	0.15	0.32	64	0.92	1.26	64	0.29	0.90	64	0.50
L*CC*M	4	0.14	64	0.97	2.70	64	**0.038**	1.02	64	0.40	0.56	64	0.69	0.26	64	0.90
Lethbridge																
Litter (L)	1	2.61	70	0.11	2.57	70	0.11	3.61	70	0.06	1.76	70	0.19	0.04	70	0.84
Clip/Roll (CC)	6	1.14	70	0.35	1.84	70	0.10	0.57	70	0.75	1.19	70	0.32	0.32	70	0.92
Month (M)	1	0.06	70	0.81	7.82	70	**0.007**	0.12	70	0.73	0.00	70	0.98	0.35	70	0.56
L*CC	6	1.07	70	0.39	0.74	70	0.62	0.49	70	0.81	1.13	70	0.36	0.36	70	0.90
L*M	1	3.24	70	0.08	0.53	70	0.47	2.07	70	0.15	1.39	70	0.24	0.35	70	0.56
CC*M	6	0.75	70	0.61	0.60	70	0.73	1.03	70	0.41	0.25	70	0.96	0.31	70	0.93
L*CC*M	4	0.57	70	0.69	0.42	70	0.80	0.59	70	0.67	0.34	70	0.85	0.65	70	0.63
Stavely																
Litter (L)	1	2.03	81	0.16	0.59	81	0.45	3.27	81	0.07	0.25	81	0.62	10.41	81	**0.002**
Clip/Roll (CC)	6	1.28	81	0.28	3.15	81	**0.008**	0.95	81	0.46	1.19	81	0.32	1.91	81	0.09
Month (M)	1	31.24	81	**< 0.001**	3.59	81	0.06	13.08	81	**0.001**	27.87	81	**< 0.001**	13.07	81	**0.001**
L*CC	6	1.87	81	0.10	1.33	81	0.25	1.89	81	0.09	1.11	81	0.37	2.82	81	**0.015**
L*M	1	3.62	81	0.06	3.51	81	0.06	5.36	81	**0.023**	1.41	81	0.24	2.97	81	0.09
CC*M	6	1.06	81	0.40	1.38	81	0.23	0.60	81	0.73	0.75	81	0.61	1.67	81	0.14

L*CC*M	6	2.05	81	0.07	1.75	81	0.12	1.88	81	0.09	1.43	81	0.21	2.09	81	0.06

Chapter 3. Individual and Combined Effects of Simulated Livestock Soil Compaction, Plant Defoliation and Litter Depletion on three Grassland Vegetation Types

3.1 Introduction

Livestock grazing is a common land use in grassland ecosystems globally that alters plant community composition and productivity (Herrero-Juregui and Oesterheld 2018, Milchunas and Lauenroth 1993). Livestock can alter grasslands through a variety of different mechanisms: removal of grass biomass, trampling, excretion and the alteration of other ecosystem properties, such as the plant litter. Most studies on livestock effects take one of two approaches: 1) *in situ* studies which examine the effects of livestock on ecosystems (e.g. Bork et al. 2019) and include all of their effects, and 2) simulated studies which almost exclusively examine only the removal of grass biomass by clipping (e.g. Carlyle et al. 2014). Rarely are the multiple mechanisms through which livestock alter vegetation isolated to examine their individual effects on plant communities despite that the degree of some of these mechanisms can be altered by different livestock management systems. Understanding these mechanisms, and how they change across grassland types, could provide insight into the differential impacts of grazing management on grassland ecosystems.

Growing interest in adaptive-multipaddock (AMP) grazing, which emphasizes trampling, evenness of defoliation and litter manipulation as key mechanisms of grazing for grassland productivity, raises questions about grazing mechanisms and how they affect vegetation communities in different climates (Briske et al. 2008, Savory and Butterfield 1998, Teague et al. 2013). Grazing systems are known to have different effects on vegetation communities depending on the intensity of grazing due to the extent of disturbance that plants experience within each system. Contrasts have been shown, for example, between effects of continuous and

deferred grazing systems on vegetation communities, as these two systems differ in the duration of grazing disturbances on individual plants. Continuous grazing at a heavy stocking rate has been criticized for allowing for selective grazing and thus altering plant communities towards more grazing tolerant species (Holechek et al. 1999, Teague et al. 2011), while deferring grazing until an optimal time in the growing season for the specific plant community has allowed for sensitive species to develop (Dormaar et al. 1997). Careful consideration of climate and evolutionary history of the area being grazed is important when managing grazing in order to preserve biodiversity and productivity (Herrero-Juregui and Oesterheld 2018, Milchunas et al. 1988, Milchunas et al. 1993).

The response of a plant community to trampling is dependent on the vegetation community; studies have found communities with prior trampling are able to recover faster than communities that have not evolutionarily adapted to trampling (Cole 1995a, Cole 1995b). Resistance to trampling was primarily dependent on plant morphological structure, where low-growing graminoids were the most resistant (Cole 1995a, Cole 1995b). The reduction in vegetation height by trampling can often cause microclimate changes in soil moisture and light availability (Kobayashi et al. 1997). Vegetation growth can additionally be inhibited by trampling due to soil compaction, reduced soil porosity and thus reduced water infiltration (Greenwood and McKenzie 2001, Naeth et al. 1990a).

The response of vegetation to defoliation is similarly tied to the environmental characteristics and history of the community; the response may vary depending on the presence of grazing in the evolutionary history of the plant community (Carlyle et al. 2014, Kobayashi et al. 1997, Milchunas et al. 1988). Grazing at high stocking rates can decrease plant richness, species cover and above-ground growth, with timing of grazing also being an important factor in

response (Herrero-Juregui and Oesterheld 2018, Wang and Wesche 2016, Wilms et al. 1985). However, grazing at light or moderate stocking rates at productive sites may increase species richness, indicating that plant community structure in grasslands is resilient to grazing at moderate levels (Herrero-Juregui and Oesterheld 2018, Wang and Wesche 2016, Wilms et al. 1985).

Litter presence alters vegetation microclimate dynamics such as soil moisture and temperature, depending on ecosystem structure and subsequent microclimate effects (Deutsch et al. 2010a, Deutsch et al. 2010b). Litter is influential on the production and species diversity of grasslands, depending on the climate of the grassland, through effects on the availability of resources such as moisture and sunlight (Foster and Gross 1998, Willms et al. 1986, Xiong and Nilsson 1999). Grazing can alter the presence of litter through increased physical decomposition and defoliation; heavy and early-season grazing can drastically decrease litter presence (Naeth et al. 1991a).

To examine the individual and combined effects of defoliation, compaction and litter removal we conducted a factorial study that manipulated these factors at three grassland sites in southern Alberta, Canada. Sites were located across a climatic gradient in order to examine their individual and combined effects on plant production and community composition. It is hypothesized that litter removal will decrease production, species richness and diversity in xeric sites, while increasing production and species richness in mesic sites. Clipping in the spring is hypothesized to decrease production and diversity in mesic sites, as this is an important time for growth, while increasing production in xeric sites due to moisture limitations later in the growing season. Trampling in the spring is hypothesized to decrease production and diversity due to

increased compaction in moist conditions, with increasing severity dependent on the moisture regime of the site.

3.2 Materials and Methods

3.2.1 Site Descriptions

Vegetation response was evaluated at three sites within southern Alberta, Canada, each representing a different subregion of the Grasslands Natural Region. Stavely Range Research Ranch (50° 12'N, 113° 57'W), operated by Alberta Environment and Parks and located within the Foothills Fescue natural subregion, is characterized by Black Chernozemic soil and a moist, cool climate, with a mean annual temperature (MAT) of 3.9 °C and mean annual precipitation (MAP) of 470 mm (Downing and Pettapiece 2006). Stavely is characterized by a *Festuca campestris* (Rydb.) and *Danthonia parryi* (Scribn.) community. Less mesic than the Stavely site, the Lethbridge Animal Disease Research Institute site (49° 43'N, 112° 57'W), operated by the Government of Canada and located within the Mixedgrass Prairie natural subregion, is characterized by Dark Brown Chernozemic soil, a MAT of 4.4 °C and a MAP of 394 mm (Downing and Pettapiece 2006). The Lethbridge site is characterized by a *Pascopyrum smithii* ((Rydb.) Á. Löve, *Nassella viridula* (Trin.) and *Hesperostipa comata* (Trin. and Rupr.) vegetation community. The Onefour site (49° 07'N, 110° 29'W), within the Dry Mixedgrass Prairie natural subregion, is operated by Alberta Environment and Parks; the Dry Mixedgrass Prairie has a MAP of 333 mm and a MAT of 4.2 °C (Downing and Pettapiece 2006). The Onefour site is characterized by a *Hesperostipa comata* (Trin. and Rupr.) and *Bouteloua gracilis* (Willd. ex Kunth) community (Downing and Pettapiece 2006).

3.2.2 Treatments and Experimental Design

In a factorial experiment, 1 m^2 plots (separated from adjacent plots by 0.5 m) were treated with litter removal, spring or fall clipping (defoliation) and spring and fall compaction (trampling) (Figure M1). Each site contained four blocks for a total of 56 plots. Litter removal was completed during initial site setup in 2016, and subsequent years of monitoring examined the effects of this single, initial litter removal. Litter removal was done once upon initial site setup in 2016; a forage harvester was used to remove standing dead, then the surface litter was raked. Site preparation was careful to remove only the litter layer, attempting to not disturb other layers of the LFH horizon for this treatment. Litter removal occured in the early spring of 2016 before the beginning of the growing season; standing dead was removed to the same height as the clipping height for each site. Litter removal was done prior to the application of clipping and compaction treatments in 2016, so litter would not be compacted and decomposed before litter removal. Litter removal was intended to simulate litter loss through grazing.

Seasonal clipping and compaction treatments were done in 2016 and 2017; spring treatments were completed in late June to early July, and fall treatments were completed in mid-September. One exception to this was the 2017 fall treatments at Stavely, which were pushed to mid-October due to a fire risk. Clipping was done by hand using sheep shears. The clipping treatment was intended to simulate seasonal, moderate (50%) defoliation via grazing. Due to the moisture differences between study sites, Stavely produces more grass biomass and was consequently clipped at a higher level (12.5 cm) than Lethbridge and Onefour (7.5 cm) (Willms et al. 1996, Willms et al. 2002).

Compaction treatments were done using a Cambridge cultipacker –different cultipackers were used in 2016 and 2017 due to equipment availability. Surface soil compaction is expected

to increase with higher ground pressure (Smith and Dickson 1990). In 2016, the cultipacker used had a weight of 215.5 kg, which was increased to 306.2 kg during treatment using sand. The pressure exerted by the cultipacker used in 2016 was not able to be calculated due to equipment availability. The cultipacker used in 2017 had an approximate pressure of 142 kPa, which is within a similar range of pressure as mechanisms used in other compaction studies (Di et al. 2001). During compaction treatment, the weighted cultipacker was pulled using an all-terrain vehicle (ATV) three times over the treated plots, always moving in the same direction throughout the site for consistency.

Any seasonal treatments that were expected to interfere with data collection were accounted for in sampling and analysis. Sampling times, temperature and precipitation data for this study are presented in Figure 3.1 (A-C).

3.2.3 Vegetation Production Measurements, Sampling and Processing

Vegetation mass was collected from a 0.2 x 1.0 m subsample of each plot at peak biomass (mid-July) in 2017 and a 0.5 x 0.5 m subsample of each plot in 2018; the area clipped in 2017 was avoided when sampling for biomass in 2018, as biomass was sampled in approximately the same area in each plot. As spring clipping treatments in 2017 occurred before biomass sampling, biomass clipped during treatment was accounted for at the time of spring treatment. The values were standardized to g/m^2 for analysis. Vegetation was clipped at ground level and separated into grasses, shrubs and forbs. Surface litter was collected by hand in the same area after clipping. All samples were dried at 60°C for at least 48 hours, then weighed.

Using the vegetation biomass samples, annual net primary production (ANPP) and standing litter mass were estimated in 2017 and 2018 using an estimate of the current and previous year's growth. Subsamples from each plot were sorted by hand into production from the

current year and previous year and were weighed separately. These proportions were applied to the total live/dead herbage biomass to estimate ANPP and standing litter mass (Willms et al. 2002). A subsample of approximately 50% of the total 2017 live/dead herbage biomass was sorted, and a subsampled area (0.2 x 0.5 m) of the 2018 biomass sample was sorted.

The following parameters are reported for vegetation production analysis (g/m^2): live/dead herbage mass, which was the accumulation of herbage mass, including both live and dead mass, plus the mass clipped in the spring (in 2017, as no treatment in 2018). Forb mass was the forbs clipped within each plot. Surface litter mass was the surface litter collected by hand within each plot. Standing litter mas was the herbage mass plus the mass clipped in the spring (in 2017, as no treatment in 2018), with the calculated proportion of dead material applied to the total value. ANPP was the herbage mass plus the mass clipped in the spring (only in 2017, as no treatment in 2018), with the calculated proportion of live material applied to the total value.

Percent cover for each species was visually estimated in the same 0.5 x 0.5 m area in each plot in July of 2017 and 2018; this included percent bare ground and ground cover, where ground cover included the areal cover of little club moss (*Selaginella densa* (Rydb.)), lichen and any gopher disturbance found. The data were used to compute species richness (S), Pielou's species evenness (J), and species diversity (Shannon-Wiener Index, H' and Simpson's Diversity index, D).

Dominant grasses tend to make up the majority of biomass in these systems and changes in their abundance can affect overall production and have effects on subordinate species so we separately examined the response of the three most abundant species at each site. These species were selected based on their cover in control plots in 2018. At Onefour, the three most abundant grasses were: *Hesperostipa comata* (Trin. & Rupr.) Barkworth (48%), *Elymus lanceolatus*

(Scribn. & J.G. Sm.) Gould (8%) and *Koeleria macrantha* (Ledeb.) Schult. (4%). At Lethbridge the dominant grasses were: *Pascopyrum smithii* (Rydb.) Á. Löve (66%), *Nasella viridula* (Trin.) Barkworth (1%) and *Agropyron cristatum* (L.) Gaertn. (1%). At Stavely, the dominant grasses were: *Danthonia parryi* Scribn. (34%), *Poa pratensis* L. (28%) and *Festuca campestris* Rydb. (17%).

3.2.4 Soil Sample Collection and Processing

Soil samples were collected in July of 2017 and 2018 for chemical analyses, to be used in this study for NMDS ordination analyses. Soil cores were taken from each plot at a depth of 0-15 cm. In 2017, a 2 cm diameter JMC Backsaver soil core was used (JMC Soil Samplers, Newton, IA, USA), and in 2018 a 2.5 cm diameter JMC Backsaver soil core (JMC Soil Samplers, Newton, IA, USA) was used. Differences in soil core size between years was due to a need for larger samples in 2018. The combined soil samples for each plot were homogenized and bagged separately for analyses.

3.2.5 Chemical Analyses

Soil from each plot was air dried, then coarsely ground to pass through a 2 mm sieve. Chemical data collected for each plot included soil pH, moisture content, non-purgeable (water extractable) C (NPOC), water extractable total N (WETN) and available P (AP).

Soil pH was determined by mixing a 2:1 liquid-to-solid ratio of air-dried soil and deionized water, which was then measured using a pH meter after settling (Orion Star A215, Thermo Fisher Scientific, Waltham, MA, USA). An automatic pH meter was used for 2018 soil samples for efficiency, which also used a 2:1 ratio of deionized water and air-dried soil. pH was measured by the automatic pH meter using an automated titration analysis system (MT-100, Mantech, Guelph, Ontario, Canada), equipped with a pH electrode. Operation and automation of

the system was controlled using PC-Titrate software (version 3). To ensure consistency between pH measurement devices, calibration and standard soil samples were used.

Gravimetric soil moisture content was evaluated by determining weight loss after drying field-moist soils at 105ºC for a minimum of 24 hours.

A method modified from Chantigny et al. (1999) was used to measure soil non-purgeable (water extractable) C and water extractable total N. A 2:1 liquid-to-solid ratio of air-dried soil and ultra-pure water was shaken for 30 minutes, after which the solution was syringe-filtered (0.45µm). The levels of NPOC and WETN in the filtrate were measured with a TOC-VCSH equipped with a TMN-1 (Shimadzu Corp. Kyoto, Japan).

A modified Olsen extractable-P method (Olsen et al. 1954) was used to determine available P in soil. A 1:10 soil/solution ratio was used to extract soil with 0.5 mol L^{-1} $NaHCO_3$, pH 8.5, after which P was measured using an Auto-Analyzer III (Bran and Leubbe, Germany).

3.2.6 Statistical Analysis

Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria). All data including diversity indices, vegetation production and site-specific dominant species cover were checked for normality and, if necessary, log transformed before analysis in order to improve normality; however, results and figures are presented as original non-transformed data to facilitate interpretation. Data that was log-transformed for analyses included: all vegetation production indices, *Koeleria macrantha* (2017), *Hesperostipa comata* (2017), *Elymus lanceolatus* (2018), *Nassella viridula* (2017 and 2018), *Agropyron cristatum* (2017 and 2018), *Festuca campestris* (2017 and 2018), *Poa pratensis* (2018) and *Danthonia parryi* (2018). All response variables were subsequently analysed using linear mixed models through use of the lmer function in the lme4 R-package (Bates et al. 2015) with analysis of

variance (ANOVA). Fixed effects included site, litter treatment and seasonal clipping/ compaction treatment, as well as all interactions. Years were analysed separately due to the legacy effect of the 2017 vegetation growth, different weather conditions between years, and differences in treatments and sampling. Block was included as a random factor. Significance of effects was assessed at $P \leq 0.05$. Additionally, post-hoc mean comparisons were conducted using Tukey's test ($\alpha = 5$).

Vegetation community composition, using species cover data, was ordinated using non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance metric. NMDS enables exploration of relationships between species composition and environmental factors, including treatments, soil chemical characteristics, vegetation biomass characteristics, and the bare ground accounted for in each plot. All environmental factors were fitted in the ordination plot as vectors using the envfit function in the R-package vegan (Oksanen et al. 2013). Permutational multivariate analysis of variance (perMANOVA) was used to understand relationships between treatments within the Bray-Curtis distance matrixes, by using the adonis function in the R-package vegan (Oksanen et al. 2013). Additionally, dominant grass species from each site were ordinated and overlayed onto NMDS ordinations from each site and year, to display treatment influence on community species composition of common species at sites.

3.3 Results

3.3.1 Biomass Responses

Live/dead herbage mass, forb mass, surface litter mass, standing litter mass and ANPP all differed between sites in 2017 and 2018 **(**Table 3.1). The fall clip/fall compact treatment had less live/dead herbage mass than the control treatment in 2017 (Figure 3.3A, $p = 0.005$, $F_{6, 117} = 3.28$).

In 2018, the fall clip/fall compact and spring clip/spring compact treatment also had less live/dead herbage mass than the control treatment (Figure 3.3B, $p = <0.001$, $F_{6, 117} = 4.01$).

An interaction between site, litter manipulation and clipping/compaction treatments was seen in the forb mass data in 2017 (Figure 3.5, $p = 0.04$, $F_{12,117} = 1.93$). Expected site differences were seen, with Onefour and Lethbridge having lower values overall than at Stavely, however higher values at Lethbridge were seen within the litter removed plots in the control and fall compaction treatments, while the highest values at Stavely were seen in the fall clipping treatments (Figure 3.5). Litter removal increased forb mass at Lethbridge in 2018 (Figure 3.2C, $p = 0.02$, $F_{2,117} = 4.15$).

As expected, the litter removal treatment reduced surface litter mass in 2017 and 2018 (2017: $p = <0.001$, $F_{1, 117} = 167.77$, 2018: $p = <0.001$, $F_{1, 117} = 75.72$). Interaction between site and litter manipulation treatment was found in surface litter mass in 2017 (Figure 3.2A**,** $p = < 0.001$, $F_{2,117} = 24.03$). Similar accumulation of surface litter mass was found in the Lethbridge litter retained plots and the Stavely litter removed plots, while the litter retained treatments at Stavely had the highest yield among the treatments and sites (Figure 3.2A). Fall clip/fall compact plots had lower surface litter yield than the spring compact and fall compact treatments in 2018 (Figure 3.3F, $p = < 0.001$, $F_{6,117} = 6.71$). An interaction was seen between site and clipping/compaction treatments in surface litter mass in 2017, where treatments were mainly separated by their site differences (Figure 3.4, Table L1, $p = 0.045$, $F_{12, 117} = 1.87$).

In 2017, standing litter mass was lower in the fall clipping treatments than all other treatments (2017: $p = < 0.001$, $F_{6, 117} = 11.09$) (Figure 3.3D). In 2018, the standing litter mass within the control and spring compaction treatments were higher than other treatments (2018: $p = < 0.001$, $F_{6, 117} = 29.33$) (Figure 3.3E). Standing litter mass was lower in the litter removal

treatment (88.41 g/m^2 ± 10.05) than the litter retained treatment (101.02 g/m^2 ± 10.85) in 2017 ($p = 0.03$, $F_{1,117} = 4.85$).

ANPP was lower in the spring clipping treatments in comparison to the control and fall clip treatments in 2017 ($p = < 0.001$, $F_{6, 117} = 4.46$) (Figure 3.3C). Litter retained plots had a higher ANPP yield than that of the litter removed treatments at Onefour in 2017 (Fig. 3.2B, $p=0.04$, $F_{2,117} = 3.29$).

3.3.2 Community Diversity Responses

Richness was lowest in Lethbridge in comparison to Onefour and Stavely in both years (Table 3.2). In 2017 richness at Stavely was higher than at Onefour and Lethbridge ($p = < 0.001$, $F_{2, 9} = 49.07$); in 2018 richness did not differ at Onefour and Stavely ($p = < 0.001$, $F_{2, 9} = 26.80$) (Table 3.2). Higher richness values were found in fall compaction treatments in contrast to spring clipping treatments (Figure 3.6, $p = 0.003$, $F_{6,117} = 3.46$) (Table 3.2).

Pielou's species evenness index was lower at Lethbridge than Onefour and Stavely in both years (2017: $p = < 0.001$, $F_{2, 9} = 21.04$, 2018: $p = 0.01$, $F_{2, 9} = 8.14$) (Table 3.2).

In both years, Shannon's diversity was lower in Lethbridge in comparison to Onefour and Stavely (Table 3.2). In 2017 Shannon's diversity at Stavely was higher than at Onefour and Lethbridge ($p = < 0.001$, $F_{2, 9} = 145.62$); in 2018 Shannon's diversity did not differ at Onefour and Stavely ($p = < 0.001$, $F_{2, 9} = 37.24$) (Table 3.2).

Simpson's diversity was lower in Lethbridge in comparison to Onefour and Stavely in both years (2017: $p = < 0.001$, $F_{2, 9} = 61.16$, 2018: $p = < 0.001$, $F_{2, 9} = 22.99$) (Table 3.2).

3.3.3 Dominant Species Areal Cover Responses

Table 3.3 displays mixed model ANOVA results for percent cover of dominant species assessed in this study.

Elymus lanceolatus showed lower cover in litter removed treatments (7 % ± 1) than litter retained treatments (9 % ± 1) at Onefour in 2017 ($p = 0.037$, $F_{1, 39} = 4.64$). Higher percent cover of *Hesperostipa comata* was found at Onefour in 2017 in control and spring compaction treatments, in comparison to spring clip treatments ($p = 0.002$, $F_{6,39} = 4.32$) (Figure 3.7A). *Koeleria macrantha* showed increase in percent cover in litter removed plots at Onefour in 2018 (litter retained: 5 % ± 1, litter removed: 8 % ± 1 $p=0.027$, $F_{1, 39} = 5.26$).

In Lethbridge, *Pascopyrum smithii* showed higher percent cover in in spring compaction treatments in comparison to spring clip/spring compact, spring clip and fall clip/fall compact treatments in 2017 (Figure 3.7B, $p=< 0.001$, $F_{6,39} = 5.96$). *Nasella viridula* in Lethbridge in 2018 showed high cover in spring compaction treatments in comparison to spring clip treatments (Figure 3.7C, $p = 0.023$, $F_{6,39} = 2.81$). Treatment effect was not seen on the cover of *Agropyron cristatum* in either year.

Danthonia parryi increased in cover in litter removed plots at Stavely in 2017 (litter retained: 28 % ± 4, litter removed: 39 % ± 4, $p = 0.021$, $F_{1,39} = 5.75$) and 2018 (litter retained: 20 % ± 3, litter removed: 40 % ± 5, $p = 0.003$, $F_{1, 39} = 10.06$). The opposite effect was seen in *Poa pratensis* at Stavely, which decreased in cover in litter removed plots in 2017 (litter retained: 35 % ± 4 litter removed: 24 % ± 3, $p = 0.01$, $F_{1, 39} = 7.39$). *Poa pratensis* displayed higher cover in fall compaction treatments in comparison to spring clip and spring clip/spring compaction treatments in 2017 ($p = 0.015$, $F_{6,39} = 3.08$) (Figure 3.8A). *Danthonia parryi* in Stavely displayed response to the clipping/compaction treatment in 2017 ($p = 0.031$, $F_{6,39} = 2.62$) (Figure 3.8B). No treatment effect was seen on the cover of *Festuca campestris* in either year.

3.3.4 NMDS: Environmental Effects on Species Community

At Onefour in 2017, associations were detected between bare ground, surface litter mass, soil NPOC and the vegetation community (Table I1). Positive visual associations were detected between the cover of *K. macrantha* and surface litter mass at the site (Figure 3.9A**)**. At Onefour in 2018, associations were detected between bare ground, forb mass and the vegetation community (Table I1).

At Lethbridge in 2017, associations were detected between soil pH, soil NPOC, soil WETN, soil AP and the vegetation community (Table I1). Positive visual associations were detected between soil NPOC, soil WETN and the cover of *A. cristatum* (Figure 3.9C). At Lethbridge in 2018, relationships were detected between bare ground, live/dead herbage mass, forb mass, surface litter mass, standing litter mass, ANPP and the vegetation community (Table I1).The effect of the litter manipulation treatments was present on the vegetation community (Figure 3.9D, Table J1); litter removed treated plots can be seen grouping together. Positive visual associations were detected between *A. cristatum*, *P. smithii*, *N. viridula* and surface litter mass and standing litter mass (Figure 3.9D). The association of surface litter mass was opposite to bareground and forb mass at Lethbridge in 2018 (Figure 3.9D).

At Stavely in 2017, effect of the litter manipulation treatment was present on the vegetation community (Figure 3.9E, Table J1); litter retained treated plots can be seen grouping together (Figure 3.9E). No association was detected between the vegetation community, environmental variables, and vegetation production characteristics. At Stavely in 2018, association was detected between soil WETN and the vegetation community (Table I1). Weak positive visual association was detected between *P. pratensis, F. campestris* and soil WETN (Figure 3.9F).

3.4 Discussion

Vegetation production generally decreased in response to clipping treatments in contrast to compaction. Our study demonstrated higher vegetation and litter accumulation in plots treated solely with compaction, in comparison to those that were clipped. These results indicate that the disturbance generated through defoliation of vegetation was greater than that of compaction in our years of study. Other studies examining compaction effects have found effect of trampling on vegetation communities; for example, a study in a tropical rangeland in Kenya found reductions in plant cover and biomass in response to trampling (Dunne et al. 2011). Compaction treatments were intended to simulate trampling by livestock, a disturbance where vegetation is physically degraded and soil is compacted. Trampling can lead to a variety of responses by the ecosystem, depending on the severity of the compaction, growing conditions and evolutionary history (Milchunas et al. 1988, Mulholland and Fullen 1991). When examining vegetation response to grazing systems, a study in Albertan Boreal ecosystems contrasting high intensity grazing systems, which have been speculated to increase pressure on the landscape through increased trampling by livestock, with low intensity grazing systems, found decreases in vegetation cover in high intensity grazing (Donkor et al. 2003). Our findings indicate that clipping treatments created a more intense disturbance on the vegetation than compaction treatments, as was seen through decreased growth and litter accumulation in plots treated with clipping in comparison to compaction. The sensitivity of the vegetation communities to defoliation in comparison to compaction indicates the dependence of the vegetation communities on existing growth in dry growing conditions.

Richness was found to decrease in response to clipping treatments in comparison to compaction; this was the only species diversity variable affected by these treatments. A study

encompassing data from many temperate grassland sites in south Alberta determined a moderate increase in species richness in response to grazing, which was attributed to adaptation of the area to grazing disturbances (Lyseng et al. 2018); this idea references the theory of adaptation to an evolutionary history of grazing by Milchunas et al. (1988). A similar response may have occurred in our study; richness increased in response to a moderate grazing disturbance such as compaction. As conditions were dry throughout this study, hydrolic properties of the ecosystem would have been affected, thus affecting the response of the vegetation community to the clipping treatment in comparision to compaction, as defoliation was generally found to decrease production in our study. From this effect, we can determine that growing conditions likely decreased for sensitive species in clipping treatments, thus decreasing richness. Sensitive grassland species have a lower tolerance to defoliation, and studies have shown that heavy grazing can alter species composition in favour of more tolerant species, which are generally less productive than less tolerant species (Broadbent et al. 2016, Willms et al. 1985, Willms and Jefferson 1993). One study, based in a forested ecosystem in Japan, reported an increase in diversity in response to defoliation in contrast to compaction, though the difference between results is likely due to the increased intensity in the trampling treatments applied by Kobayashi et al. (1997). The reduction of species richness in response to clipping, as seen in our study, demonstrates the varying tolerance of species to defoliation (Ferraro and Oesterheld 2002, McNaughton 1983); this indicates the importance of moderate grazing to preserve diversity in a grassland community (Lyseng et al. 2018, Willms et al. 1985).

The change in conditions at each site due to treatment altered the plant community at each site, depending on the specific growth conditions and grazing tolerance of each species. Species examined are known to increase or decrease in abundance in response to grazing

(referred to here as “increaser” or “decreaser” species, respectively); a regional guidebook by Tannas (2003) provided response classifications for species. An understanding of species response to grazing has been determined throughout decades of study, thus changing dynamics within a vegetation community can be predicted (Vesk and Westoby 2001, Weaver and Hansen 1941). Most dominant grass species examined at each site showed response to treatment.

Higher cover of increaser species, *K. macrantha* and *D. parryi*, were observed at Onefour and Stavely, respectively, in plots treated with litter removal. These responses may indicate some of the adaptive advantages of increaser species, such as capitalizing on dry, exposed soil for establishment. Litter removal is known to decrease soil moisture in grassland communities, to which the arid prairie regions of Alberta are particularly susceptible (Deutsch et al. 2010b, Willms and Jefferson 1993). One exception to this observation is the response of *P. pratensis*, an increaser at Stavely; the decreased cover of *P. pratensis* in litter removed plots is likely due to the preference of this species for moist conditions, where it is a highly aggressive invasive species (Tannas 2003, Willms and Quinton 1995). NMDS ordinations on the structure of the observed vegetation community found pH, surface litter mass, and bare ground as common influencing factors on the observed activity of the above-mentioned species. This may indicate the sensitivity of species with different moisture preferences towards soil pH or soil exposure.

Clipping reduced the cover of three decreaser species (*H. comata* at Onefour, *N. viridula* and *P. smithii* at Lethbridge), while they were not reduced by compaction. A higher tolerance to compaction in comparison to clipping likely indicates that these species are more sensitive to defoliation than trampling within a disturbance such as grazing. This response is probable because individuals are intact after a compaction treatment, whereas they most likely have biomass removed after being clipped, reducing opportunity for photosynthesis and further

growth. Additionally, due to the dry conditions at both sites during treatment, compaction treatments likely did not cause decreased soil water infiltration, lessening the intensity of the effect. Interaction of *P. smithii* with vegetation production factors in the Lethbridge NMDS ordinations may indicate the dependence of this species on vegetation and ground cover to maintenance of soil moisture and nutrient conditions. Additionally, *P. pratensis*, an increaser with preference for moist conditions, also showed greater cover under compaction treatment.

Limited response of species at sites likely indicates different effects at each. Limited response from *A. cristatum* in Lethbridge likely indicates its adaptation to harsh conditions in its role as an invasive species in Alberta prairies (Tannas 2003). The absence of response to treatment from *F. campestris* may indicate that disturbance from treatment was not severe or conducted at a long enough duration at Stavely, as *F. campestris* is a decreaser species that is sensitive to grazing but recovers from defoliation with increased aboveground biomass growth (McInley et al. 2010, Tannas 2003).

Vegetation production was marginally affected by litter removal, particularly in forb production at Lethbridge, which showed increase in litter removal treatments, as well as in ANPP at Onefour, which saw decrease in litter removal treatments. The expected reduction of both surface and standing litter was found in litter removed treatments, and litter retention increased the amount of litter accumulation on plots. The limited effect of litter retention found in this study is in contrast to the literature, as litter retention is known to be generally positive on above ground biomass in grasslands (Xiong and Nilsson 1999). A study conducted in the Mixedgrass Prairie natural subregion by Willms et al. (1993) reported increases in herbage production in response to litter retention, and decreases in production in response to litter removal, though the effect of litter is thought to be most influential in times of water scarcity, as

litter contributes to moisture conservation (Deutsch 2010a, Deutsch 2010b). A previous study conducted in the Foothills Fescue natural subregion and in the Dry Mixedgrass Prairie natural subregion reported a decrease in production in response to litter removal in the Dry Mixedgrass Prairie, while production in the Foothills Fescue marginally increased due to litter removal (Willms et al. 1986). Litter removal has also been shown to decrease grassland productivity in other parts of the world, such as in Mongolian grasslands (Wang et al. 2011). Due to the dry conditions in sampling years, litter removal was expected to decrease ANPP, while litter retention was expected to aid in increases in vegetation mass, as litter aids in soil moisture conservation and soil temperature moderation (Deutsch 2010a, Deutsch 2010b, Facelli and Pickett 1991). The observed increase of forb mass in response to litter removal possibly influenced the affect of litter removal on ANPP; this effect is likely due to the drier conditions within the litter removal plots, where drought tolerant species could establish in the harsh conditions of the treatment. To determine response of herbage production to litter removal, a longer length of study and thorough examination of plant growth form dynamics are recommended, as effects from the litter manipulation treatment were not seen in our study.

The litter manipulation treatment had no effect on the diversity indices measured in this study. This result was unexpected, as litter is known to be an important component of moisture retention for soil and vegetation at xeric sites, though removal of litter is known to increase herbage yield in mesic grasslands (Deutsch et al. 2010a, Deutsch et al. 2010b, Willms et al. 1986, Willms et al. 1993). The influence of litter on grasslands in contrasting climates has been widely studied. The importance of litter for moisture was observed by Bansal et al. (2014), who determined that high-intensity litter removal (>75 %) was found to decrease plant cover in mixed grassland communities in Oregon during hot, dry years. Litter accumulation is known to limit the

resources, mainly sunlight, available for plants, thus decreasing species diversity in productive grasslands (Xiong and Nilsson 1999). As effects from the litter manipulation treatment on species diversity were not seen in our study, it is likely that the vegetation community composition would have to be observed for a longer length of time to determine response.

A decrease in vegetation production was found in response to the fall clipping treatment in both years of sampling. This effect was seen in both vegetation and litter variables, particularly at more xeric sites. Lower production in fall clipping treatments was unpredicted but not unexpected, due to the timing of both treatment and sampling within the growing season. Late-season grazing on native grasslands at appropriate stocking rates is a recommended practice for Canadian grasslands as vegetation is dormant in these seasons, thus resources are not removed for future growth (Bailey et al. 2012). Additionally, early-season grazing can increase soil compaction and thus lower water infiltration, due to the higher moisture content of soil in the spring, which can decrease growing conditions (Evans et al. 2012, Greenwood and McKenzie 2001). Late season defoliation, particularly when combined with compaction, may have lowered vegetation production due to the shorter recovery time in comparison to plots clipped in the spring, which had greater time to recover within the growing season. Fall defoliation also removed senesced plant material, lowering the amount of standing dead mass found in those plots. Additionally, xeric sites consistently showed decreased production in response to fall defoliation; this is likely due to differences in water availability within the growing season. Increased herbage yield was found in early-season defoliation treatments in an Alberta boreal aspen forest, which was attributed to early season growth in a study by Donkor et al. (2003). Prevalent moisture in the spring at more xeric sites may allow for greater recovery throughout the growing season (Broadbent et al. 2016, Willms and Jefferson 1993). Recovery time

differences between treatments is likely the cause of lower productivity seen within fall clipping treatments, where moisture-deprived sites were affected more heavily than mesic sites.

Due to the differences in growth patterns between species, seasonal disturbances may have varying effect on species (Briske and Richards 1993, Ferraro and Oesterheld 2002). Our study showed little effect of seasonal treatments within dominant species at sites, which may also be due to species presence within certain treatments.

3.5 Conclusion

This study revealed the localized effects of grazing mechanisms on grassland production and diversity. Results from our study show that defoliation was more influential on production and species diversity than trampling, particularly when defoliation occurred in the fall. A limited effect of litter presence on vegetation production and vegetation diversity was observed in this study. Species more tolerant to grazing were observed to have a higher sensitivity to litter manipulation, while species that were less tolerant to grazing were found to have a higher sensitivity to clipping. These findings have important implications for understanding the impacts of grazing on grasslands; the immediate effect of litter manipulation on vegetation species response illustrates moisture and exposure effects. Further study may examine these mechanisms long-term, to aid in further understanding of grazing mechanism influences on vegetation in grasslands.

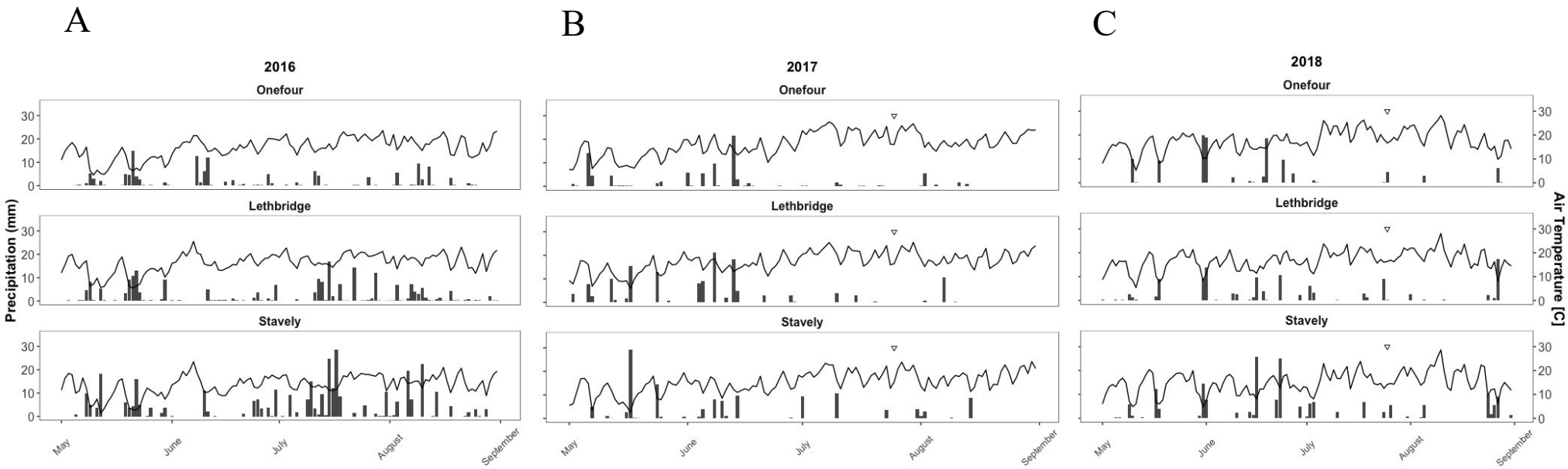

Figure 3.1: (A-C): Precipitation and temperature record for all sites; precipitation events are displayed as bars, daily temperature as a line (Alberta Climate Information Service). Approximate biomass sampling events are indicated with a downward triangle. A precipitation event of 45.6 mm that occurred at Stavely on June 13, 2017 was removed from this plot to optimize axes.

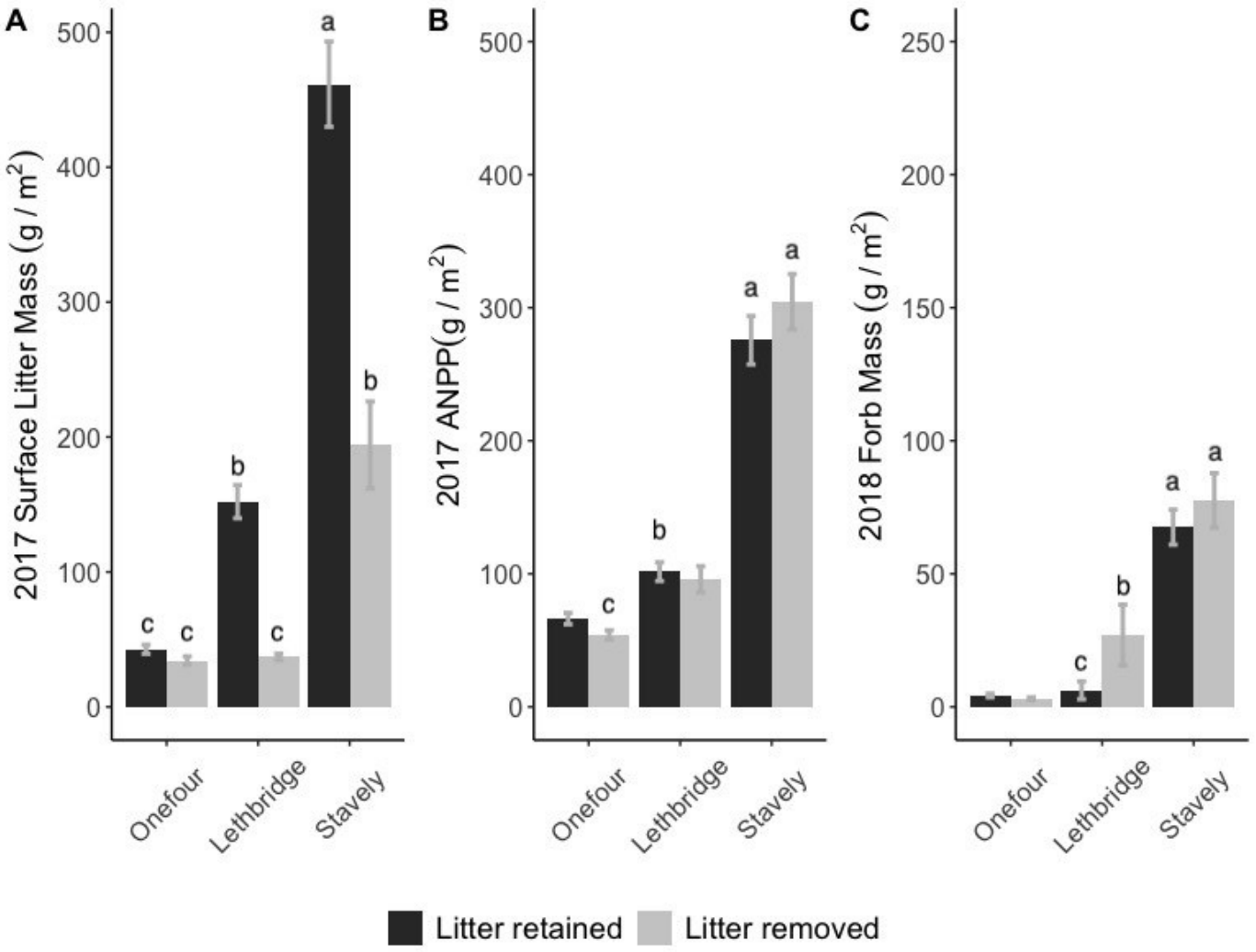

Figure 3.2 (A-C): Mean surface litter mass (A), mean ANPP (B) and mean forb mass (C) for each site in 2017 within each litter manipulation treatment. Groups that did not share a letter are significantly different at $p \leq 0.05$.

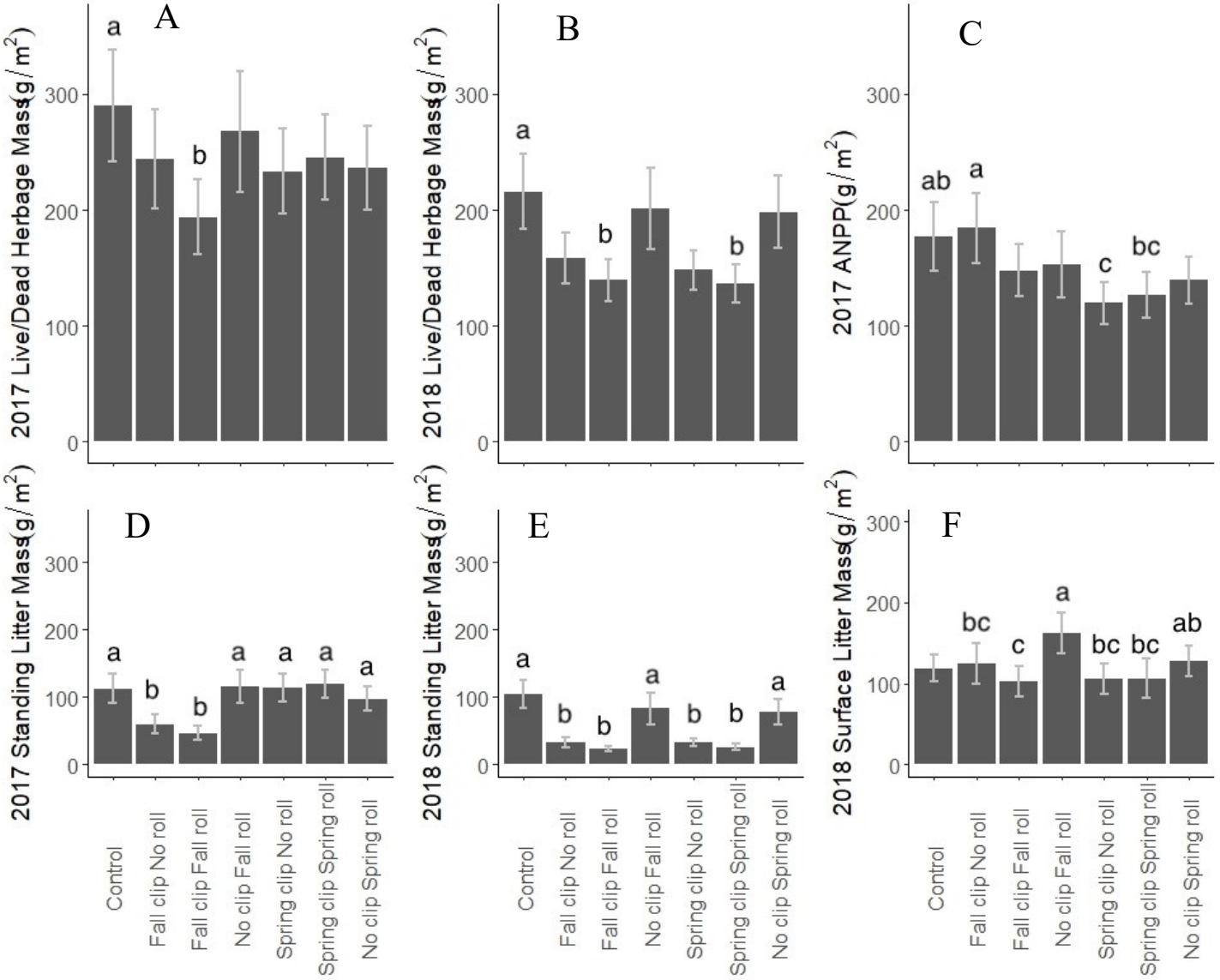

Figure 3.3 (A-F): Mean vegetation production for all sites in both years of study within each clipping/compaction treatment. Variables represented by each graph area as follows: 2017 and 2018 live/dead herbage mass (A and B, respectively), 2017 ANPP (C), 2017 and 2018 standing litter mass (D and E respectively), 2018 surface litter mass (F). Groups that did not share a letter are significantly different at $p \leq 0.05$.

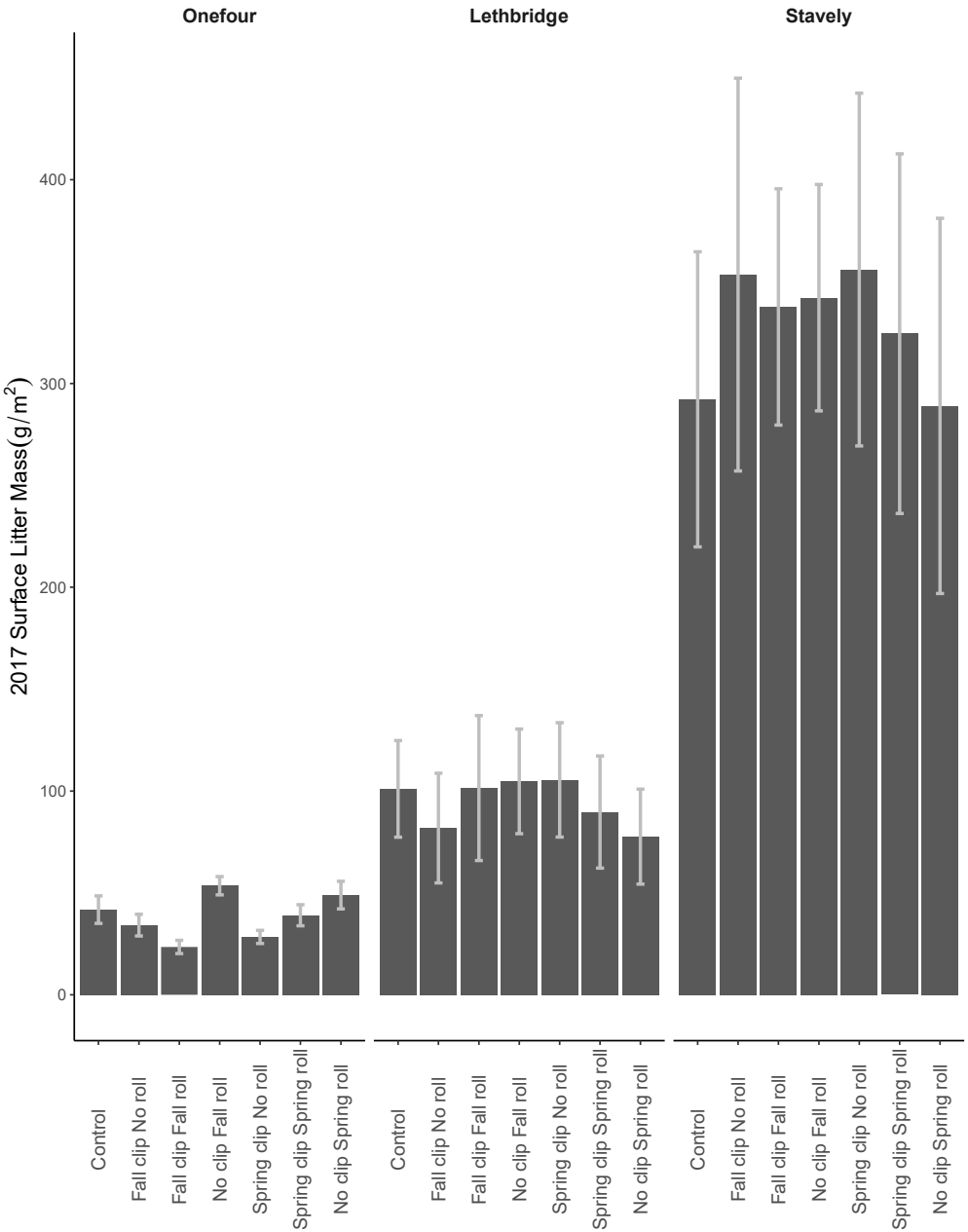

Figure 3.4: Mean 2017 surface litter mass for each site within each clipping/compaction treatment. Treatment contrasts (letters) were too complex to include, see Table K1 for contrast letters.

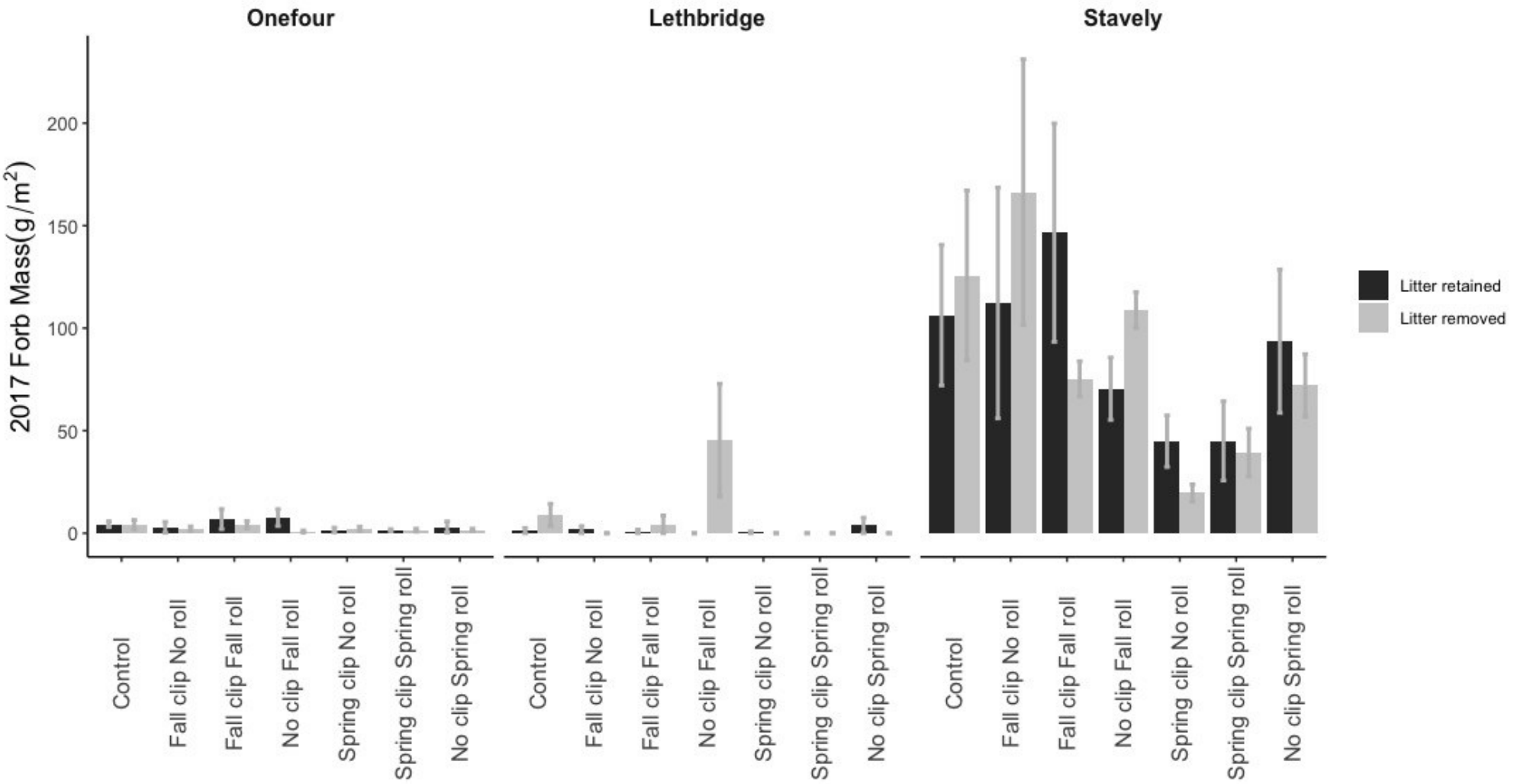

Figure 3.5: Mean 2017 forb mass for each site within each litter manipulation and clipping/compaction treatment. Treatment contrasts (letters) were too complex to include, see Table L1 for contrast letters.

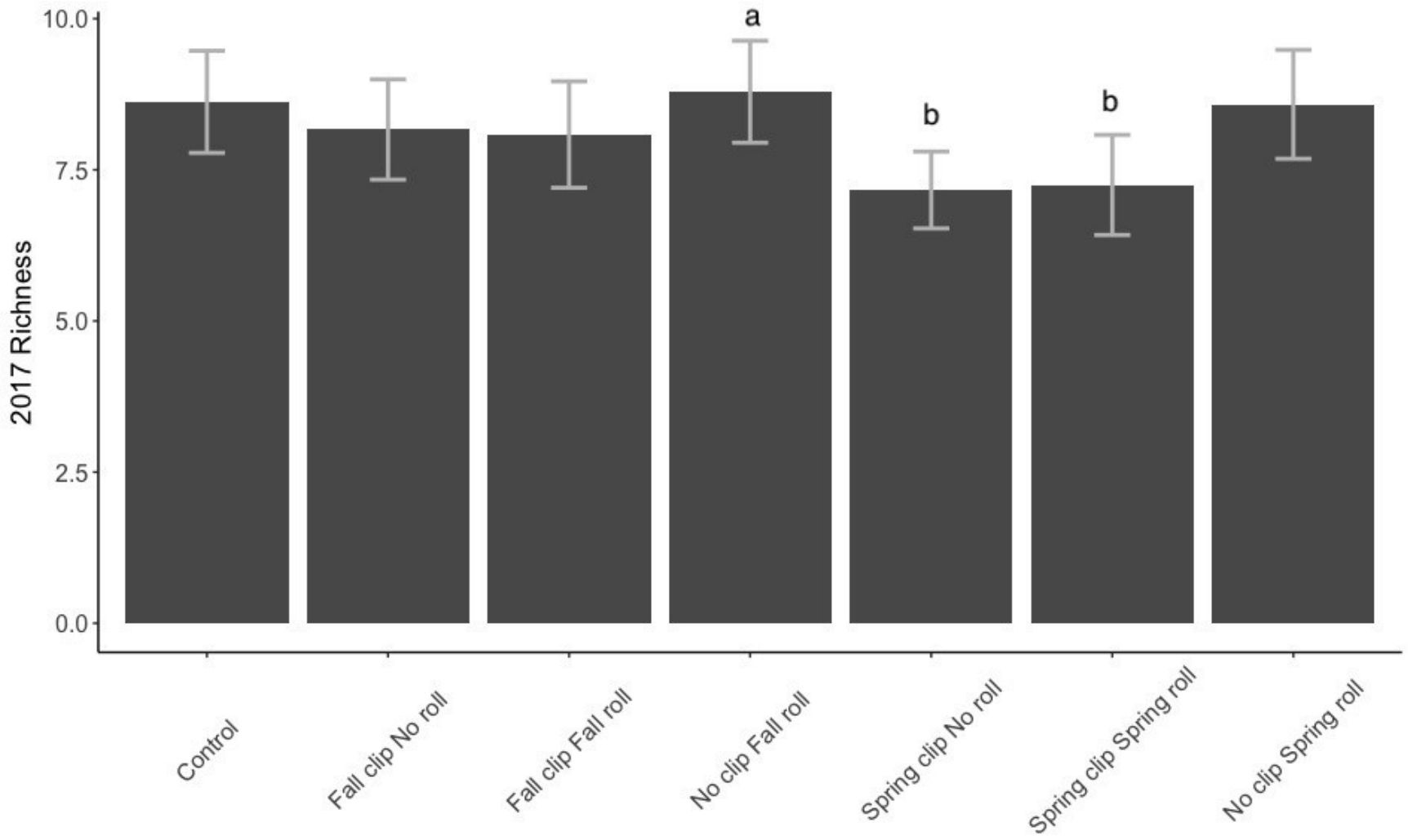

Figure 3.6: Species richness at all sites in 2017 within each clipping/compaction treatment. Groups that do not share a letter are significantly different at $p \leq 0.05$.

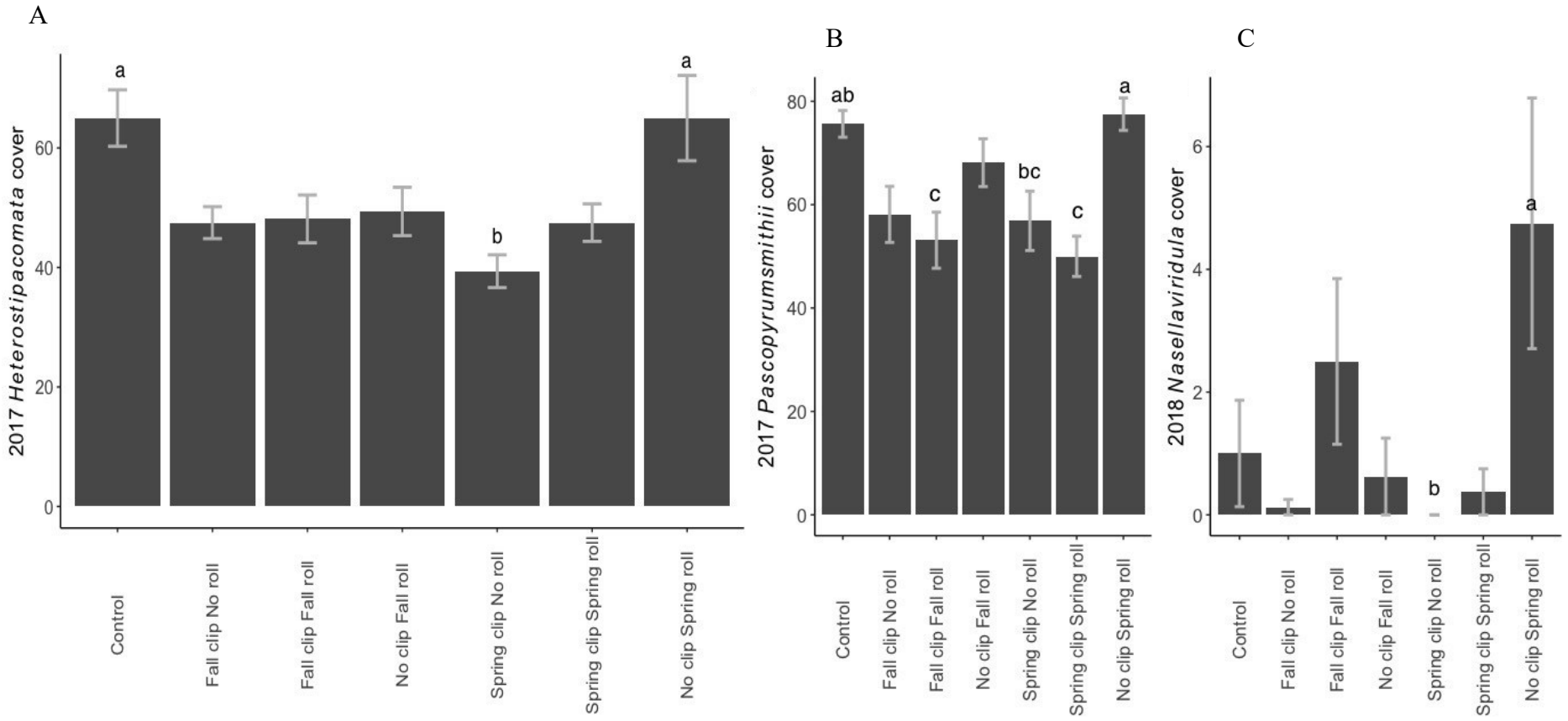

Figure 3.7 (A-C): Species cover response from Onefour (A) and Lethbridge (B and C). Plot (A) represents 2017 H. comata cover, (B) represents 2017 P. smithii cover and (C) represents 2018 N. viridula cover. Groups that did not share a letter are significantly different at $p \leq 0.05$.

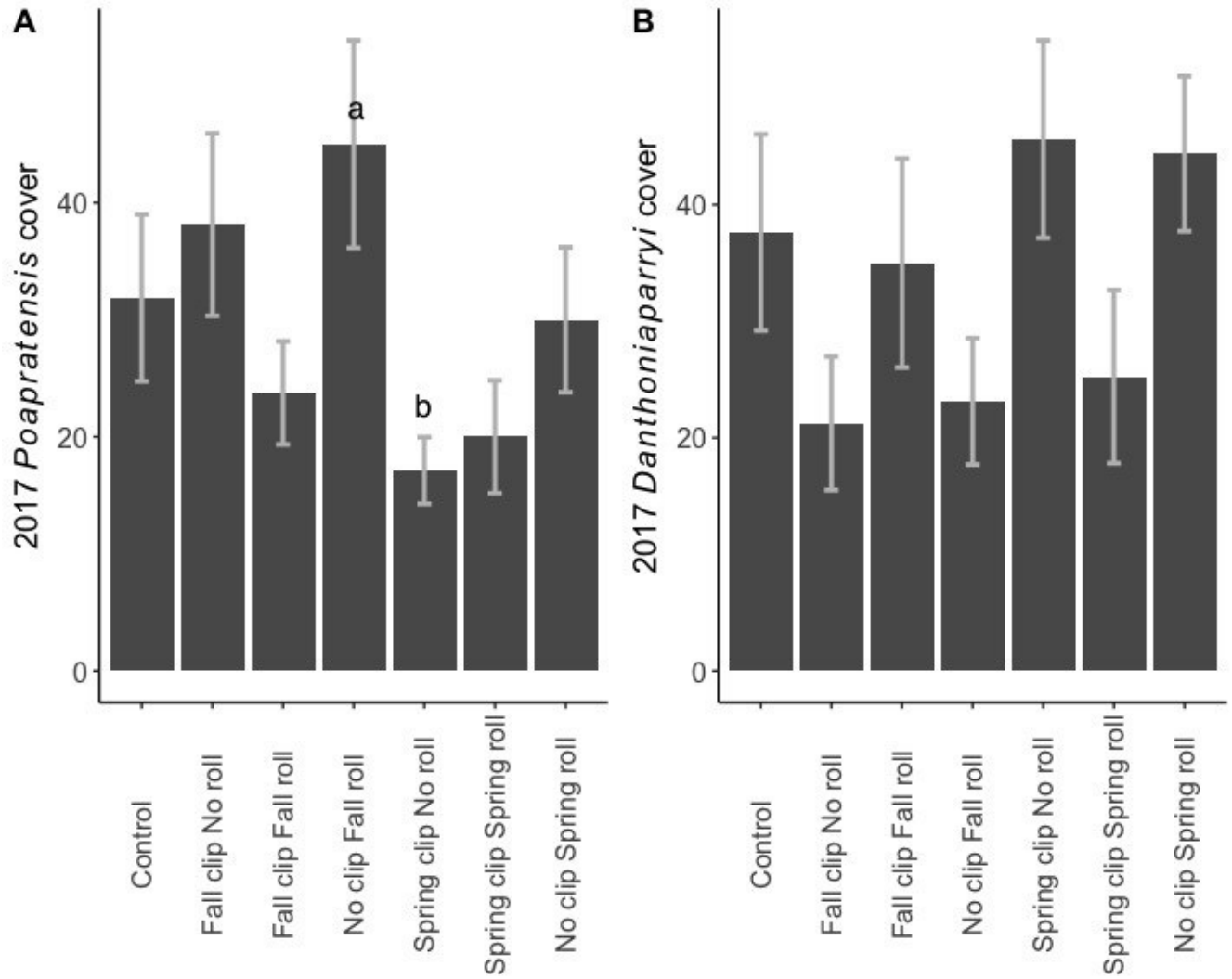

Figure 3.8 (A-B): Species cover response from Stavely. Plot (A) represents 2017 P. pratensis cover, (B) represents 2017 D. parryi cover. Groups that did not share a letter are significantly different at $p \leq 0.05$.

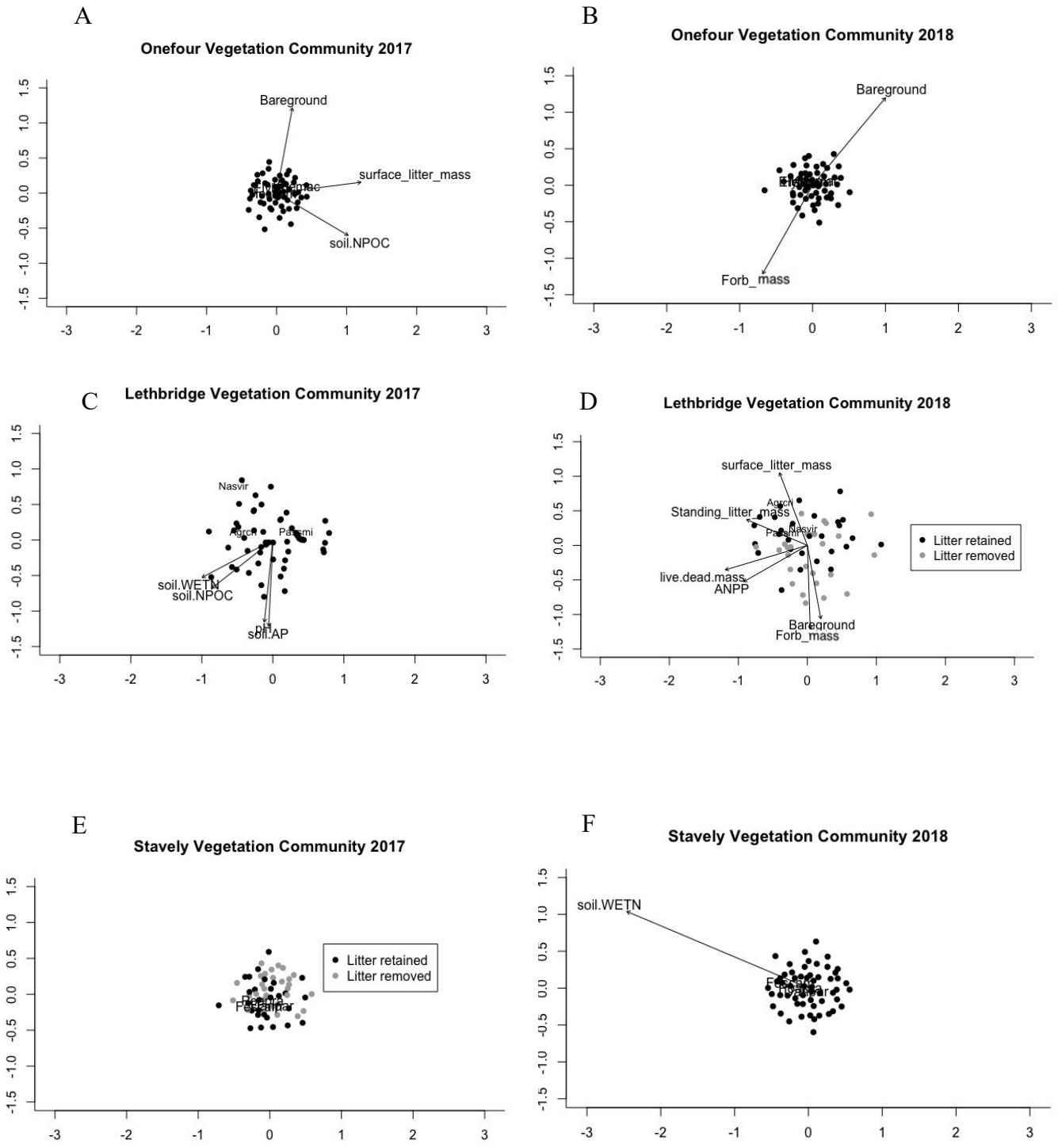

Figure 3.9 (A-F): Non-metric multidimensional scaling (NMDS) ordination of the vegetation community at each site and year in relation to soil and vegetation production characteristics. Significant environmental variables (soil AP – soil Available Phosphorous, soil.NPOC – soil Non-Purgeable Organic Carbon, soil.WETN – soil Water Extractable Total Nitrogen, litter.MC – Litter Moisture Content) and vegetation production variables are shown as vectors, and significant treatment factors (litter manipulation or clip/compaction) are shown on legends.

Table 3.1: Mixed model ANOVA results of vegetative productivity in 2017 and 2018 among study sites.

2017 Results												
			Live/Dead Herbage Mass (g/m^2)		Forb Mass (g/m^2)		Surface litter mass (g/m^2)		Standing litter mass (g/m^2)		ANPP (g/m^2)	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Site	2	9	62.33	**< 0.001**	77.56	**< 0.001**	40.46	**< 0.001**	52.94	**< 0.001**	57.90	**< 0.001**
Litter (L)	1	117	3.60	0.06	0.30	0.59	167.77	**< 0.001**	4.85	**0.03**	1.89	0.17
Clip / Compact (CC)	6	117	2.40	**0.03**	4.63	**< 0.001**	1.75	0.12	11.09	**< 0.001**	4.46	**< 0.001**
Site:L	2	117	1.80	0.17	1.15	0.32	24.03	**< 0.001**	0.89	0.41	3.29	**0.04**
Site:CC	12	117	1.40	0.18	0.80	0.65	1.87	**0.04**	1.71	0.07	0.41	0.96
L:CC	6	117	0.41	0.87	0.81	0.57	1.33	0.25	0.51	0.80	0.30	0.93
Site:L:CC	12	117	1.51	0.13	1.93	**0.04**	1.29	0.24	0.99	0.46	1.67	0.08

Table 3.1 continued: Mixed model ANOVA results of vegetative productivity in 2017 and 2018 among study sites.

2018 Results												
			Live/Dead Herbage Mass (g/m^2)		Forb Mass (g/m^2)		Surface litter mass (g/m^2)		Standing litter mass (g/m2)		ANPP (g/m^2)	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Site	2	9	75.68	**< 0.001**	21.23	**< 0.001**	86.57	**< 0.001**	73.72	**< 0.001**	40.51	**< 0.001**
Litter (L)	1	117	2.76	0.10	1.18	0.28	75.72	**< 0.001**	0.45	0.51	2.10	0.15
Clip / Compact (CC)	6	117	4.01	**< 0.001**	1.73	0.12	6.71	**< 0.001**	22.57	**< 0.001**	0.16	0.99
Site:L	2	117	0.03	0.98	4.15	**0.02**	2.02	0.14	0.93	0.40	0.09	0.91
Site:CC	12	117	1.06	0.40	0.93	0.52	1.18	0.31	1.25	0.26	1.07	0.39
L:CC	6	117	0.36	0.90	0.52	0.79	1.02	0.42	0.81	0.57	0.18	0.98
Site:L:CC	12	117	0.66	0.78	0.98	0.47	1.33	0.21	1.16	0.32	0.57	0.86

Table 3.2: Mixed model ANOVA results of diversity indices in 2017 and 2018 among three sites.

		2017											
		Richness			**Evenness**			**Simpson's**			**Shannon's**		
Treatment	**df**	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
Site (S)	2	49.07	9	**< 0.001**	21.04	9	**< 0.001**	61.16	9	**< 0.001**	145.62	9	**< 0.001**
Litter (L)	1	0.20	117	0.66	0.0005	116	0.98	0.07	117	0.79	0.48	117	0.49
Clip/Compact (CC)	6	3.46	117	**< 0.001**	1.78	116	0.11	1.54	117	0.17	1.71	117	0.13
S:L	2	0.07	117	0.93	1.08	116	0.34	0.30	117	0.75	0.64	117	0.53
S:CC	12	1.43	117	0.16	1.30	116	0.23	1.10	117	0.37	1.64	117	0.09
L:CC	6	0.42	117	0.87	0.76	116	0.60	0.40	117	0.88	0.40	117	0.88
S:L:CC	12	0.45	117	0.94	0.76	116	0.69	0.55	117	0.88	0.61	117	0.83

Table 3.2 continued: Mixed model ANOVA results of diversity indices in 2017 and 2018 among three sites.

		2018											
		Richness			Evenness			Simpson's			Shannon's		
Treatment	**df**	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***	**F**	**df.res**	***P***
Site (S)	2	26.80	9	**< 0.001**	8.14	9	**0.01**	22.99	9	**< 0.001**	37.24	9	**< 0.001**
Litter (L)	1	0.78	117	0.38	0.01	117	0.94	0.06	117	0.81	1.13	117	0.29
Clip/Compact (CC)	6	1.06	117	0.39	0.97	117	0.45	1.17	117	0.33	0.80	117	0.57
S:L	2	0.27	117	0.76	1.40	117	0.25	0.41	117	0.67	0.09	117	0.92
S:CC	12	1.26	117	0.25	0.56	117	0.87	0.88	117	0.57	0.67	117	0.78
L:CC	6	0.48	117	0.82	1.46	117	0.20	1.12	117	0.36	1.07	117	0.38
S:L:CC	12	0.17	117	1.00	0.35	117	0.98	0.43	117	0.95	0.50	117	0.91

Table 3.3: Mixed model ANOVA results for dominant species at three sites in 2017 and 2018.

2017								
			Onefour					
			Elymus lanceolatus		*Hesperostipa comata*		*Koeleria macrantha*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	4.64	**0.037**	1.47	0.23	0.35	0.56
Clip/Compact (CC)	6	39	0.49	0.81	4.32	**0.002**	1.65	0.16
L:CC	6	39	1.87	0.11	0.69	0.66	0.65	0.69
			Lethbridge					
			Pascopyrum smithii		*Nassella viridula*		*Agropyron cristatum*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	0.003	0.96	1.87	0.18	0.03	0.87
Clip/Compact (CC)	6	39	5.96	**< 0.001**	1.19	0.33	0.66	0.68
L:CC	6	39	0.63	0.70	0.78	0.59	1.00	0.44
			Stavely					
			Festuca campestris		*Poa pratensis*		*Danthonia parryi*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	0.10	0.75	7.39	**0.01**	5.75	**0.021**
Clip/Compact (CC)	6	39	1.01	0.43	3.08	**0.015**	2.62	**0.031**
L:CC	6	39	1.14	0.36	1.06	0.40	2.10	0.07

Table 3.3 continued: Mixed model ANOVA results for dominant species at three sites in 2017 and 2018.

2018								
			Onefour					
			Elymus lanceolatus		*Hesperostipa comata*		*Koeleria macrantha*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	0.15	0.70	1.76	0.19	5.26	**0.027**
Clip/Compact (CC)	6	39	0.77	0.60	1.31	0.28	0.77	0.60
L:CC	6	39	1.14	0.36	1.30	0.28	1.68	0.15
			Lethbridge					
			Pascopyrum smithii		*Nassella viridula*		*Agropyron cristatum*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	1.75	0.19	0.01	0.94	3.28	0.08
Clip/Compact (CC)	6	39	2.23	0.06	2.81	**0.023**	0.42	0.86
L:CC	6	39	1.21	0.32	0.51	0.80	0.59	0.74
			Stavely					
			Festuca campestris		*Poa pratensis*		*Danthonia parryi*	
Treatment	**df**	**df.res**	**F**	***P***	**F**	***P***	**F**	***P***
Litter (L)	1	39	1.36	0.25	0.51	0.48	10.06	**0.003**
Clip/Compact (CC)	6	39	1.09	0.38	0.83	0.56	1.74	0.14
L:CC	6	39	0.55	0.76	0.55	0.77	2.15	0.07

Chapter 4. Synthesis

Grassland production and function provide immeasurable ecosystem goods and services (EG&S), but their levels can be subject to management grazing management. Cattle affect grasslands in a number of ways, such as defoliation of plants, input of nutrients through defecation and urination, as well as trampling of the soil with hooves, all of which can alter plant growth, litter accumulation and biogeochemical cycles. The mechanisms through which grazing affects grasslands have become a focus of attention, as some grazing systems, such as adaptive multipaddock grazing, have placed emphasis on their benefits (Briske et al. 2008, Savory and Butterfield 1998, Teague et al. 2013). However, it is not clear how these grazing mechanisms impact the production and function of grasslands or contribute to their success, particularly in western Canada, but understanding how different grazing systems increase grassland function could lead to improved grassland management.

The object of this study was to test the effects of simulated seasonal trampling, defoliation and litter manipulation across a moisture gradient in native grasslands, with a primary focus on soil and litter extracellular enzyme activity, as well as vegetation production and diversity.

Due to defoliation effects and alteration of soil moisture through litter manipulation during grazing, the effect of grazing mechanisms on nutrient cycling in the soil and surface litter is a key area of study when considering the actions of grazing livestock. Extracellular enzyme activity (EEA) is an important aspect of ecosystem function and nutrient cycling; enzymes are highly specific protein complexes produced by soil microbes and plants that catalyse the breakdown of organic compounds. Enzymes are highly sensitive to a number of environmental factors, including substrate availability, temperature, pH and moisture (Allison and Vitousek

2005, German et al. 2011, Gianfreda and Ruggerio 2006, Sinsabaugh 1994, Sinsabaugh and Moorhead 1994), making them a good indicator of microbial functional response to environmental change.

An important finding of this study is the increase of litter enzyme activity with higher moisture levels. Increased sensitivity of litter EEA is expected to be the cause of greater fluctuations in temperature and moisture in the litter, due to its increased exposure in comparison to soil (Ge et al. 2017, Papa et al. 2014). The differences in magnitude and response of EEA found between litter and soil emphasizes the sensitivity of microbial activity to disturbances at varying ecosystem levels. This has important implications for land managers when considering the impact that surface litter has on the nutrient cycling within their landscape. Additionally, in the increasing variation of precipitation cycles with climate change, variation of EEA may occur due to moisture changes.

This study found that litter manipulation primarily affected soil and litter EEA; this effect is likely due to moisture differences caused by litter manipulation. Litter has a large influence in moisture regulation in grassland ecosystems due to its role in decreasing soil exposure, thus decreasing evapotranspiration and temperature increase (Deutsch et al. 2010a, Deutsch et al. 2010b, Willms et al. 1993). Both soil and litter EEA have been documented in various ecosystems as sensitive to moisture (Brockett et al. 2012, Ge et al. 2017, Hewins et al. 2016a, Olivera et al. 2014). The response to litter removal was subject to variation between sites based on average precipitation, suggesting that there may be variation in the sensitives of microbial communities between sites of different moisture regimes. This finding suggests the importance of litter presence for enzyme activity in grasslands through its role in moisture regulation, though this relationship may vary between moisture regimes.

The production and structure of the vegetation community in grasslands is essential for the preservation of EG&S in grasslands, such as biodiversity. Response of the vegetation community to grazing is known to change with intensity, dependent on the climate and grazing history of the landscape. The effect of grazing on grassland ecosystems has been studied at length, however, inspection of the effects of isolated grazing mechanisms on grassland vegetation is widely unknown.

Findings from this study suggest that particular grazing mechanisms affect species depending on grazing tolerance; in particular, grazing tolerant species were affected by litter manipulation, while species with a lower tolerance to grazing were affected by defoliation treatments. In grasslands, litter has a key role in preserving soil moisture, reducing soil erosion and temperature regulation in the soil (Deutsch 2010a, Deutsch 2010b). Litter presence is a known positive influence on vegetation production in grassland ecosystems, particularly when water is scarce (Willms et al. 1993). The removal of above-ground biomass reduces the photosynthetic ability of plant individuals (Briske and Richards 1993), making some species more likely to survive than others based on morphology and reproduction strategy. These results emphasize the importance proper grazing management, chiefly management of plant litter for moisture retention and soil cover, as well as defoliation, for the establishment of a biodiverse plant community.

This study found that plant production and diversity decreased in response to seasonal defoliation. This response was greater than the response to seasonal compaction, which may have a detrimental impact on grasslands in moist conditions, as soil compaction can increase soil bulk density and decrease water infiltration, negatively impacting growth and establishment of vegetation communities (Dunne et al. 2011, Evans et al. 2012, Greenwood and McKenzie 2001,

Naeth et al. 1990a). However, due to dry conditions seen during this study, defoliation likely had a greater influence on the existing vegetation and its capacity to grow through removal of photosynthetic material (Briske and Richards 1993). Change in composition of Canadian grassland communities due to the impact of defoliation was documented by Broadbent et al. (2016), who found an increase in shorter-statured grasses, while dominant cover grasses decreased. These patterns suggest that vegetation structure and production struggle to recover from defoliation due to influences on photosynthetic potential and increase of grazing-tolerant species.

Results from our study found a greater decrease in production in response to fall defoliation in comparison to spring defoliation. This is in contrast to many studies in these subregions which recommend grazing later in the season to protect sensitive early season growth and prevent soil compaction in wet conditions (Naeth et al. 1990b, Naeth et al. 1991a, Naeth et al. 1991b). Due the relatively short Canadian growing season, native Canadian grasslands are largely comprised of cool-season grasses, which grow mainly between late spring and early summer, making native prairie highly vulnerable to overgrazing during the early growing season (Bailey et al. 2012). Decreased growth in response to fall defoliation was found in this study; this could be due to numerous factors, such as decreased recovery time in comparison to spring defoliation (when biomass is measured the following growing season) or an increased amount of biomass removed, as fall treated plots had more time for growth than spring treated plots. Decreased standing dead or surface litter mass in fall treated plots in comparison to those treated in the spring is likely because sensced growth that would have been accounted for as standing dead or surface litter would be removed in the fall treatment, therefore decreasing those masses in the following measurement period. With these factors in consideration, as well as the many

years of rangeland research to determine best management practices (Bailey et al. 2012, Naeth et al. 1990b, Naeth et al. 1991a, Naeth et al. 1991b), it is the recommendation of this study to continue with established best management practices in Alberta grasslands, which generally encourage grazing later in the growing season.

When considering the implications of this study, reflection on the limitations of simulated grazing mechanisms in comparison to live grazing cattle is key. The simulated grazing treatments used in this study do not encapsulate the variability, selectivity and other behaviours that influence grazing cattle. These treatments were designed to simulate particular mechanisms occurring during grazing, not the full process of grazing. Studies simulating compaction use a range of methods, from a mechanical hoof, to pressure from sources such as poles placed into the ground or trampling from human feet (Di et al. 2001, Dunne et al. 2011, Sorenson et al. 2009). The cultipackers used in the trampling treatments in this study were chosen to apply pressure to the majority of the area of the treated plots, whereas trampling by cattle will have more localized pressure from hooves and would be subject to the selectivity of cattle behaviour. These limitations must be considered when reflecting on the results found in this study.

Weather conditions during study years must be considered when among the limitations of study results. Dry growing conditions were seen in both years of measurement (2017 and 2018), which may have affected response to the applied treatments of this study. In different conditions, such as a year with greater than average precipitation, response to trampling treatment may have varied, due to the expected increase in compaction in moist conditions (Naeth et al. 1990b). It is expected that results would have varied in response to defoliation treatments if study conditions had allowed for greater precipitation, due to the expected increase in growth in these moisture-limited ecosystems. The role of litter in moist conditions is different than that of litter in dry

conditions (Deutsch et al. 2010b), as the role of litter in conserving moisture would not have been as key in moist conditions, therefore it is assumed that results would vary in response to litter removal in years with higher precipitation.

With the growing urgency of climate change, land management decisions have an increasing impact on both the longevity of the function and diversity of a landscape. In the context of land management decisions, my research aids in understanding response of grassland enzyme activity and vegetation to grazing systems. To further develop our knowledge, it would be constructive to explore the response of soil nutrient fluctuations and plant root exudates to grazing mechanisms to understand the influence on observed enzyme activity. Knowledge of the response of below ground growth to grazing mechanisms could aid in understanding of response of vegetation dynamics. Examination of other environmental variables when considering enzyme activity may also be advantageous in understanding results.

Findings from both the EEA study (Chapter 2) and the vegetation study (Chapter 3) emphasise the paramount importance of moisture in grassland communities, both for nutrient cycling and for the dynamics of the vegetation community. Litter was related to increased grazing tolerant species and EEA, likely due to its effect on moisture retention in both studies, while clipping and compaction displayed varied responses. Sites, chosen for their variation in precipitation regimes and vegetation community, varied in their response to treatment, demonstrating the importance of considering environmental conditions when implementing land management. Findings from this study indicate the effect of grazing mechanisms on moisture retention in grasslands, particularly the influence of litter presence in northern temperate grasslands. The results of this research provide information on the impacts of isolated

mechanisms occurring during grazing, to aid in a more complete understanding of the response of grasslands to grazing systems.

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Appendices

Appendix A: Summary of envfit Results for Ordination of Extracellular Enzyme Data

Table A1: envfit results testing the relationships of soil and litter characteristics on litter and soil extracellular enzyme activities at each site and year. Significant values ($P < 0.05$) are in bold.

Soil Results					**Litter Results**				
	2017		**2018**			**2017**		**2018**	
	r2	**Pr(>r)**	**r2**	**Pr(>r)**		**r2**	**Pr(>r)**	**r2**	**Pr(>r)**
Variable	**Onefour**				**Variable**	**Onefour**			
soil pH	0.23	**0.001**	0.31	**0.001**	litter pH	0.03	0.25	0.18	**0.001**
soil NPOC	0.04	0.11	0.12	**0.002**	soil NPOC	0.06	0.10	0.03	0.23
soil WETN	0.04	0.12	0.01	0.50	soil WETN	0.07	0.06	0.00	0.86
soil AP	0.01	0.48	0.10	**0.003**	soil AP	0.01	0.79	0.03	0.24
soil moisture content	0.26	**0.001**	0.04	0.11	litter moisture content	0.06	0.08	0.11	**0.008**
Lethbridge					**Lethbridge**				
soil pH	0.18	**0.001**	0.19	**0.001**	litter pH	0.001	0.97	0.07	**0.025**
soil NPOC	0.02	0.42	0.28	**0.001**	soil NPOC	0.04	0.39	0.39	**0.001**
soil WETN	0.05	**0.082**	0.07	**0.022**	soil WETN	0.02	0.70	0.13	**0.002**
soil AP	0.0007	0.973	0.17	**0.001**	soil AP	0.04	0.42	0.16	**0.002**
soil moisture content	0.23	**0.001**	0.29	**0.001**	litter.MC	0.04	0.37	0.26	**0.001**
Stavely					**Stavely**				
soil pH	0.05	**0.073**	0.25	**0.001**	litter pH	0.12	**0.006**	0.29	**0.001**
soil NPOC	0.02	0.33	0.00	0.95	soil NPOC	0.003	0.85	0.02	0.41
soil WETN	0.02	0.43	0.01	0.56	soil WETN	0.02	0.41	0.03	0.18
soil AP	0.13	**0.003**	0.02	0.44	soil AP	0.0001	1.00	0.01	0.48
soil moisture content	0.12	**0.002**	0.02	0.30	litter.MC	0.03	0.29	0.07	**0.018**

Appendix B: Summary of perMANOVA Results for Ordination of Extracellular Enzyme Data.

Table B1: Statistical results of perMANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil and litter extracellular enzyme activities at each site. Significant values (P < 0.05) are in bold.

Soil Results						
	2017			**2018**		
	df	**R2**	***P***	**df**	**R2**	***P***
Treatment	**Onefour**					
Litter (L)	1	0.01	0.18	1	0.04	**0.007**
Clip/Compact (CC)	6	0.03	0.87	6	0.06	0.09
Month	1	0.07	**0.001**	1	0.21	**0.001**
*L*CC*	6	0.01	1.00	6	0.06	0.16
*L*M*	1	0.02	0.10	1	0.003	0.69
*CC*M*	6	0.03	0.73	6	0.04	0.50
*L*CC*M*	6	0.03	0.85	6	0.03	0.55
Residuals	81			82		
Total	108			109		
Lethbridge						
Litter (L)	1	0.002	0.83	1	0.01	0.14
Clip/Compact (CC)	6	0.005	1	6	0.04	0.253
Month	1	0.20	**0.001**	1	0.33	**0.001**
*L*CC*	6	0.02	0.97	6	0.03	0.49
*L*M*	1	0.003	0.68	1	0.004	0.55
*CC*M*	6	0.01	1.00	6	0.03	0.72
*L*CC*M*	6	0.01	0.98	6	0.03	0.50
Residuals	81			83		
Total	108			110		
Stavely						

Litter (L)	1	0.01	0.31	1	0.00	0.49
Clip/Compact (CC)	6	0.06	0.43	6	0.01	0.89
Month	1	0.06	**0.001**	1	0.77	**0.001**
*L*CC*	6	0.05	0.54	6	0.01	0.64
*L*M*	1	0.02	0.14	1	0.00	0.49
*CC*M*	6	0.02	1.00	6	0.01	0.75
*L*CC*M*	6	0.04	0.83	6	0.01	0.74
Residuals	78			84		
Total	105			111		

Table B1 continued: Statistical results of perMANOVAs testing the effects of month sampled, litter manipulation as well as clipping and compaction treatments, on soil and litter extracellular enzyme activities at each site. Significant values (P < 0.05) are in bold.

Litter Results						
	2017			**2018**		
	df	**R2**	***P***	**df**	**R2**	***P***
Treatment	**Onefour**					
Litter (L)	1	0.03	**0.042**	1	0.01	**0.044**
Clip/Compact (CC)	6	0.07	0.461	6	0.07	**0.044**
Month	1	0.04	**0.004**	1	0.06	**0.001**
*L*CC*	6	0.06	0.58	6	0.09	0.47
*L*M*	1	0.02	0.13	1	0.02	0.12
*CC*M*	6	0.10	0.09	6	0.06	0.43
*L*CC*M*	6	0.08	0.21	4	0.04	0.57
Residuals	55			65		
Total	82			90		
Lethbridge (note: month not used in 2017 perMANOVA, na.omit omitted large amount of July samples)						
Litter (L)	1	0.01	0.84	1	0.03	**0.011**
Clip/Compact (CC)	6	0.14	0.37	6	0.06	0.18
Month	**NA**	**NA**	**NA**	1	0.24	**0.001**
*L*CC*	6	0.16	0.19	6	0.04	0.57
*L*M*	**NA**	**NA**	**NA**	1	0.01	0.19
*CC*M*	**NA**	**NA**	**NA**	6	0.06	0.10
*L*CC*M*	**NA**	**NA**	**NA**	4	0.03	0.62
Residuals	35			73		
Total	48			98		
Stavely						
Litter (L)	1	0.03	**0.014**	1	0.05	**0.005**

Clip/Compact (CC)	6	0.08	**0.036**	6	0.04	0.69
Month	1	0.08	**0.001**	1	0.04	**0.017**
*L*CC*	6	0.05	0.55	6	0.09	**0.041**
*L*M*	1	0.02	0.15	1	0.02	0.06
*CC*M*	6	0.03	0.88	6	0.05	0.38
*L*CC*M*	6	0.05	0.44	6	0.06	0.16
Residuals	75			84		
Total	102			111		

Appendix C: Summary of Pairwise perMANOVA Results Displaying Interactions between Treatments seen in Litter Extracellular Enzyme Activity

Table C1: Statistical results of pairwise perMANOVAs testing significant treatment effects on litter extracellular enzyme activities at applicable sites (see Table 6 for significant treatment effects). Significant values ($P < 0.05$) are in bold.

Pairwise comparisons using permutation MANOVAs on a distance matrix (using *pairwise.perm.manova* function, using 999 permutations)										
Sample type	**Site**	**Year**	**Interaction**	**pairwise comparisons**						
					fallclip fallroll	noclip fallroll	fallclip noroll	noclip noroll	spring clip noroll	noclip spring roll
				noclip fallroll	**0.017**	-	-	-	-	-
				fallclip noroll	0.30	0.21	-	-	-	-
				noclip noroll	0.19	0.35	0.56	-	-	-
				springclip noroll	0.36	0.18	0.58	0.19	-	-
				noclip springroll	0.89	0.48	0.83	0.25	0.94	-
Litter	Onefour	2018	Clipping/Compaction	springclip springroll	0.30	0.23	0.58	0.99	0.24	0.31
					fallclip fallroll	noclip fallroll	fallclip noroll	noclip noroll	spring clip noroll	noclip spring roll
				noclip fallroll	**0.012**	-	-	-	-	-
				fallclip noroll	0.13	0.24	-	-	-	-
Litter	Stavely	2017	Clipping/Compaction	noclip noroll	0.08	0.26	0.14	-	-	-

				springclip noroll	0.70	**0.031**	0.32	0.50	-	-									
				noclip springroll	0.17	0.18	1.00	0.17	0.42	-									
				springclip springroll	**0.044**	0.59	0.20	0.92	0.43	0.31									
					fallclip fallroll * Litter	fallclip fallroll * Litter removed	noclip fallroll * Litter	noclip fallroll * Litter removed	fallclip noroll * Litter	fallclip noroll * Litter removed	noclip noroll * Litter	noclip noroll * Litter removed	spring clip noroll * Litter	spring clip noroll * Litter removed	noclip spring roll * Litter	noclip spring roll * Litter removed	spring clip springroll * Litter		
				fallclip fallroll * Litter removed	0.73	-	-	-	-	-	-	-	-	-	-	-	-		
				noclip fallroll * Litter	0.54	0.60	-	-	-	-	-	-	-	-	-	-	-		
				noclip fallroll* Litter removed	0.73	0.39	0.29	-	-	-	-	-	-	-	-	-	-		
				fallclip noroll * Litter	0.85	0.40	0.28	0.90	-	-	-	-	-	-	-	-	-		
				fallclip noroll * Litter removed	0.22	0.36	0.87	0.11	0.15	-	-	-	-	-	-	-	-		
				noclip noroll * Litter	0.73	0.52	0.54	0.43	0.45	0.31	-	-	-	-	-	-	-		
Litter	Stavely	2018	Clipping / Compaction * Litter	noclip noroll * Litter removed	0.10	0.16	0.25	**0.02**	0.05	0.56	**0.03**	-	-	-	-	-	-		

				springclip noroll * Litter	0.59	0.59	0.62	0.32	0.37	0.43	0.22	0.71	-	-	-	-	-
				springclip noroll * Litter removed	0.46	0.78	0.73	0.25	0.27	0.52	0.18	0.71	1.00	-	-	-	-
				noclip springroll * Litter	0.51	0.21	0.11	0.79	0.73	**0.039**	0.51	**0.007**	0.16	0.06	-	-	-
				noclip springroll * Litter removed	0.09	0.15	0.23	**0.058**	0.05	0.59	**0.03**	0.68	0.44	0.32	**0.01**	-	-
				springclip springroll * Litter	0.33	0.10	**0.034**	0.70	0.66	**0.014**	0.19	**0.005**	0.11	**0.04**	0.63	**0.007**	-
				springclip springroll * Litter removed	0.29	0.31	0.64	0.21	0.16	0.55	0.09	0.46	0.73	0.64	0.07	0.63	**0.029**

Appendix D: Means and Standard Errors of Observed Soil Characteristics within Clipping/Compaction Treatments.

Table D1: Calculated means (n=16) and standard errors of soil characteristics at each site and year for each clipping and compaction treatment. Note: “se” denotes standard error.

Site	Year	ClipxRoll	Soil pH	Soil pH se	NPOC (mg/kg)	NPOC (mg/kg) se	WETN (mg/kg)	WETN (mg/kg) se	AP (mg/L)	AP (mg/L) se	Soil Moisture Content (%)	Soil Moisture Content se (%)
Onefour	2017	fallclip fallroll	7.02	0.11	54.46	4.92	4.63	0.45	1.22	0.29	5.77	0.43
Onefour	2017	fallclip noroll	6.95	0.10	51.51	3.71	3.99	0.27	1.17	0.20	5.85	0.44
Onefour	2017	noclip fallroll	6.90	0.07	54.15	7.09	4.71	0.50	1.63	0.47	5.34	0.35
Onefour	2017	noclip noroll	7.03	0.12	55.84	6.41	4.09	0.44	1.32	0.41	5.93	0.53
Onefour	2017	noclip springroll	7.15	0.12	55.78	6.34	3.54	0.35	1.48	0.41	5.63	0.46
Onefour	2017	springclip noroll	6.83	0.09	58.10	5.81	3.87	0.34	1.27	0.30	5.33	0.45
Onefour	2017	springclip springroll	6.84	0.09	59.38	3.67	5.02	1.01	1.60	0.48	5.25	0.65
Onefour	2018	fallclip fallroll	7.01	0.05	47.62	4.09	4.09	0.29	0.16	0.02	5.95	0.39
Onefour	2018	fallclip noroll	6.94	0.04	44.14	4.16	4.33	0.32	0.18	0.03	5.06	0.32
Onefour	2018	noclip fallroll	6.90	0.04	41.65	4.13	3.72	0.33	0.17	0.03	4.74	0.72
Onefour	2018	noclip noroll	6.99	0.07	46.03	7.82	3.63	0.37	0.19	0.02	5.30	0.47
Onefour	2018	noclip springroll	7.16	0.08	46.71	5.36	3.76	0.26	0.20	0.02	5.61	0.40
Onefour	2018	springclip noroll	6.85	0.03	47.43	3.83	4.20	0.27	0.15	0.02	4.99	0.40
Onefour	2018	springclip springroll	6.95	0.03	44.62	3.95	3.98	0.26	0.15	0.02	4.99	0.40
Lethbridge	2017	fallclip fallroll	7.80	0.06	116.87	12.39	9.90	0.72	1.22	0.21	9.72	0.96
Lethbridge	2017	fallclip noroll	7.78	0.06	126.57	23.74	10.41	1.63	1.22	0.21	9.52	0.86
Lethbridge	2017	noclip fallroll	7.77	0.03	107.40	7.14	11.13	1.44	1.16	0.24	9.63	0.96
Lethbridge	2017	noclip noroll	7.77	0.07	120.21	16.09	10.40	1.59	1.28	0.24	10.28	0.97
Lethbridge	2017	noclip springroll	7.81	0.05	97.67	7.43	9.08	1.33	1.16	0.23	9.35	0.92
Lethbridge	2017	springclip noroll	7.76	0.04	101.72	5.64	8.62	0.37	1.09	0.15	9.01	1.04

Lethbridge	2017	springclip springroll	7.71	0.05	105.94	6.11	8.94	0.52	1.09	0.19	9.23	1.02
Lethbridge	2018	fallclip fallroll	7.94	0.05	80.18	12.41	9.90	1.22	0.22	0.03	9.38	0.82
Lethbridge	2018	fallclip noroll	7.95	0.06	81.10	10.42	9.58	0.87	0.21	0.03	8.84	0.77
Lethbridge	2018	noclip fallroll	8.01	0.04	80.78	13.20	8.44	0.79	0.20	0.03	9.24	0.67
Lethbridge	2018	noclip noroll	8.03	0.05	85.53	14.31	8.73	0.89	0.20	0.03	8.82	0.57
Lethbridge	2018	noclip springroll	7.96	0.06	76.80	10.99	8.16	0.75	0.21	0.03	8.89	0.61
Lethbridge	2018	springclip noroll	8.00	0.04	79.36	12.60	9.29	0.99	0.22	0.03	8.86	0.65
Lethbridge	2018	springclip springroll	7.96	0.05	75.27	9.13	9.12	0.87	0.19	0.03	8.62	0.66
Stavely	2017	fallclip fallroll	6.11	0.05	484.20	52.83	28.40	2.24	4.27	0.56	20.43	1.13
Stavely	2017	fallclip noroll	6.30	0.09	442.87	50.05	30.09	2.72	6.29	1.20	19.87	0.93
Stavely	2017	noclip fallroll	6.20	0.05	457.02	35.24	33.77	2.62	4.88	0.51	20.55	1.24
Stavely	2017	noclip noroll	6.08	0.04	439.68	32.03	28.25	1.87	3.79	0.50	20.72	1.10
Stavely	2017	noclip springroll	6.16	0.04	425.97	31.34	27.76	2.47	5.16	0.70	21.94	1.28
Stavely	2017	springclip noroll	6.11	0.04	426.72	37.47	29.50	2.44	3.99	0.51	21.24	0.94
Stavely	2017	springclip springroll	6.05	0.04	361.41	35.67	24.89	2.49	3.02	0.33	20.66	1.11
Stavely	2018	fallclip fallroll	6.33	0.04	182.35	22.37	14.00	1.26	0.39	0.03	20.32	0.72
Stavely	2018	fallclip noroll	6.51	0.10	180.98	17.82	15.79	1.48	0.50	0.04	21.36	0.61
Stavely	2018	noclip fallroll	6.52	0.08	180.71	13.53	16.33	0.91	0.53	0.04	22.48	0.60
Stavely	2018	noclip noroll	6.35	0.04	155.39	17.21	16.24	1.73	0.37	0.03	23.56	1.30
Stavely	2018	noclip springroll	6.39	0.04	177.62	14.01	15.19	1.13	0.44	0.04	22.40	0.58
Stavely	2018	springclip noroll	6.38	0.05	158.64	15.99	14.63	1.26	0.39	0.04	22.12	0.54
Stavely	2018	springclip springroll	6.31	0.04	150.80	9.15	12.74	0.88	0.37	0.03	21.83	0.49

Appendix E: Means and Standard Errors of Observed Litter Characteristics within Clipping/Compaction Treatments.

Table E1: Calculated means (n=16) and standard errors of litter characteristics at each site and year for each clipping and compaction treatment. Note: “se” denotes standard error.

Site	Year	ClipxRoll	Litter Moisture Content (%)	Litter Moisture Content se (%)	Litter pH	Litter pH se
Onefour	2017	fallclip fallroll	4.98	0.62	4.98	0.06
Onefour	2017	fallclip noroll	6.72	1.32	5.01	0.05
Onefour	2017	noclip fallroll	5.48	0.92	4.84	0.04
Onefour	2017	noclip noroll	6.08	1.19	4.84	0.05
Onefour	2017	noclip springroll	5.25	0.93	5.07	0.05
Onefour	2017	springclip noroll	5.34	0.91	5.02	0.05
Onefour	2017	springclip springroll	5.34	0.84	5.07	0.05
Onefour	2018	fallclip fallroll	16.69	3.24	5.13	0.04
Onefour	2018	fallclip noroll	14.25	3.07	5.26	0.06
Onefour	2018	noclip fallroll	9.21	1.57	4.98	0.06
Onefour	2018	noclip noroll	10.47	2.23	4.99	0.05
Onefour	2018	noclip springroll	9.53	1.83	5.01	0.05
Onefour	2018	springclip noroll	17.48	3.73	5.18	0.05
Onefour	2018	springclip springroll	11.10	2.10	5.00	0.05
Lethbridge	2017	fallclip fallroll	7.39	1.55	5.08	0.06
Lethbridge	2017	fallclip noroll	9.37	1.99	5.17	0.06
Lethbridge	2017	noclip fallroll	7.57	1.76	5.17	0.06
Lethbridge	2017	noclip noroll	8.05	1.52	5.20	0.04

Lethbridge	2017	noclip springroll	7.98	1.24	5.25	0.05
Lethbridge	2017	springclip noroll	7.90	1.51	5.27	0.05
Lethbridge	2017	springclip springroll	8.51	1.71	5.20	0.04
Lethbridge	2018	fallclip fallroll	2.74	0.94	5.57	0.05
Lethbridge	2018	fallclip noroll	3.10	0.86	5.62	0.05
Lethbridge	2018	noclip fallroll	4.05	0.56	5.58	0.04
Lethbridge	2018	noclip noroll	4.37	0.72	5.55	0.04
Lethbridge	2018	noclip springroll	3.21	0.65	5.59	0.04
Lethbridge	2018	springclip noroll	3.01	0.90	5.52	0.05
Lethbridge	2018	springclip springroll	3.86	0.64	5.52	0.04
Stavely	2017	fallclip fallroll	16.93	3.17	5.55	0.08
Stavely	2017	fallclip noroll	16.03	2.32	5.69	0.09
Stavely	2017	noclip fallroll	19.45	3.05	5.83	0.09
Stavely	2017	noclip noroll	17.60	2.74	5.82	0.07
Stavely	2017	noclip springroll	18.96	2.67	5.79	0.08
Stavely	2017	springclip noroll	18.18	2.82	5.55	0.07
Stavely	2017	springclip springroll	15.63	2.06	5.69	0.06
Stavely	2018	fallclip fallroll	11.90	1.80	5.62	0.04
Stavely	2018	fallclip noroll	12.50	2.34	5.72	0.06
Stavely	2018	noclip fallroll	13.67	1.99	5.73	0.09
Stavely	2018	noclip noroll	12.70	2.10	5.79	0.07
Stavely	2018	noclip springroll	13.51	1.87	5.75	0.08
Stavely	2018	springclip noroll	13.40	2.37	5.57	0.04
Stavely	2018	springclip springroll	12.01	1.69	5.63	0.07

Appendix F: Means and Standard Errors of Soil and Litter Extracellular Enzyme Activity within Clipping/Compaction Treatments.

Table F1: Calculated means (n=16) and standard errors of soil and litter extracellular enzyme activities at each site and year for each clipping and compaction treatment. Note: "se" denotes standard error.

Soil EEA Statistical Data (EEA reported in nmol/g/h)												
Site	**Year**	**Treatment**	**Cello**	**Cello.se**	**Xylo**	**Xylo.se**	**Gluco**	**Gluco.se**	**NAG**	**NAG.se**	**Phos**	**Phos.se**
Onefour	2017	fallclip fallroll	32.00	5.17	22.85	3.72	181.70	21.74	78.52	22.29	558.29	70.95
Onefour	2017	fallclip noroll	35.44	5.01	22.92	2.90	189.51	21.32	68.23	10.73	594.37	74.53
Onefour	2017	noclip fallroll	26.02	3.53	19.09	1.94	168.25	15.70	51.05	5.38	578.28	64.24
Onefour	2017	noclip noroll	32.35	5.25	24.69	3.45	188.84	16.16	50.29	5.70	585.67	72.82
Onefour	2017	noclip springroll	32.93	5.24	24.00	2.41	175.20	17.02	56.45	7.27	555.07	84.70
Onefour	2017	springclip noroll	42.29	6.26	28.16	3.73	213.68	18.37	55.55	5.38	736.27	81.30
Onefour	2017	springclip springroll	37.38	5.39	22.82	3.34	185.72	21.35	57.34	5.72	584.64	91.58
Onefour	2018	fallclip fallroll	22.40	2.79	16.47	2.04	101.71	9.12	34.62	3.73	287.71	19.68
Onefour	2018	fallclip noroll	19.16	1.60	14.33	1.24	88.88	6.46	33.04	2.07	289.20	18.43
Onefour	2018	noclip fallroll	21.05	2.33	16.03	1.30	93.48	7.95	29.28	2.83	298.02	18.67
Onefour	2018	noclip noroll	22.84	4.59	16.26	2.94	91.76	10.76	38.55	8.99	310.22	49.27
Onefour	2018	noclip springroll	20.94	2.97	14.65	1.80	92.86	13.10	31.69	4.18	241.79	26.54
Onefour	2018	springclip noroll	24.84	2.46	17.81	1.63	105.68	8.96	33.73	2.55	331.61	23.39
Onefour	2018	springclip springroll	26.66	5.59	21.22	5.27	114.36	21.11	39.53	9.12	343.22	55.38
Lethbridge	2017	fallclip fallroll	37.33	11.50	34.38	8.61	249.14	70.96	45.81	11.97	600.35	156.25
Lethbridge	2017	fallclip noroll	34.66	10.34	34.75	9.87	254.88	79.27	42.62	12.38	621.08	173.19
Lethbridge	2017	noclip fallroll	24.83	6.94	31.54	6.50	193.75	45.82	35.58	9.10	693.64	195.64
Lethbridge	2017	noclip noroll	29.04	6.76	36.95	8.54	207.79	49.68	37.43	9.15	788.96	302.02
Lethbridge	2017	noclip springroll	26.33	6.82	28.68	5.40	219.34	60.37	33.89	7.73	827.51	289.05
Lethbridge	2017	springclip noroll	30.44	8.76	31.89	7.14	215.61	58.51	42.21	12.58	691.84	204.53
Lethbridge	2017	springclip springroll	29.99	8.85	30.81	7.02	208.21	59.27	43.96	12.13	622.53	181.72
Lethbridge	2018	fallclip fallroll	25.23	3.82	25.47	3.14	127.55	14.50	25.32	4.32	204.32	35.52

Lethbridge	2018	fallclip noroll	24.78	3.07	28.50	2.82	125.58	10.52	28.13	2.83	201.15	27.13
Lethbridge	2018	noclip fallroll	22.40	5.32	32.14	7.23	125.53	15.04	23.55	3.98	219.76	30.65
Lethbridge	2018	noclip noroll	24.35	2.80	31.46	3.16	137.33	12.40	24.03	3.92	220.84	26.80
Lethbridge	2018	noclip springroll	14.71	2.30	22.69	2.19	112.79	11.19	21.60	4.49	228.78	25.31
Lethbridge	2018	springclip noroll	18.51	4.22	25.68	4.33	109.86	17.46	20.88	3.32	167.38	26.57
Lethbridge	2018	springclip springroll	18.23	3.04	26.67	3.21	129.04	12.76	28.99	5.36	184.91	26.88
Stavely	2017	fallclip fallroll	109.13	8.70	61.65	3.65	355.00	19.81	178.19	16.81	589.61	44.92
Stavely	2017	fallclip noroll	122.97	16.93	85.72	11.61	411.35	44.29	239.00	69.12	678.07	59.51
Stavely	2017	noclip fallroll	145.14	20.49	102.17	14.28	465.36	61.37	254.30	44.86	707.03	46.44
Stavely	2017	noclip noroll	111.94	10.87	71.68	6.14	362.63	27.32	178.87	17.71	664.92	75.92
Stavely	2017	noclip springroll	112.17	10.08	78.52	6.47	380.05	21.75	173.65	15.86	659.10	47.50
Stavely	2017	springclip noroll	120.81	11.41	75.09	7.08	397.15	26.21	182.57	17.95	637.18	52.30
Stavely	2017	springclip springroll	130.42	11.51	80.07	7.16	408.01	32.52	190.18	21.16	750.76	63.74
Stavely	2018	fallclip fallroll	254.01	51.96	165.46	34.16	833.48	168.81	416.49	79.05	1847.37	356.78
Stavely	2018	fallclip noroll	283.30	58.00	188.37	35.62	915.88	175.92	425.67	76.64	1968.37	342.04
Stavely	2018	noclip fallroll	333.60	65.25	208.52	39.09	1052.32	194.18	537.13	106.73	2120.66	371.42
Stavely	2018	noclip noroll	316.91	78.40	186.98	40.23	962.46	198.84	439.74	96.80	1917.36	346.45
Stavely	2018	noclip springroll	304.43	58.00	212.31	40.83	1053.01	191.08	472.44	93.36	2081.66	350.76
Stavely	2018	springclip noroll	297.09	57.60	217.24	42.31	1079.36	201.21	534.52	105.63	2181.65	378.83
Stavely	2018	springclip springroll	254.93	54.02	172.29	34.51	934.94	168.05	450.52	92.82	2049.25	352.48

Table F1 continued: Calculated means (n=16) and standard errors of soil and litter extracellular enzyme activities at each site and year for each clipping and compaction treatment. Note: “se” denotes standard error.

Litter EEA Statistical Data (EEA reported in nmol/g/h)												
Site	**Year**	**Treatment**	**Cello**	**Cello.se**	**Xylo**	**Xylo.se**	**Gluco**	**Gluco.se**	**NAG**	**NAG.se**	**Phos**	**Phos.se**
Onefour	2017	fallclip fallroll	2192.22	380.91	608.67	94.32	2704.33	332.44	1544.42	296.55	3719.05	698.97
Onefour	2017	fallclip noroll	1871.21	329.85	686.91	114.09	3630.13	761.62	1637.11	332.78	3658.42	528.78
Onefour	2017	noclip fallroll	1444.02	155.58	776.79	109.82	3421.57	421.31	2359.68	233.36	3652.03	405.73
Onefour	2017	noclip noroll	1964.85	367.71	770.35	134.88	5193.96	1080.24	1893.57	259.17	3329.00	512.13
Onefour	2017	noclip springroll	1907.00	339.41	588.22	64.96	3047.97	348.31	2030.51	187.62	3269.36	480.17
Onefour	2017	springclip noroll	2476.76	715.25	759.25	139.47	3567.96	628.39	1386.29	146.47	3559.53	395.87
Onefour	2017	springclip springroll	1363.91	138.65	792.32	110.75	6119.92	3554.84	2207.04	484.75	3115.20	538.74
Onefour	2018	fallclip fallroll	1562.17	372.82	657.09	139.70	2732.93	638.11	1231.36	274.15	2104.81	407.09
Onefour	2018	fallclip noroll	1331.58	274.21	640.94	138.12	3096.96	514.78	1422.26	268.68	2702.01	308.58
Onefour	2018	noclip fallroll	1260.47	210.38	533.36	103.92	2306.25	280.56	1509.70	213.92	3155.57	324.59
Onefour	2018	noclip noroll	1402.05	239.77	791.51	155.26	3484.81	563.01	1569.17	283.50	3967.88	527.23
Onefour	2018	noclip springroll	1771.79	261.49	805.17	191.67	2959.17	240.85	1673.90	251.07	3132.61	360.91
Onefour	2018	springclip noroll	1555.17	364.89	755.82	183.85	2797.39	459.04	1710.59	348.69	2802.16	440.55
Onefour	2018	springclip springroll	1340.34	236.17	743.22	148.12	3230.55	522.79	1223.46	184.76	3798.52	761.15
Lethbridge	2017	fallclip fallroll	2710.57	380.51	539.44	106.35	3336.39	625.85	2833.24	575.96	6682.71	1515.07
Lethbridge	2017	fallclip noroll	2418.99	405.05	1081.10	270.69	4395.64	597.68	3071.46	560.86	5205.97	1154.26
Lethbridge	2017	noclip fallroll	2337.55	358.08	861.14	186.13	5324.79	1154.48	2942.50	443.09	5138.12	564.71
Lethbridge	2017	noclip noroll	2935.26	450.00	1066.49	170.13	4040.96	397.46	3044.76	739.45	5809.48	791.18
Lethbridge	2017	noclip springroll	2764.73	528.31	781.77	141.23	4717.45	719.41	2195.45	333.75	4674.63	903.94
Lethbridge	2017	springclip noroll	2408.28	504.85	686.04	72.73	4668.55	325.46	2628.78	452.60	5215.21	710.88
Lethbridge	2017	springclip springroll	2327.99	389.41	651.35	102.36	5150.90	788.57	2031.15	361.50	5096.40	853.79
Lethbridge	2018	fallclip fallroll	1666.52	189.78	671.44	120.08	3564.18	598.70	1635.75	147.06	2976.41	484.83
Lethbridge	2018	fallclip noroll	2107.44	182.78	895.36	116.44	3302.17	220.73	2009.34	162.57	3161.62	198.62

Lethbridge	2018	noclip fallroll	1888.74	143.30	965.16	109.84	3247.51	161.88	2006.25	83.31	3341.92	349.68
Lethbridge	2018	noclip noroll	1952.75	134.25	897.89	84.42	3236.86	172.92	2107.61	99.93	3287.70	275.84
Lethbridge	2018	noclip springroll	2354.36	397.75	908.51	97.10	3408.45	228.60	2142.56	111.14	3386.66	134.24
Lethbridge	2018	springclip noroll	2593.57	604.45	919.68	94.53	3343.30	271.12	2040.69	188.74	3295.68	312.57
Lethbridge	2018	springclip springroll	2065.71	275.06	898.62	83.65	4133.93	904.40	1847.12	158.29	3679.54	532.94
Stavely	2017	fallclip fallroll	1629.38	158.76	846.71	101.30	3649.47	332.19	1578.21	178.92	5172.58	675.57
Stavely	2017	fallclip noroll	1643.28	198.72	773.98	81.74	4021.69	447.02	2017.97	166.74	3611.05	439.70
Stavely	2017	noclip fallroll	1796.29	269.28	888.54	171.32	5305.74	823.78	2362.91	390.19	3539.89	444.21
Stavely	2017	noclip noroll	1271.20	142.05	599.00	46.19	3714.74	257.78	2410.03	555.21	4341.50	740.09
Stavely	2017	noclip springroll	1541.67	187.36	704.06	66.83	3806.60	279.30	1889.59	159.96	3710.36	309.84
Stavely	2017	springclip noroll	1508.68	288.15	886.24	171.68	4505.63	747.98	2187.96	336.51	4820.26	512.55
Stavely	2017	springclip springroll	1416.01	198.13	601.21	80.99	4082.22	311.23	2123.56	294.70	3965.96	377.43
Stavely	2018	fallclip fallroll	755.19	101.79	345.78	41.44	2244.71	311.85	1285.06	205.51	2395.45	373.56
Stavely	2018	fallclip noroll	624.87	118.65	239.71	30.99	1840.22	298.00	1084.76	216.14	1979.63	358.82
Stavely	2018	noclip fallroll	618.26	86.53	254.35	28.36	1721.96	231.43	1022.55	148.56	1910.16	305.15
Stavely	2018	noclip noroll	765.17	140.43	279.58	37.95	2061.00	281.69	1148.55	207.41	2178.76	345.82
Stavely	2018	noclip springroll	854.02	189.99	282.87	39.68	1964.11	289.78	1030.21	173.81	2188.57	374.45
Stavely	2018	springclip noroll	1023.15	190.96	415.45	41.60	2491.60	309.74	1677.71	290.97	3143.17	336.51
Stavely	2018	springclip springroll	950.31	208.73	363.76	56.94	2074.45	323.59	1851.16	670.34	2690.52	522.02

Appendix G: Linear regressions displaying relationships between litter moisture content and litter β-1,4-Xylosidase activity.

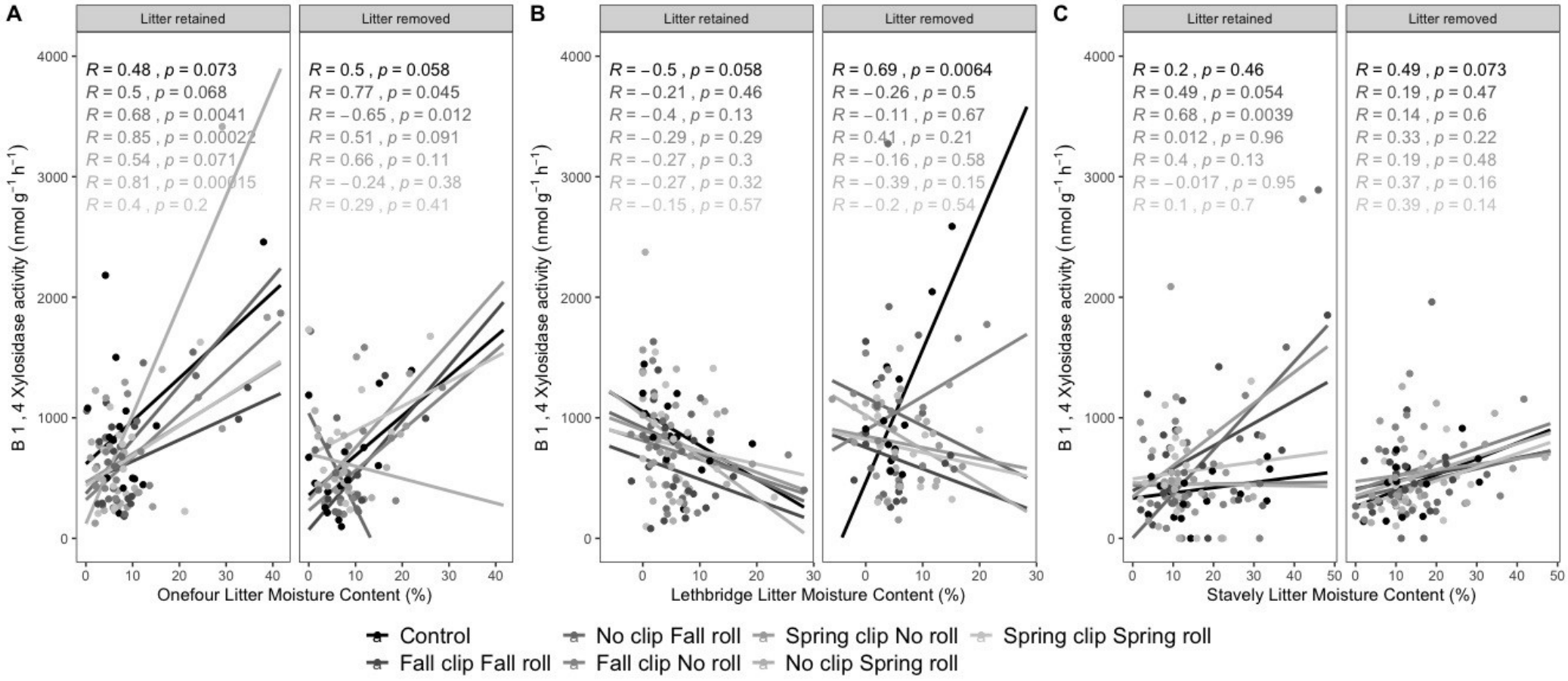

Figure G1: Linear regressions displaying relationships between litter moisture content and litter β-1,4-Xylosidase activity at Onefour (A), Lethbridge (B) and Stavely (C).

Appendix H: Control Fluorescence Readings between both Microplate Readers used in Extracellular Enzyme Study.

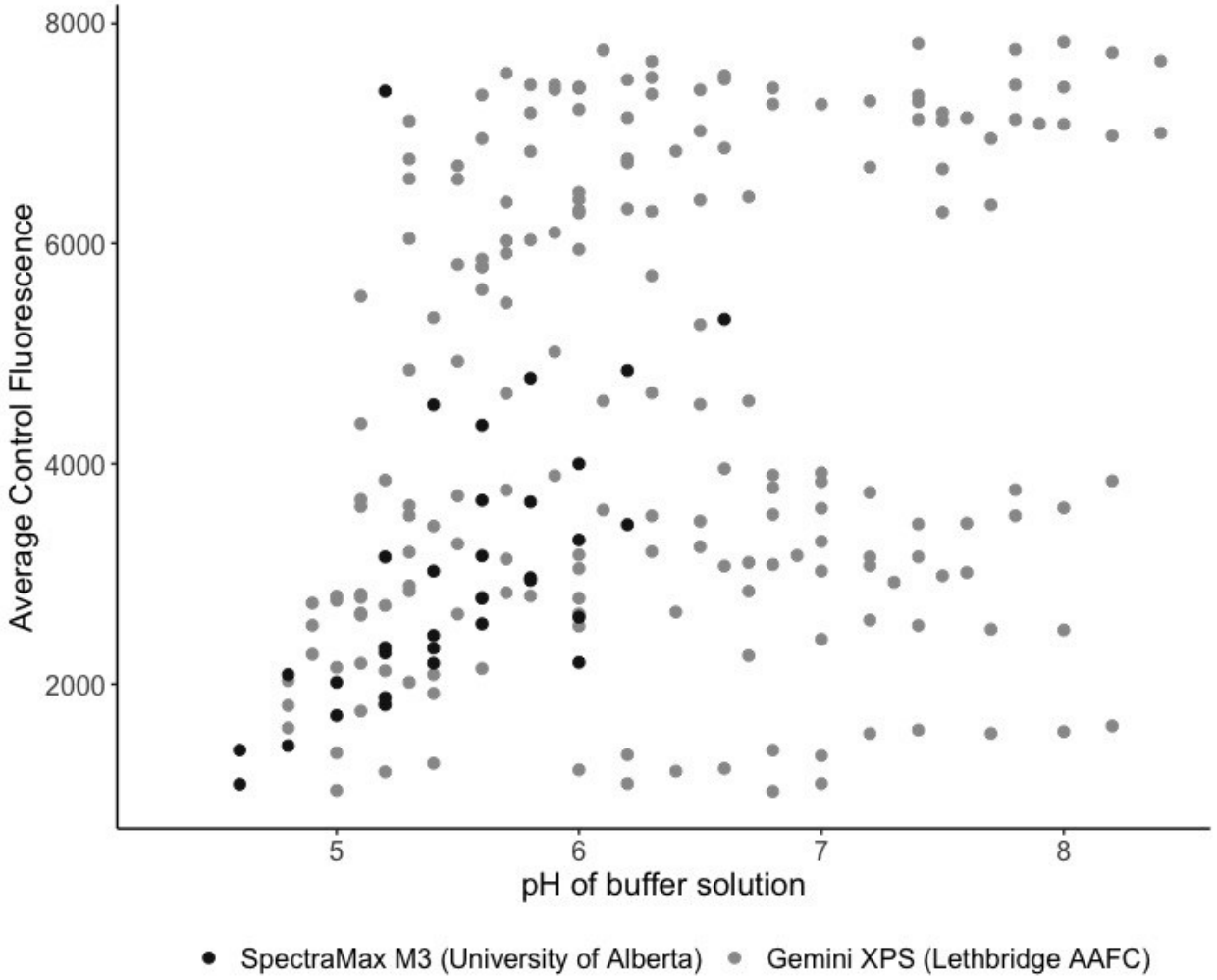

Figure H1: Fluorescence readings of standard methylumbelliferone control solution for microplate readers used. Data shows fluorescence readings from both microplate readers are within the same range. Absorbance readings varied depending on pH of the buffer.

Appendix I: Summary of envfit Results for Ordination of Vegetation Community Data.

Table I1: Envfit output for environmental vectors from NMDS for all sites in both years sampled.

2017			2018	
Environmental Variable	**r2**	**Pr(>r)**	**r2**	**Pr(>r)**
Onefour				
Live/dead herbage mass	0.03	0.40	0.05	0.24
Surface litter mass	0.13	**0.018**	0.04	0.29
Forb mass	0.08	0.11	0.11	**0.039**
ANPP	0.02	0.55	0.06	0.18
Standing litter mass	0.05	0.27	0.01	0.76
Bareground	0.16	0.01	0.14	**0.024**
Soil NPOC	0.13	**0.013**	0.00	0.96
Soil WETN	0.09	0.08	0.01	0.82
Soil AP	0.04	0.34	0.07	0.13
pH	0.03	0.50	0.05	0.29
Soil Moisture Content	0.08	0.11	0.02	0.52
Lethbridge				
Live/dead herbage mass	0.032	0.42	0.294	**0.001**
Surface litter mass	0.042	0.35	0.242	**0.002**
Forb mass	0.024	0.54	0.282	**0.001**
ANPP	0.080	0.13	0.215	**0.004**

Standing litter mass	0.009	0.78	0.175	**0.004**
Bareground	0.001	0.98	0.223	**0.005**
Soil NPOC	0.112	**0.046**	0.037	0.39
Soil WETN	0.119	**0.041**	0.005	0.88
Soil AP	0.139	**0.028**	0.004	0.90
pH	0.127	**0.033**	0.019	0.58
Soil Moisture Content	0.068	0.16	0.013	0.70
Stavely				
Live/dead herbage mass	0.01	0.75	0.04	0.31
Surface litter mass	0.08	0.13	0.02	0.59
Forb mass	0.00	0.90	0.10	0.06
ANPP	0.01	0.86	0.01	0.75
Standing litter mass	0.01	0.68	0.05	0.27
Bareground	0.06	0.24	0.00	1.00
Soil NPOC	0.06	0.25	0.10	0.07
Soil WETN	0.03	0.50	0.12	**0.03**
Soil AP	0.02	0.54	0.01	0.72
pH	0.03	0.42	0.01	0.72
Soil Moisture Content	0.05	0.27	0.05	0.24

Appendix J: Summary of perMANOVA Results for Ordination of Vegetation Community Data.

Table J1: perMANOVA output for NMDS results at three sites in both years sampled.

2017 NMDS perMANOVA output																
		Onefour					**Lethbridge**					**Stavely**				
Treatment	df	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)
Litter (L)	1	0.10	0.10	1.46	0.03	0.12	0.07	0.07	0.55	0.01	0.66	0.26	0.26	1.87	0.03	**0.019**
Clip / Compact (CC)	6	0.44	0.07	1.08	0.12	0.20	0.71	0.12	0.90	0.11	0.37	0.92	0.15	1.08	0.12	0.10
L:CC	6	0.34	0.06	0.82	0.09	0.65	0.38	0.06	0.49	0.06	0.96	0.63	0.10	0.74	0.08	0.77
Residuals	42	2.89	0.069	0.766	NA	NA	5.54	0.13	0.83	NA	NA	5.93	0.14	0.77	NA	NA

Table J1 continued: perMANOVA output for NMDS results at three sites in both years sampled.

2018 NMDS perMANOVA output																
		Onefour					**Lethbridge**					**Stavely**				
Treatment	df	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)	SumsOfSqs	Mean Sqs	F.Model	R2	Pr(>F)
Litter (L)	1	0.14	0.14	1.59	0.03	0.06	0.32	0.32	1.65	0.03	**0.028**	0.19	0.19	1.19	0.02	0.13
Clip / Compact (CC)	6	0.31	0.05	0.59	0.07	0.96	0.78	0.13	0.68	0.08	0.60	0.96	0.16	0.98	0.11	0.14
L:CC	6	0.32	0.05	0.61	0.07	0.95	0.47	0.08	0.41	0.05	0.99	0.84	0.14	0.86	0.10	0.39
Residuals	42	3.67	0.09	0.83	NA	NA	8.05	0.19	0.84	NA	NA	6.83	0.16	0.77	NA	NA

Appendix K: Summary of Tukey Contrasts for Figure 3.4.

Table K1: Surface litter (g/m^2) tukey letter contrasts within each clipping/compaction treatment by site. Groups that did not share a letter are significantly different at $p \leq 0.05$.

Site	Clipping/compaction treatment	emmean	Standard error	df	lower CL	upper CL	Tukey letter
Onefour	fall clip fall compact	3.14	0.21	35.40	2.44	3.84	a
Onefour	spring clip no compact	3.34	0.21	35.40	2.64	4.04	ab
Onefour	fall clip no compact	3.48	0.21	35.40	2.78	4.18	ab
Onefour	spring clip spring compact	3.63	0.21	35.40	2.93	4.33	ab
Onefour	control	3.66	0.21	35.40	2.96	4.36	ab
Onefour	no clip spring compact	3.84	0.21	35.40	3.15	4.54	ab
Onefour	no clip fall compact	3.97	0.21	35.40	3.27	4.67	abc
Lethbridge	no clip spring compact	4.03	0.21	35.40	3.33	4.73	abc
Lethbridge	fall clip no compact	4.14	0.21	35.40	3.44	4.84	abcd
Lethbridge	spring clip spring compact	4.23	0.21	35.40	3.53	4.93	abcd
Lethbridge	fall clip fall compact	4.30	0.21	35.40	3.60	5.00	abcde
Lethbridge	spring clip no compact	4.40	0.21	35.40	3.70	5.09	bcde
Lethbridge	control	4.40	0.21	35.40	3.70	5.10	bcde
Lethbridge	no clip fall compact	4.40	0.21	35.40	3.70	5.10	bcdef
Stavely	no clip spring compact	5.09	0.21	35.40	4.39	5.79	cdefg
Stavely	control	5.29	0.21	35.40	4.59	5.99	defg
Stavely	fall clip no compact	5.40	0.21	35.40	4.70	6.10	efg
Stavely	spring clip spring compact	5.46	0.21	35.40	4.76	6.16	efg
Stavely	spring clip no compact	5.57	0.21	35.40	4.87	6.27	fg
Stavely	fall clip fall compact	5.65	0.21	35.40	4.96	6.35	g
Stavely	no clip fall compact	5.72	0.21	35.40	5.02	6.42	g

Appendix L: Summary of Tukey Contrasts for Figure 3.5.

Table L1: Forb mass (g/m^2) tukey letter contrasts within each litter manipulation and clipping/compaction treatment by site. Groups that did not share a letter are significantly different at $p \leq 0.05$.

Site	Litter manipulation treatment	Clipping/ compaction treatment	emmean	SE	df	lower.CL	upper.CL	Tukey letter
Lethbridge	Litter removed	no clip spring compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter removed	fall clip no compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter removed	spring clip spring compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter retained	spring clip spring compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter removed	spring clip no compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter retained	no clip fall compact	0.00	0.46	88.20	-1.54	1.54	a
Lethbridge	Litter retained	spring clip no compact	0.24	0.46	88.20	-1.30	1.78	ab
Lethbridge	Litter retained	fall clip fall compact	0.38	0.46	88.20	-1.16	1.92	ab
Lethbridge	Litter retained	control	0.45	0.46	88.20	-1.09	1.99	ab
Onefour	Litter removed	no clip fall compact	0.47	0.46	88.20	-1.07	2.01	abc
Lethbridge	Litter retained	fall clip no compact	0.52	0.46	88.20	-1.02	2.06	abc
Onefour	Litter retained	spring clip no compact	0.67	0.46	88.20	-0.87	2.21	abc
Lethbridge	Litter retained	no clip spring compact	0.69	0.46	88.20	-0.85	2.23	abc
Lethbridge	Litter removed	fall clip fall compact	0.73	0.46	88.20	-0.81	2.27	abc
Onefour	Litter retained	fall clip no compact	0.73	0.46	88.20	-0.81	2.27	abc

Onefour	Litter removed	spring clip spring compact	0.75	0.46	88.20	-0.79	2.29	abc
Onefour	Litter retained	spring clip spring compact	0.82	0.46	88.20	-0.72	2.35	abc
Onefour	Litter removed	no clip spring compact	0.84	0.46	88.20	-0.70	2.37	abc
Onefour	Litter retained	no clip spring compact	0.84	0.46	88.20	-0.70	2.38	abc
Onefour	Litter removed	fall clip no compact	0.93	0.46	88.20	-0.61	2.47	abc
Onefour	Litter removed	spring clip no compact	0.94	0.46	88.20	-0.60	2.48	abc
Onefour	Litter removed	control	1.29	0.46	88.20	-0.25	2.83	abcd
Onefour	Litter removed	fall clip fall compact	1.42	0.46	88.20	-0.12	2.95	abcd
Onefour	Litter retained	control	1.49	0.46	88.20	-0.05	3.03	abcd
Onefour	Litter retained	fall clip fall compact	1.51	0.46	88.20	-0.03	3.05	abcd
Lethbridge	Litter removed	control	1.55	0.46	88.20	0.01	3.09	abcd
Onefour	Litter retained	no clip fall compact	1.67	0.46	88.20	0.14	3.21	abcde
Lethbridge	Litter removed	no clip fall compact	2.25	0.46	88.20	0.71	3.79	bcdef
Stavely	Litter removed	spring clip no compact	2.94	0.46	88.20	1.40	4.48	cdefg
Stavely	Litter removed	spring clip spring compact	3.56	0.46	88.20	2.02	5.10	defg
Stavely	Litter retained	spring clip no compact	3.60	0.46	88.20	2.06	5.14	defg
Stavely	Litter retained	spring clip spring compact	3.61	0.46	88.20	2.07	5.14	defg
Stavely	Litter retained	fall clip no compact	4.06	0.46	88.20	2.52	5.59	efg
Stavely	Litter retained	no clip fall compact	4.17	0.46	88.20	2.63	5.71	fg

Stavely	Litter removed	no clip spring compact	4.21	0.46	88.20	2.67	5.75	fg
Stavely	Litter removed	fall clip fall compact	4.32	0.46	88.20	2.78	5.86	fg
Stavely	Litter retained	no clip spring compact	4.37	0.46	88.20	2.83	5.91	fg
Stavely	Litter retained	control	4.41	0.46	88.20	2.87	5.95	fg
Stavely	Litter removed	control	4.68	0.46	88.20	3.14	6.22	fg
Stavely	Litter removed	no clip fall compact	4.69	0.46	88.20	3.15	6.23	fg
Stavely	Litter retained	fall clip fall compact	4.80	0.46	88.20	3.26	6.33	g
Stavely	Litter removed	fall clip no compact	4.93	0.46	88.20	3.39	6.47	g

Appendix M: Diagram of Example of Experimental Design.

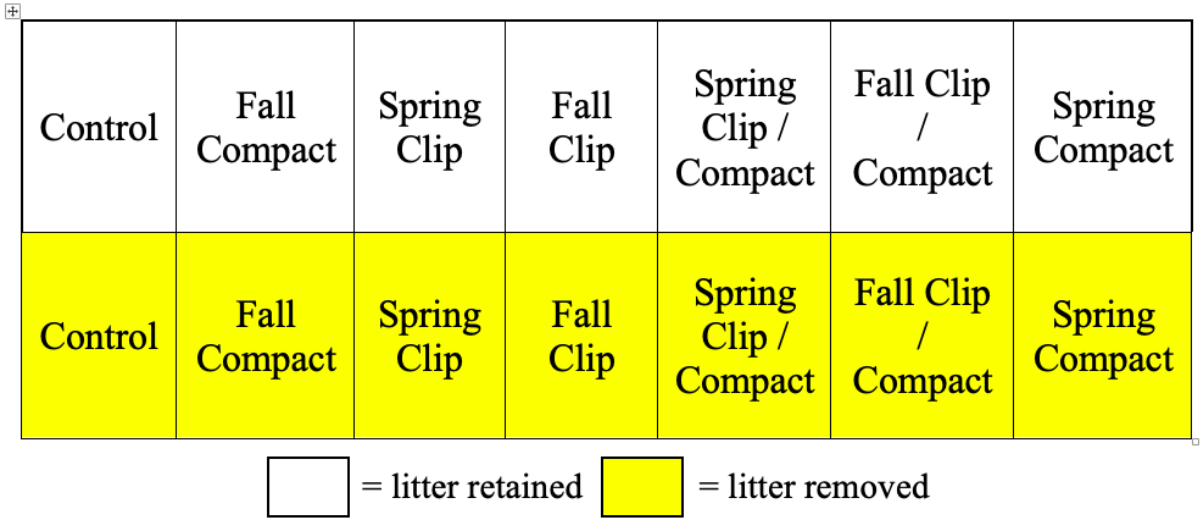

Figure M1: Example of the layout of one block of treatment in the study. Each box represents one 1.5 x 1.5 m plot, with the text inside denoting one of seven seasonal clipping or compaction treatments. Rows of plots with different colours denote different litter manipulation treatments.