Effect of Grazing on Litter Decomposition and Extracellular Enzyme Activity across Agro-climatic Subregions in Alberta

by

Xiaozhu Chuan

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Rangeland and Wildlife Resources

Department of Agriculture, Food & Nutritional Science
University of Alberta

Abstract

Grasslands cover approximately 40% of the Earth's terrestrial surface and provide a wide range of ecologically and economically important services such as forage production, carbon (C) and nitrogen (N) storage, and wildlife habitat. Livestock grazing is a ubiquitous use of grasslands around the globe; however, the direct and indirect effects of grazing on ecosystem processes including C and nutrient cycling, are still overlooked. To better understand how livestock grazing and associated shifts in plant community compositions affect ecosystem function, we studied decomposition of litter of different grass species and extracellular enzyme activity (EEA) over 18-months using litterbags, placed inside and outside of long-term grazing exclosures at 15 sites across three grassland subregions, including the Central Parkland, Foothills Fescue and Mixedgrass Prairie. Overall, livestock grazing increased litter decomposition rates and EEAs in litter samples, though this response varied among subregions and individual enzymes; decomposition was most rapid in the Foothills Fescue followed by the Parkland and Mixedgrass subregions. While grazing enhanced enzymes activities regulating C cycling, it decreased those associated with N and P cycling decomposing litter. No effects of grazing on soil EEAs were detected. Litter types also altered litter decomposition rates and EEAs regardless of subregion or grazing effects. *P. pratensis* had particularly high decomposition rates and EEAs, especially in the Foothills, and a similar pattern existed for *B. gracilis* in the Mixedgrass, suggesting increases in these grazing tolerant species may alter biogeochemical cycling, and therefore C accumulation. Results from this study suggest that grazing could play a critical role in regulating litter decomposition and C cycling in the northern temperate grassland.

"The most exciting phrase to hear in science, the one that heralds the most discoveries, is not 'Eureka!'(I found it!) but 'That's funny …'"

- Isaac Asimov

To my beloved parents and grandparents,
who offered unconditional love and support throughout my life.
To Xiaoyu Sun,
who makes me be a better man.

Acknowledgements

This thesis represents the culmination of three years of hard work and involving the assistance of numerous people. This work could not have been completed without the contribution of my committee members, who guided me through my graduate program. I would like to extend my thanks first and foremost to my super co-supervisor Drs. Edward Bork and Cameron Carlyle, for their guidance, patience, and willingness to share their knowledge and experience. I would also like to thank all members of my supervisory committee – Drs. Scott Chang and Daniel Hewins – for taking the time to consider being my committee member and to offer valuable feedback and suggestions for improvement. I would also like to take this opportunity to extend a special thanks to Dr. Daniel Hewins, as my mentor, committee member and true friend. It has been an honor to share time with him in the lab and field. In particular, you have provided me with valuable insight into my research, and your consistent enthusiastic help and encouragement (put more hairs on my chest!!) when I felt as though I had lost my way. Through this experience I gained the confidence to always move forward to reach my goals. I also thank Dr. Miles Dyck for kindly agreeing to be my external examiner for my thesis defense.

This study would not have been possible without the help of many people who assisted with sample collection in the field, preparation of soil and vegetation materials, and sample processing for lab analyses. These individuals include Mike Alexander, Barry Adams, Laura Blonski, Lisa Raatz and Dr. Barry Irving. Laboratory staff members who provided help throughout included Robin Miles, Drs Kelvin Lien and Urmila Basu. My thanks to fellow graduate students including Mark Lyseng, Ashton Sturm, Angela Phung, Kassia James, Faezeh Najafi, Carly Moore, Ekaterine Stolnikova, Lysandra Pyle, Monica Kohler and Donald Schoderbek. I am particularly grateful to my field assistants HongJing Feng, Diana Nguyen, Leah Rodvang, Mariem Oloroso, Grace Carscallen, Karen Linquist, Claire Kisko,

Janelle King and Sean Morstad, such that it is almost impossible to thank everyone working in this project appropriately.

This project would not have been possible without the generous financial and technical support from the following institutions and organizations, for which I am grateful: the Alberta Livestock and Meat Agency Ltd. (ALMA) – Grant # 2014E007R, Alberta Innovates – Bio Solutions, the Rangeland Research Institute (RRI) at the University of Alberta, and Alberta Environment and Parks (AEP).

Last, but most important, my heartfelt gratitude goes out to my family: my parents, my grandparents, my aunt and uncle and my cousin; my friend – Xin Cao. In particular my parents, whose unwavering and consummate faith in me to complete a project of this magnitude always motivated and encouraged me to persevere in the face of my own doubts and reservations.

Table of Contents

Abstract ii
Acknowledgements v
List of Figures ix
List of Tables xi
Chapter 1. An Introduction to the Effects of Livestock Grazing on Carbon Cycling in Alberta Grasslands 1
1.1 Introduction 1
1.1.1 Grasslands 1
1.1.2 Grassland in Canada 1
1.1.3 Grasslands in Alberta 2
1.1.4 Dry Mixedgrass Prairie 3
1.1.5 Central Parkland 4
1.1.6 Foothills Fescue 4
1.2 Grassland and Carbon Storage 5
1.3 Litter Decomposition 8
1.3.1 Controls on Decomposition 9
1.3.2 Grazing and Decomposition 11
1.4 Extracellular Enzyme Activity 13
1.4.1 Important Enzymes in Terrestrial Ecosystems 13
1.4.2 Controls of Extracellular Enzyme Activity 16
1.4.3 Extracellular Enzyme Activity Modeling 18
1.5 Synthesis 18
1.6 Research Objectives 19
Chapter 2. Long-term Grazing and Regional Climate Effects on Litter Decomposition Rates in Northern Temperate Grasslands 22
2.1 Introduction 22
2.2 Materials and Methods 24
2.2.1 Study Area 24
2.2.2 Study Species 25
2.2.3 Experimental Design 26
2.2.4 Litterbags 27
2.2.5 Litterbag Collection and Processing 27
2.2.6 Statistical Analysis 28
2.3 Results 30
2.3.1 Litter Quality and Mass Loss 30
2.3.2 Grazing and Subregion Effects on Decomposition 30
2.3.3 Grazing and Litter Type Effects in Each Subregion 31
2.3.4 Decay Constant k for Litter C 32
2.4 Discussion 33
2.4.1 Grazing Impacts on Decomposition 33
2.4.2 Litter Type Impacts on Decomposition 35
2.5 Conclusions 40
Chapter 3. Grazing Effects on Soil and Litter Extracellular Enzyme Activity across Northern Temperate Grasslands 49
3.1 Introduction 49
3.2 Materials and Methods 52
3.2.1 Study Area and Experimental Design 52
3.2.2 Litter Species 53
3.2.3 Litterbag Preparation 53

3.2.4 Sample Collection and Processing 54
3.2.5 Extracellular Enzyme Assays (EEA) 55
3.2.6 Statistical Analysis 56
3.3 Results 58
3.3.1 Grazing and Subregion Effects on Common Litter cEEAs 58
3.3.2 Grazing Effects on Litter cEEAs in Each Subregion 59
3.3.3 Grazing and Subregion Effects on Soil cEEAs 60
3.3.4 Environmental Effects on cEEAs 60
3.4 Discussion 61
3.4.1 Grazing Effects on EEAs 61
3.4.2 Litter Type Effects on EEAs 65
3.5 Conclusions 68
Chapter 4. Synthesis 81
4.1 Introduction 81
4.2 Summary of Key Results 82
4.3 Future Direction 84
4.4 Conclusion 85
Bibliography 87
Appendices 106
Appendix A: Agroclimatic Conditions 106
Appendix B: Natural Subregions of Alberta 107
Appendix C: Study Site Information 108
Appendix D: Litterbag Deployment and Retrieval Schedule 109
Appendix E: Litterbag Processing 111
Appendix F: Litter Mass Summary Data 112
Appendix G: Mass Loss and *k* Value Summary 113
Appendix H: Summary ANOVA Results for the 3-Way Assessment of Litter Mass and *k* Values 114
Appendix I: Summary of Litter Enzyme Activties among 18 Months 115
Appendix J: Supplementary Information for Chaters 2 and 3. 118
Appendix K: Summary ANOVA Results for the 3-Way Assessment of Enzyme Activties on Four Common Litter Types 120

List of Figures

Figure 2.1. Mean (±SE) ash-free dry mass remaining (%) of each grazing treatment in the (A) Foothills and (B) Parkland subregions. Decay curves were fitted for each dataset using single-pool exponential decay function (Eqn. 1). Mean *k* values of different grazing treatments with different letters differ ($P < 0.05$). 44

Figure 2.2. Ash-free mean (±SE) dry mass of litter remaining (%) of each species with each of the (A) Foothills, (B) Mixedgrass and (C) Parkland subregion. Mean *k* values of litter types with different letters differ ($P < 0.05$).....45

Figure 2.3. Mean (±SE) k_{carbon}/k_{lit} ratios for each of (A) *P. smithii*, (B) *B. gracilis*, (C) *F. campestris*, (D) *F. hallii*, (E) *H. comata*, (F) *K. macrantha*, (G) *P. pratensis*, and (H) the community litter mix, in relation to grazing and/or subregion. 46

Figure 2.4. Relationship between k_{carbon} and k_{lit} of (A) *P. smithii*, (B) *B. gracilis*, (C) *F. campestris*, (D) *F. hallii*, (E) *H. comata*, (F) *K. macrantha*, (G) *P. pratensis*, and (H) the community litter mix within each subregion. Data are combined across grazing treatments. Regression lines are dashed and 1:1 line is solid.....47

Figure 2.5. Relationship between decay constant k_{lit} of different litter types and (A) initial C:N ratio, (B) initial lignin (%) concentration, and (C) lignin:N ratios of litter. Regressions are best fit lines across all litter sources.....48

Figure 3.1. The effect of grazing treatment on mean (±SE) cEEA of acid phosphatase (Phos), β-xylosidase (Xylo), β-glucosidase (BG), N-Acetyl-glucosaminidase (NAG), and β-Cellobiosidase (Cello) of four common litter types. Error bars represent standard error. Upper case letters represent differences between grazing treatments at $P < 0.05$ and lower cases represent differences between grazing treatments at $P = 0.07$. 75

Figure 3.2. The effect of subregion on mean (±SE) cEEAs of acid phosphatase (Phos), β-xylosidase (Xylo), β-glucosidase (BG), N-Acetyl-glucosaminidase (NAG), and β-Cellobiosidase (Cello) of four litter types common to all study sites. Error bars represent standard error. Different letters represent differences among subregion means within each EEA ($P < 0.05$).....76

Figure 3.3. The interaction effects between subregion and grazing on mean (±SE) cumulative (A) β-glucosidase (BG) and (B) β-Cellobiosidase (Cello) activity of four litter types common to all study sites. Different letters represent significance between means ($P < 0.05$). 77

Figure 3.4. Mean (±SE) cumulative EEAs of (A) acid phosphatase (Phos), and (B) N-Acetyl-glucosaminidase (NAG) for each of *P. smithii*, *F. campestris*, *K. macrantha* and *P. pratensis* in the Foothills subregion during the 18-month study. Different letters represent significant differences among means ($P < 0.05$).....78

Figure 3.5. Non-metric multidimensional scaling (NMDS) ordinations showing patterns of cEEAs in the the 0-5 cm mineral layer (left) and LFH mulch layer (right) of soil. Vector lengths indicate the strength of relationships between environmental factors and the activity of enzymes. 79

Figure 3.6. Non-metric multidimensional scaling (NMDS) ordination showing patterns of cEEAs for each of four litter types common to all study sites. Vector lengths indicate the strength of relationships between environmental factors and the activity of enzymes. MG: Mixedgrass, PK: Parkland, FH: Foothills; PS: *P. smithii*, KM: *K. macrantha*, CE: Cellulose and CM: Community mix. 80

List of Tables

Table 1.1. Extracellular enzymes used in the study and their functions. 21

Table 2.1. Mean (±SE) initial concentration of lignin, carbon (C) and nitrogen (N), and the initial lignin to N ratio and C to N ratio for each litter species. 41

Table 2.2. Two-way ANOVA results showing the effects of grazing, litter type, and their interaction on decay constant *k* and the final litter mass remaining, assessed by subregion. Bold values indicate significant effects ($P < 0.05$). 42

Table 2.3. Statistical tests comparing the k_{carbon} and k_{lit} intercept to the 0 origin. Significant coefficients are shown in bold ($P < 0.05$). 43

Table 3.1. Mean (±SE) cumulative enzyme activities (cEEAs) over an 18 month period for litter types common to all study sites. Cumulative activity was calculated by integrating enzyme activity over time; results are presented in units of micro-moles of substrate per g of litter sample (μmol g^{-1}). Phos: acid phosphatase, Xylo: β-xylosidase, BG: β-glucosidase, NAG: N-Acetyl-glucosaminidase and Cello: β-Cellobiosidase. 70

Table 3.2. Results of the ANOVA tests for the effect of specific litter types, grazing treatments and their interactions, on the cumulative 18 month activity of each of five extracellular enzymes, within each of three subregions. Bold indicates effects that are significant ($P < 0.10$). 71

Table 3.3. Mean (±SE) aggregate activity over an 18 month period for each of five EEAs (μmol g^{-1} h^{-1}) associated with different litter types (i.e., plant species) in each of three subregions in Alberta, Canada. 72

Table 3.4. Non-metric multidimensional scaling (NMDS) results showing relationships between soil cEEAs and grazing, mean annual precipitation (MAP), mean annual temperature (MAT), annual heat moisture index (AH:M), soil pH, carbon (C), nitrogen (N), C to N ratio (C:N) and soil organic matter (SOM). Significant environmental variables are in bold ($P < 0.10$). 73

Table 3.5. Non-metric multidimensional scaling (NMDS) results showing relationships between litter cEEAs and grazing, mean annual precipitation (MAP), mean annual temperature (MAT), annual heat moisture index (AH:M), environmental pH, and soil organic matter (SOM). Significant environmental variables are in bold ($P < 0.05$). 74

List of Symbols and Abbreviations

°C – Degrees Celsius
^{14}C – Carbon-14
AB – Alberta
ABMI – Alberta Biodiversity Monitoring Inistitue
AEP – Alberta Environment and Parks
AESRD – Alberta Environment and Sustainable Resource Development
AH:M – Annual Heat/Moisture Index
ANOVA – Analysis of Variance
AUM – Animal Unit Month
BG – β-glucosidase
C – Carbon
CA – California
CDI – Climate Decomposition Index
cEEA – Cumulative Extracellular Enzyme Activity
Cello – β-Cellobiosidase
cm – centimeter
CO_2 – Carbon Dioxide
CP – Central Parkland
DMG – Dry Mixedgrass
DNA - Deoxyribonucleic Acid
e – Natural Logarithm
EE – Extracellular Enzyme
EEA – Extracellular Enzyme Activity
EG&S – Ecological Goods and Services
FF – Foothills Fescue
g – gram
GDP – Gross Domestic Product
Gt – Gigaton
H_2O_2 – Hydrogen Peroxide
ha – hectare
hr – hours
IA – Iowa
k value – Decay Constant
k_{carbon} – Decay Constant of Carbon
k_{lit} – Decay Constant of Litter
L-DOPA – L-3, 4-dihydrophenylalanine
LFH – Litter-Fibric-Hemic
LIDET – Long-term Intersite Decomposition Experiment
m – meter
M – Million
MAP – Mean Annual Precipitation
MAT – Mean Annual Temperature
MI – Michigan
ml – milliliter
mm – millimeter
mM – millimolar
MO – Missouri
MUB – Methylumbelliferone
N – Nitrogen

NAG – N-acetyl-glucosaminidase
NAU – Net Absorbance Units
NFU – Net Fluorescence Units
nm – nanometer
NMDS – Non-metric Multidimensional Scaling
NY – New York
OM – Organic Matter
ON – Ontario
P – Phosphorus
PA – Pennsylvania
Pero – Peroxidase
Pg – Petagram
pH – Potential of Hydrogen
Phos – Acid Phosphatase
ppm – Parts per million
PPO – Phenol Oxidase
RRA – Rangeland Reference Area
SE – Standard Error
SOM – Soil Organic Matter
USA – United States of America
Xylo – β-xylosidase
yr – years
μM – micromolar
μmol – micromole

Chapter 1. An Introduction to the Effects of Livestock Grazing on Carbon Cycling in Alberta Grasslands

1.1 Introduction

1.1.1 Grasslands

Grasslands are dominated by herbaceous plants with less than 10% tree and shrub cover (White 1983) and occupy approximately 40% of the Earth's terrestrial surface (Holechek et al. 1994). Grasslands are known for their role in providing ecological goods and services (EG&S), including biodiversity, forage and carbon (C) storage (Wang et al. 2014). Grasslands also play a pivotal role in supporting the global livestock industry, which contributes an estimated $1.4 trillion of global revenue (Thornton 2010) making up an estimated 2.5% of global GDP (FAO, 2015). These effects are likely to be more pronounced in rural, arid or developing areas, where the livestock industry contributes a greater proportion to GDP (Delgado et al. 2008). However, high livestock densities and associated rangeland use levels can affect grassland ecosystems, including reducing the EG&S they provide (Jones et al. 2004).

1.1.2 Grassland in Canada

In Canada, grasslands have a long history of use by humans and animals. Bison grazed the Canadian prairies starting approximately 10,000 years ago during the Holocene (Dyck 1983). Aboriginals started to immigrate to these areas around 16,000 years ago (Goebel et al. 2008). Since then, indigenous tribes have used bison as a food source (Binnema 2006). Around 1600, new European settlers appeared on grasslands as the demand for trade in beaver fur increased (Willms et al. 2011). Much of the prairies were settled during the 19th century by Europeans, with the population rising from a few thousand in the early 19th century to over seven million by 1910 (Bailey et al. 2010). Rapid settlement of western

Canada facilitated the extirpation of bison from the Canadian prairies by the late 1800s (Morgan 1980) and widespread conversion of grasslands to cropland.

Settlers introduced cattle and sheep as the primary livestock to Alberta and the remaining grasslands were generally overgrazed during the 19th and early 20th centuries, with stocking rates and densities exceeding their carrying capacity (AESRD, 2013). The combination of high stocking rates, excessive cultivation and severe drought resulted in substantial degradation events, including the aptly named Dust Bowl, during the 1920s and 1930s, which led to major negative economic and ecological impacts across the Canadian prairies. Since then, ranchers have progressively reduced their livestock stocking rates on both private and public lands. Acceptable stocking rates on public land have been lowered from 20 ha/AU to 36 to 40 ha/AU at present in the dry mixedgrass prairie (Adams et al. 2004). Other management improvements, such as the change of grazing season and infrastructure to enhance livestock distribution, have also improved grassland condition.

1.1.3 Grasslands in Alberta

Alberta grasslands are home to a wide variety of ecological diversity, including a reliable forage base that supports the livestock industry. The Alberta livestock industry in 2016 has about 1.8 M breeding cattle (e.g., cows, heifers and bulls), and together with their calves and stocker animals, rely on grasslands for much of their forage. The total beef industry contributed ~$5.23 billion in farm cash receipts in 2015 (Government of Alberta, 2016).

Native grasslands cover roughly 16% of Alberta (9.6 M ha), extending west to the Rocky Mountains and north to the southern edge of the boreal forest, and are considered a part of the Great Plains of North America (Castelli et al. 2005). Alberta's grasslands are roughly grouped into several subregions, including the: Dry Mixedgrass Prairie, Mesic

Mixedgrass, Foothills Fescue, Foothills Parkland, Central Parkland, Northern Fescue, Montane, Subalpine and Alpine (See Appendix A and B), which are each differentiated based on climate, soil and vegetation (Downing and Pettapiece 2006). The research done in this thesis took place in the Dry Mixedgrass Prairie, Central Parkland and Foothills Fescue grassland regions.

1.1.4 Dry Mixedgrass Prairie

The Dry Mixedgrass (DMG) Prairie occupies southeastern Alberta and 7% of the entire province, and comprises 47.5% of the Grassland Natural Region area (ASIC 2000). The DMG is also the driest area of the province. The DMG has a continental climate, with mean annual precipitation of 355 mm. Mean annual temperature of the region is 4°C, with an average daily high of 19°C in July, and a low of -14°C in January (Downing and Pettapiece 2006).

The dominant soil type in the DMG is a Brown Chernozem, with Brown Solodized Solonetz soils on poorly drained areas, and Regosolic soils on unstable ecosites, such as steep slopes, and areas such as sandy dunes subject to wind erosion (AESRD 2013). The *Stipa-Agropyron* community is the most common plant community found in more productive areas of the DMG (i.e., on Dark Brown soils) (Coupland 1961), while dominant community types on brown soils are the *Stipa-Bouteloua, Stipa-Bouteloua-Agropyron* and *Agropyron-Koeleria*, with *Bouteloua gracilis* abundant under heavy grazing (Smoliak et al. 1972). Intensive grazing in the mixedgrass prairie can have negative impacts on the plant community and microenvironment, because it is difficult to accumulate litter in such arid regions, which in turn, can lead to lower production, retrogression in plant community composition and soil surface erosion (Willms et al. 1986; Frank et al. 1995).

1.1.5 Central Parkland

The Central Parkland (CP) is one of three Parkland Natural Subregions, along with the Foothills Parkland and Peace River Parkland, and occupies over 50,000 km^2 in east-central Alberta, geographically forming an arc surrounding the northern and western edges of the Dry Mixedgrass Prairie and Foothills Fescue. It is one of the wetter grassland regions in Alberta, with moderate precipitation (mean annual precipitation 422 mm; Achuff 1994; Downing and Pettapiece 2006) and low evaporation due to cooler temperatures, with most precipitation falling during the growing season (AESRD 2013). The mean annual temperature of the CP is 2°C, with a mean high summer temperature of 17°C in July and low winter temperature of -15°C in January (Downing and Pettapiece 2006). Black and Dark Brown Chernozemic soils predominate in the CP, and Dark Grey Chernozems can be found in areas where aspen encroachment has occurred (AESRD 2013). Historically, native grasslands were dominated by plains rough fescue (*Festuca hallii*), accompanied by a diverse mix of other grasses and forbs. Ecotonal transitions are also abundant, particularly in the absence of fire, and can lead to increasing amounts of aspen (*Populus tremuloides*) and dense shrubs such as *Symphoricarpos occidentalis* and *Elaeagnus commutata*. Fescue grasslands are known to be grazing sensitive, and intensive defoliation over time often results in *Festuca hallii* being replaced by *Agropyron smithii*, *Hesperostipa curtiseta* and *Bouteloua gracilis*, as well as introduced grasses such as *Poa pratensis* and *Bromus inermis* (AESRD 2013).

1.1.6 Foothills Fescue

The Foothills Fescue (FF) is situated in southwestern Alberta, just east of the Rocky Mountains. It is an area well-known for its ability to support winter grazing due to the warm Chinook winds that periodically remove snow during winter. The FF is characterized by relatively high topographic relief, and markedly greater elevation than other prairie

grasslands (Achuff 1994). The FF is wetter and warmer than the adjacent Central Parkland, with a mean annual precipitation of 550 mm (AESRD 2013). Mean annual temperatures in the FF are 5°C, with a high of 17°C in summer and a low of -17°C in winter (Downing and Pettapiece 2006). The dominant soil type in the FF is well-drained Black Chernozems with deep, richly humified surface horizons. Gleysols are found in poorly-drained or periodically wet areas, while Regosols are present on unstable locations such as slopes or eroded surfaces (AESRD 2013). The native grass *Festuca campestris* dominates many communities, particularly those with rich Black Chernozemic soils, with the most common being the *Fesctuca campestris-Danthonia parryii* association, which extends from Montana into southwestern Alberta Foothills (AESRD 2013). Intensive grazing will lead *Festuca campestris* being replaced by more *Danthoania parryii* and *F. idahoensis*, reduce forage yields, and eventually replace the native grasses with *Taraxacum officinale* and *Antennaria parvifolia* (Willms et al. 1985; Dormaar and Willms 1998), as well as various introduced grasses. In many areas of the FF, native fescue communities have been partly or wholly replaced by *P. pratensis*, *Phleum pratense*, and *Bromus inermis* (Willoughby 1997). Thus, the native fescue community is relatively vulnerable to deterioration and sensitive to grazing pressure.

1.2 Grassland and Carbon Storage

Atmospheric carbon dioxide (CO_2) has dramatically increased from 268 to 400 ppm over the past 150 years, as a net result of fossil fuel combustion and human industrial and agricultural activity (Forster et al. 2007). Soil has the capacity to store as much as three times the C found in atmosphere (Davidson et al. 2000). While a portion of this C is inorganic, the vast majority of soil C is transformed into organic matter through decomposition from organisms (Izaurralde et al. 2001). Globally, soil contains an estimated 1500 Gt of organic C

to a depth of 1 m, and 10% of this C is equivalent to all the CO_2 released over the last 30 years (Kirschbaum 2000). These anthropogenic activities have impacted the global climate by increasing the average land and ocean surface temperature by 0.85°C (IPCC 2001).

Grasslands are particularly vulnerable to global change including higher temperatures and more variable rainfall (Polley et al. 2013). Recently grasslands have been recognized for their roles in conserving large C stocks, as they contain a similar amount of C to the Earth's forested ecosystems (Hamilton et al. 2002; Schuman et al. 2002). Most C stored by grasslands is in soil (Scurlock and Hall 1998; Schuman et al. 2002), and converting grassland to cultivated land can lead to loss of 30 to 50% of soil C (Lal 2002). Therefore, conserving C in grassland has the potential to impact the economic and ecological role of native grasslands to society in a global context (Joyce et al. 2013).

Despite the important role of grasslands in C storage, the effect of livestock grazing, one of the most common land uses in grasslands, on C stocks remains limited. Several studies indicate that grazing can increase soil C (Schuman et al. 2009; Dormaar et al. 1984), either by mixing mineral and organic materials in the upper humus (Schuman et al. 1999) or by increasing root biomass (Schuman and Reeder 2002). For example, a study completed at Onefour, Alberta in the DMG by Smoliak et al. (1972) found a 25% increase in soil C after 10 years of heavy grazing relative to the nongrazed exclosure. Moderate grazing led to a smaller increase of 5% in soil C, while light grazing increased soil C by only 4%. The authors attributed the increase in soil C to increased root development under heavy grazing, specifically an increase in *Bouteloua gracilis*. Dormaar et al. (1984) conducted another study at Onefour and in the FF at Stavely, AB, examining a variety of extracellular enzyme assays in soil, and concluded that grazing generated approximately 25% greater soil organic C at both locations, albeit with much higher seasonal variation at Stavely. This finding was attributed primarily to an over-winter microbial and litter buildup as the winter is longer at

Stavely.

Another study conducted on the mixedgrass prairie in Wyoming, USA, found C in the top 0-15 cm of soil increased 24 and 27% under light and intensive grazing, respectively (Schumann et al. 1999), mainly due to the presence of modified plant community composition under long term grazing. Notably, this response was associated with compensatory root growth under grazing, as these sites were dominated by the warm-season increaser grass *Bouteloua gracilis*. A similar positive effect of grazing on soil C was found in the mixedgrass prairie (Henderson et al. 2004).

Despite the for-mentioned studies finding favorable responses in soil C, overall patterns of grazing and soil C remain unpredictable. Several studies have showed no net effect of grazing on soil C. For example, a study at Stavely, AB, of continuous grazing effects on fescue patch dynamics showed a statistically non-significant 4% increase in soil organic matter (SOM) under a low stocking rate relative to a high stocking rate (Willms et al. 1988). Similarly, Henderson et al. (2004) found that while soil C inside grazing exclosures of the DMG in southern AB remained slightly elevated compared to adjacent grazed areas, these differences were not statistically significant. These findings may reflect the removal of standing dead biomass, mitigated by the effects of livestock trampling on soil mixing, and beneficial impacts of litter on microclimate.

Yet other research has shown a decrease in soil C under livestock grazing. Naeth et al. (1991) studied the impact of grazing on soil C and litter at three sites across Alberta, and showed that intensive early season grazing had more negative effects on soil C than light grazing late in the season, though both grazing methods had negative effects on soil C compared to non-grazed areas. A similar study at Stavely, Alberta, also found higher soil C inside livestock exclosures, with 29% less total soil C under moderate grazing, and 46% less soil C under heavy grazing (Dormaar and Willms 1998). These results were associated with a

transformation of soil properties, such as decreased soil moisture and available N% outside the exclosures under livestock grazing.

1.3 Litter Decomposition

Plant litter is comprised of all dead plant material above the ground surface, which is also interchangeable with other terms, such as standing dead or old-dead (Odum 1960; Golley 1965; Coupland 1979). Plant litter is a primary contributor to terrestrial C and nutrient (e.g., organic nitrogen and phosphorus) cycling, and is a critical driver of ecosystem processes (Cou'teaux et al. 1995). Litter can also benefit ecosystem function by increasing soil aggregation, decreasing evaporation, stabilizing soil temperature and moisture, and providing habitat for microbes and microfauna (McGinnies 1987; Holland and Coleman 1987; Risser et al. 1984). The amount of litter in grasslands of western Canada has been closely coupled with herbage production (Willms et al. 1986; Sinton 1980).

Decomposition of litter by microbes into SOM is the process that links litter to nutrient cycling and the ecosystem (Chapman et al. 2005). Litter decomposition determines the amount of nutrients returning to the ecosystem from dead organisms (Chapin et al. 2002; Swift et al. 1979; Woodwell 1994). Raich and Schlesinger (1992) demonstrated that around 70% of annual total CO_2 emissions, which is approximately 68 Pg C, is attributed to litter decomposition.

Litter decomposition is a complex ecological process that involves chemical, physical and biological actions. In general, decomposition consists of three stages (Perez-Suarez et al. 2012). The early phase of decomposition is initiated by leaching of simple and water-soluble labile compounds, such as low-molecular-weight disaccharides. At this stage, rainfall will enhance soluble breakdown, which may lead to a pulse of soluble chemicals (i.e., mineral nutrients) that are necessary for plants growth (Salamanca et al. 2003; Schlesinger et al.

2006). Thereafter, more complex carbohydrates, including cellulose and hemicellulose, will be decomposed at intermediate phases. In the later stage of decomposition, recalcitrant substances such as lignin will be the predominant residue remaining within litter fractions (Berg and Laskowski 2006). Lignin requires more energy to break down due to the stable carbohydrate bond and is one of the slowest molecules to decompose (Couteaux et al. 1995). At this stage, decomposition may be very slow due to a lack of decomposable compounds (Berg and Ekbohm 1991).

1.3.1 Controls on Decomposition

Decomposition is regulated by both abiotic (e.g., temperature and moisture) and biotic factors (e.g., microbial biomass, extracellular enzymes, inherent litter chemistry). Environmental factors, especially climate, can lead to marked variation in decomposition rates from regional to global scales. Studies on single-species litter materials have revealed that litter mass loss and nutrient cycling are closely related to temperature and precipitation, such that litter mass loss is greater in wetter and cooler areas (Meentemeyer 1978; Aerts 1997; Trofymow et al. 2002). Variation in climatic conditions across regions therefore explains differences in decomposition rates, mainly due to the direct effects of climate on temperature and moisture (Hobbie 1996; Gholz et al. 2000). The long-term inter-site decomposition experiment (LIDET) has focused on the effects of substrate quality and global climate on litter decomposition and nitrogen dynamics (Gholz et al. 2000; Parton et al. 2007; Harmon et al. 2009). Models generated from this study suggest that climatic factors are the key drivers of leaf and root decomposition, with the climate decomposition index (CDI) being the single strongest predictor of decomposition rate (Cusack et al. 2009).

Other abiotic factors such as environmental pH and soil texture can also control litter decomposition (Daubenmire and Prusso 1963; Logue and Lindstrom 2010; Legg et al. 2012).

These factors influence decomposition by regulating nutrient cycling and altering microbial community composition. Results of a 10-year study using ^{14}C labelled ryegrass to determine the effects of soil properties on decomposition, showed that after decomposition, high clay soil contained greater litter-converted C than sandy soils, and as a result, soil organic matter remained lower within the sandy soil (Jenkinson 1977). A short-term incubation study evaluating 11 different soil factors on cellulose degradation suggested a negative relationship existed between clay soil and cellulolytic activity, while a positive relationship was evident between soil pH and cellulolytic activity (Schmidt and Ruschmeyer 1958); these findings demonstrate that decomposition processes can be regulated by soil physical characteristics.

Litter chemical composition can also influence decomposition rates (Meentemeyer 1978; Aerts et al. 1997; Chapman et al. 1988; Taylor et al. 1989; Salamanca et al. 1998). There are a number of chemical attributes that affect the rate at which plant matter breaks down, including initial cellulose concentrations. Leaves with high cellulose are preferred by microbes and disappear more rapidly. In contrast, leaves with more lignin are less degradable (Kirk 1983; Harbone 1997). Additionally, high concentrations of secondary plant compounds, such as polyphenolics (e.g., tannins), can form complex compounds with extracellular enzymes, which inhibit the enzymes evolved in reaction and therein slow down decomposition (Harrison 1971; Hättenschwiler and Vitousek 2000). Several studies have also found either no or only weak significance in decomposition rates among different litter species (Klemmedson 1992; Prescott et al. 2000b), while others have found unexplainable patterns of litter chemistry relative to the pattern in litter decomposition (Anderson and Hetherington 1999). Further investigation is needed of the interactions of litter chemistry on litter decomposition.

Plant community composition can also affect decomposition by altering the microclimate surrounding litter material, due both to the physical structure of the litter layer

and mediating effects of plant species identity on initial litter quality, such as phenolic content. A study conducted in Hulun Buir meadows of Inner Mongolia found that mixed litter had strong effects on decomposition, such that the latter was faster than in any one single species alone (Zhang et al. 2013). A mixture of litter from different species changes the physio-chemical environment at the litter surface, which in turn, affects microorganism abundance and activity (McArthur et al. 1994; Kaneko and Salamanca 1999; Hansen and Coleman 1998; Wardle 2002). In addition, heterogeneous litter types provide greater habitats for more microarthropod species than a litter medium of monoculture origin (Hansen and Coleman 1998).

1.3.2 Grazing and Decomposition

Despite the known importance of litter decomposition for ecosystem function, and our understanding of the abiotic (e.g., climate) and biotic controls (e.g., litter chemistry) of decomposition, relatively little is known of how human activities affect this integral process. On grassland, livestock grazing has inherently changed the abiotic (e.g., soil structure) and biotic environment by altering plant community composition (including diversity) and structure, which in turn, may alter the average quality of plant litter entering the soil, and eventually affect the belowground microbial community (Holland and Detling 1990; Wardle et al. 2002).

Grazing can affect vegetation by changing plants morphologically and physiologically at the individual plant scale, as well as altering plant species abundance at the population and community level (Coughenour 1991). Previous studies show grazing alters plant communities in a manner that increases the relative abundance and composition of grazing tolerant species and those species avoided by herbivores, aptly named increasers, and which can have different nutrient demands and inherent chemical structure, such as lignin content, compared

to plants more susceptible to grazing (i.e., decreasers) (Adler et al. 2001). Consequently, grazing-induced plant community shifts will influence the quality of litter returned to soil (Holland et al. 1992; Bardgett and Wardle 2003). In addition, removal of the litter layer by livestock and direct soil trampling by animals may alter soil characteristics, such as soil bulk density, moisture and pH (Lavado and Taboada 1988). Better direct contact of litter with soil following grazing events may accelerate the gradual transition of litter into mineral horizons within the soil (Naeth et al. 1991). Meanwhile, grazing also has the potential to promote the abundance and growth of plants, in part by enhancing the dominance of nitrogen-rich litter while increasing soil available nitrogen, which in turn, can increase litter decomposition rates and plant regrowth rates (Holland and Detling 1990; Augustine and McNaughton 1998; Olofsson and Oksanen 2002). Plant regrowth following herbivory often contains greater nutrient concentrations, and can contribute to increased decomposition of shoots (Ruess and McNaughton 1987; Jaramillo and Detling 1988).

Large herbivores also affect decomposition by altering soil microbial communities and the associated environment for decomposers (Shariff et al. 1994; Bardgett et al. 1998). The latter occurs via deposition of dung and urine, which increases available nutrient pools for microbes and vegetation communities (Seagle and McNaughton 1992; Tracy and Frank 1998; Sankaran and Augustine 2004). Several studies have found that soil microbial diversity increased under low and moderate grazing (Bardgett et al. 2001).

In summary, litter decomposition does not respond to human disturbance in a consistent and predictable manner, with decomposition influenced by a diverse array of environmental factors. Thus, to quantify biogeochemical cycling within various argo-ecosystems, there is a need to better understand the effect of human land use (i.e., livestock grazing) and its associated abiotic and biotic effects (i.e., plant community change and microclimate conditions) on litter decomposition.

1.4 Extracellular Enzyme Activity

Extracellular enzymes (EEs), secreted by both microbes and plants, catalyze the decomposition of organic substrates (Allison et al. 2007). EEs have been studied for more than a hundred years with a focus on linking mechanisms of litter decomposition to biogeochemical cycling (Skujins 1978). Our current understanding of EEs show that they have many functions, including serving as the main catalysts in reactions necessary for litter decomposition (Waring 2013), enhancing soil stability and physio-chemical properties (Amador et al. 1997), changing microbial community structure and composition (Waldrop et al. 2000, Kourtev et al. 2002), regulating the vegetation community (Sinsabaugh et al. 2002), helping organic matter formation, and aiding the acquisition of vital nutrient resources for the decomposer (Dick 1997; Sinsabaugh et al. 2009; Brzostek and Finzi 2011; Allison et al. 2010).

By releasing EEs into the surrounding environment, soil microbes break down complex organic matter into smaller soluble molecules, which allows plants and microbes to take up nutrients necessary for growth from the soil, including nitrogen (N) and phosphorus (P) (German et al. 2003). For example, hydrolytic enzymes such as cellulase break down carbohydrates (i.e., cellulose and hemicellulose) into simple sugars (i.e., glucose) that are critical for microbial growth and metabolism (Katz and Reese 1968).

1.4.1 Important Enzymes in Terrestrial Ecosystems

Within terrestrial ecosystems, there is a group of enzymes important for soil metabolic processes (McLaren 1975) that contribute to biochemical reactions and nutrient cycling in soil (Kiss et al. 1978). These enzymes include β-glucosidase, cellulase, xylosidase, phosphatase, and acetyl-glucosaminidase, all of which are released from plants, animals or

microorganisms (Kanfer et al. 1974; James et al. 1991; Richmond 1991; Gianfreda 2015). The detailed characteristics of enzymes included in this research are listed in Table 1.1. The following paragraphs will discuss some important enzymes relative to nutrient cycling.

Glucose is an important energy source for sustaining microbial growth (Bauchop and Elsden 1960). The EE β-glucosidase is one of the most common and predominant enzymes in the environment (Eivazi and Tabatabai 1988) as it catalyzes the hydrolysis of the glycosidic bonds in β-glucosides that are abundant in plant litter, and therefore release glucose, which is an important C source for microbes (Ajwa and Tabatabai 1994; Esen 1993). It is also an important soil quality indicator, and thus, is a powerful tool to manage soil responses to disturbance, including measuring ecological changes (Bandick and Dick 1999).

Cellulose is the most abundant compound in ecosystems, and consists of up to 50% of the biomass of vegetation photosynthesized from CO_2 (Eriksson et al. 1990). However, cellulose can not be directly metabolized by microbes and first has to be broken down into small-molecule-weight compounds, such as glucose and cellobiose (White 1982). Enzymes that catalyze cellulose degradation are known as cellulase. In general, there are two subgroups of cellulase: one subgroup targets terminal glucosidic bonds in cellulose, while the other breaks down the hydrolysis of internal bonds (Ghose 1977). A broad variety of fungi and bacteria produce cellulase, such as white-rot fungi including *Trichoderma ressei* and *Trichoderma viride* (Nakari-Setälä and Penttilä 1995; Hayashi et al. 1997).

As a fundamental component of proteins and nucleic acids, N supports life and controls primary productivity in terrestrial and aquatic ecosystems (Canfield et al. 2010; Vitousek and Howarth 1991). Microbes have been suggested to primarily contribute to N cycling, including nitrogen fixation, nitrification and denitrification. In general, small-molecule-weight N monomers, including amino acids and amino sugars, are degraded from more complex compounds, such as proteins and aminopolysaccharides (Sylvia et al. 2005). For example,

aminopolysaccharides are broken down by chitinase. Chitinase splits chitin, which is an essential component to cell walls, into dimers of chitobiose. The EE N-acetyl-glucosaminidase then depolymerizes chitobiose into the product of N-acetyl-glucosamine, which in turn, is taken up by microbes (Isobe and Ohte 2014). These N-related enzymes play a pivotal role in regulating N cycling and provide important nutrients for microbial growth.

Another important element in nutrient cycling is P, as studies have shown that this element is a major component of microbial DNA assemblages and plant growth (Speir and Ross 1978). Many studies have explored the relationship between the supply of P and enzyme activity, revealing that P production and activity are linked to the biological demand of microbes (Juma and Tabatabai 1978; Spiers and McGill 1979; Sinsabaugh et al. 1993; Tadano et al. 1993; Clarholm 1993). Levels of P are also an important indicator of soil fertility (Dick et al. 2000). Plants can increase their secretion of acid phosphatase to enhance P solubilization and remobilization (Mudge et al. 2002; Versaw and Harrison 2002).

Unlike other substrates, lignin is the most challenging substrate for enzymes to break down. The amount and composition of lignin in the latter stages of decomposition acts as a primary control to enzyme activity, since lignin retards enzymes from lysing cell wall polysaccharides (Tallbot et al. 2012), and also influences the quantity of C fixed into soil organic matter from litter (Theuerl and Buscot 2010). Degradation of lignin is an oxidative process that requires phenol oxidases and peroxidases. Phenol oxidases use oxygen as an electron acceptor to oxidize phenolic compounds in litter, whereas peroxidases contain a haeme group in the activity sites that use H_2O_2 as an electron acceptor (Sinsabaugh 2010). Enzyme assay normally measures phenol oxidase activities at the rate of substrate oxidation, while peroxidase activity is measured at the rate of substrate oxidation when H_2O_2 is added. Oxidase activities are more variable than activities of hydrolytic EE, which is mainly due to

their broad functions, including phenolics and metal detoxification, antimicrobial defense and lignin degradation (Sinsabaugh et al. 2008; Burke and Cairney 2002).

1.4.2 Controls of Extracellular Enzyme Activity

Since EEs are the primary drivers of microbial nutrient cycling, their activities are often considered indicators of microbial community activity (Schimel and Weintraub 2003; Caldwell 2005; Moorhead and Sinsabaugh 2006). Different ratios in the abundance of C-, N- and P-related enzymes could shed light on microbial community responses to different nutrient inputs and environmental changes, including disturbance and management regimes. Enzyme activities and levels in soil might be highly variable due to different biophysical and chemical characteristics of soil, including the amount of soil organic matter, soil microorganisms, moisture regime, pH and bulk density (Steinweg et al. 2013; Turner 2010). For example, C- and P-related enzyme activities have been found to be greater in the acidic, P-limited upper soil than in the neutral, base cation-rich lower soil (Caldwell et al. 1999).

Enzymes are also sensitive to pH, and only function effectively under specific pH ranges. Most enzymes are stable in a range of pH from 4 to 8, but not those in response to chitinase (4-Methylumbelliferyl-N-acetyl-β-D-glucosamide) and phosphatase (4- Methylumbelliferyl phosphate), which remain stable above a pH of 6.5 and below a pH of 5, respectively (Niemi and Vepsalainen 2005). This is mainly because the concentration of protons will influence enzymes and substrates by altering ionization and solubility (Tabatabai 1994; Turner 2010; German et al. 2011). For example, phosphomonoesterases and proteases show optimal activity at acid and neutral pH, respectively (Eivazi and Tabatabai 1977; Kamimura and Hayano 2000). Moreover, investigation of soil acid and alkaline phosphatase activity suggests that enzyme activity can be used as an indicator of soil fertility and nutrient needs for microbes (Dick et al. 2000).

Similar to litter decomposition, enzymes can be affected by temperature and moisture regime. Moreover, temperature and moisture sensitivity of enzymes across environmental gradients vary with altitude and season within a given geophysical area (Luxhoi et al. 2002; Fenner et al. 2005; Koch et al. 2007; Wallenstein et al. 2009; Kang and Freeman 2009), and are likely to change in response to patterns of temperature and precipitation. For example, drought can strongly limit enzyme activity in soil and govern the effect of temperature (Steinweg et al. 2012). Several studies in old-field environments have found no significant long-term effects of temperature on enzyme activities (Bell and Henry 2011). Other studies have found similar patterns in boreal forest, where although microbial biomass decreased when soil temperature was raised 0.5°C, little effect was found on enzyme activities (Allison and Treseder 2008). However, short-term weather or long-term climate changes will affect enzyme activities in complex ways. Microbes may decrease enzyme secretion in response to warming (Allison 2005), and more rapid denaturing of enzymes in warmer areas could change the enzyme pools available to microbial communities (Wallenstein et al. 2011).

Other factors, including litter quality, soil organic matter, and microbial community composition, are also important to consider when coupling enzyme activities and microbial community characteristics with the biogeochemical cycle (Amin et al. 2013; Rietl et al. 2016). Soluble C compounds are broken down before large complex substrates, no matter the size or type of litter, or soil environmental characteristic such as pH and moisture in litter decomposition (Rinkes et al. 2014). In general, microbes colonize litter rapidly during early decomposition, and the primary production of enzymes may not be as effective compared to the latter stages of decomposition, with lower microbial production associated with higher enzyme efficiency (Amin et al. 2013). It is likely that some microbes will intercept and retard enzyme releases during the different stages of litter decomposition (Allison 2005).

1.4.3 Extracellular Enzyme Activity Modeling

Results collected from EEs are the foundations for developing comprehensive conceptual models to understand microbial dynamics in ecosystems (Schimel and Weintraub 2003). Several modeling studies (Davidson et al. 2012; Moorhead et al. 2012; Wang et al. 2012) illustrate the importance of how future work can use enzyme activity as a proxy to understand ecosystem response to change, since EEs are cost and time effective compared to other measurements. For example, models suggest a negative relationship exists between microbial growth and cumulative enzyme activity, and highlights that the microbial community could be limited when resources become insufficient despite an increase in enzyme production (Moorhead et al. 2013). Other studies also suggested microbial EE production is highly correlated with spatial C dynamics (Schimel and Weintraub 2003; Moore et al. 2013). Coupling of EEA with models can help better predict biogeochemical cycling by identifying microbial community responses, measuring soil organic matter degradation, and quantifying C turnover.

In conclusion, understanding the effect of land use and management on EEA is a key to understanding the links between global biogeochemical cycling and ecosystem function across scales. Additionally, understanding the dynamics of EEA in ecosystems is one of the essential methods available to predict interactions between ecosystem structure and function. Enzyme modeling research can increase predictive power and help to better understand how litter and soil biogeochemistry influence nutrient cycling, and allows comparison of enzymes with functional and physiological groupings of soil microbial and vegetation communities (Caldwell 2005; Amin et al. 2013).

1.5 Synthesis

Grasslands cover a large portion of the Earth's terrestrial surface and provide a broad

range of EG&S, including forage for livestock, water and C storage. Alberta grasslands provide forage that supports a large and economically important livestock industry. Our work focuses on three contrasting natural subregions in central and southern Alberta, including the Dry Mixedgrass, mesic Central Parkland and Foothills Fescue.

Despite the importance of grasslands for livestock grazing, an overlooked component of this ecosystem is how microbial communities and associated nutrient cycles are effected by grazing. Livestock grazing is a key landscape disturbance that shapes the function and properties of grassland (McNaughton 1985). Livestock grazing causes complicated modifications of the grassland ecosystem, with effects on plant growth and chemical composition, nutrient cycling, and community composition, succession, diversity and abundance (Schlesinger et al. 1990; Coughenour 1991; Aguiar et al. 1996), as well as alterations to litter inputs (Naeth et al. 1991, Barger et al. 2004). Grazing can also physically alter ground cover through defoliation and trampling, and chemically alter the flow of energy and nutrients by changing the soil microclimate (Deutsch et al. 2011).

Plant community shifts under grazing may alter grassland ecosystem function. Grazing induced shifts in vegetation may also affect microbes directly or indirectly (Sala et al. 1988). In order to better understand how grazing and associated changes in the plant community affect ecosystem function and nutrient cycling, studies are needed quantifying concurrent litter decomposition and EEAs in grasslands of varied composition and exposure to long-term grazing.

1.6 Research Objectives

The main goal of this study is to investigate the effect of long-term livestock grazing on litter decomposition and EEA across a variety of Alberta grassland subregions, including the Central Parkland, Foothills Fescue and Dry Mixedgrass. This research will shed light on

fundamental patterns of decomposition and enzyme activities in Alberta grasslands. Assessing EEA response to defined perturbations is an effective way to assess microbial functions within the *in-situ* environment. Results from this study will generate insight into how microbial communities and EEA patterns can be used as a tool to assess grassland health and maintain grassland function, including providing important EG&S such as forage production and C storage. The specific objectives for this study include:

1. Measuring the decomposition rates of nine litter types, including 8 common litter sources and an *in-situ* community litter source, from paired grazed and non-grazed treatments, in sites stratified across three subregions in Alberta, and explore if there is a significant effect of grazing, subregions (i.e., soil, climate and vegetation type) and litter types on decomposition (Chapter 2),
2. Assessing seven extracellular enzyme activities associated with litter decomposition on these nine litter types, and investigate if grazing, subregion and litter types alter enzyme activities (Chapter 3),
3. Contrasting seven extracellular enzyme activities on two soil sources (i.e., the LFH mulch layer, and the 0-5 cm mineral horizon), and test if EEAs therein are effected by grazing and subregions, as well as whether EEAs can be linked to local environment and soil biophysal characteristic (e.g., temperature, precipitation, pH and moisture) (Chapter 3).

Chapter 4 summarizes the overall findings, including the key results and highlights of this study. It also proposes future work to address some of the newly identified information gaps. A short discussion is also provided of how existing knowledge and future study can be used to better inform grassland management.

Table 1.1. Extracellular enzymes used in the study and their functions.

Enzyme	Enzyme Class	Functions
β-1,4-glucosidase (Glu)	Cellulase	C Cycling: mediates cellulolysis, releases glucose from litter
Cellobiohydrolase (Cello)		
β-1,4-xylosidase (Xylo)	Hemicellulase	C Cycling: mediates xylolysis, releases xylose from litter
Acid phosphatase (Phos)	Phosphatase	P Cycling: hydrolyze phosphate, releases P from litter
β-N-acetylglucosaminidase (NAG)	Glucosaminidase	N Cycling: hydrolyze aminosaccharides, releases N
L-dihydroxyphenylalanine (L-DOPA)	Ligninase	C Cycling: mediates ligninolysis, releases lignin from litter

Chapter 2. Long-term Grazing and Regional Climate Effects on Litter Decomposition Rates in Northern Temperate Grasslands

2.1 Introduction

Litter decomposition plays a critical role in nutrient (including carbon (C)) cycling at local, regional and global scales (Raich & Schlesinger 1992; Robinson 2002). Litter decomposition is affected by a range of factors, both abiotic and biotic, such as temperature, moisture availability, litter quality, and the size and composition of microbial populations. Climate change caused by anthropogenic emission of CO_2 to the atmosphere affects the fate of plant-derived C during decomposition, which may in turn, alter the sequestration of C in soils (Karlen et al. 1997). In agro-ecosystems, management practices can dramatically alter the factors affecting litter decomposition and associated C cycling. Therefore, understanding how routine management practices affect litter decomposition could help develop strategies to maximize biological C stores, and thereby offset rising CO_2 levels.

Litter decomposition can be influenced by a wide range of environmental factors, including moisture regime and temperature (Meentemeyer 1978; Gartner and Cardon 2004). Previous studies identify temperature as a key factor controlling litter decomposition rates (Aerts 1997; Gholz et al. 2000; Adair et al. 2008). For example, increased temperatures during summer can accelerate chemical reactions (Salah and Scholes 2010). In addition, precipitation is known to control litter decomposition, with greater rainfall accelerating litter breakdown (Swift et al. 1979).

Temperate grasslands are productive ecosystems that contain large amounts of litter derived from annual additions of senesced plant biomass (Lamb 2008). These grasslands are widely utilized by livestock, which through grazing, may alter plant community composition (Jones and Donnelly 2004; Tanentzap and Coomes 2012), and therefore alters the plant chemical inputs into litter pools (Garibaldi et al. 2007). This raises questions about how

livestock grazing induced changes in plant community composition alter key ecosystem processes such as litter decomposition, which closely regulate soil C dynamics and storage.

Plant litter quality (e.g., lignin:N ratio) is an important driver of litter decomposition rates (Heal et al. 1997; Zhang et al. 2008; Prescott 2010). Decomposition has been linked to the microbial community and inherent litter chemistry, including the concentration of nitrogen (N) (Parton et al. 2007), P (Coulson and Butterfield 1978), and lignin (Aerts and De Caluwe 1997). In general, litter decomposes faster when plant litter has higher concentrations of N (Parton et al. 2007) and soluble compounds (Hobbie et al. 2010), while decomposition rates decrease with higher lignin concentration or C:N ratios (Silver and Miya 2001). Strong relationships have also been found between litter decomposition and plant morphological traits, such as leaf area and dry matter content (Cornelissen 1996; Cornelissen and Thompson 1997; Cornwell et al 2008; Kazakou et al. 2009).

Grazing by both wildlife and livestock is pervasive in grasslands globally and can alter a number of grassland ecosystem properties that affect litter decomposition. Grazing removes above ground plant biomass, and can alter soil characteristics (i.e., bulk density and microclimate) as well as nutrient cycling (Bardgett et al. 1998; Jones and Donnelly 2004; Tanentzap and Coomes 2012). Livestock grazing of vegetation reduces surface transpiration and water loss (Moretto et al. 2001; Klumpp et al. 2011), which creates a warm and mesic soil environment that favors decomposition.

Grazing is also well known for altering plant community composition over time, for example, by increasing the relative abundance of avoided or grazing-tolerant plant species (Alder et al. 2001). Inherent differences in the chemical or physiological characteristics of grazing-tolerant plant species, such as low N content in shoots and nutrient re-allocation to root systems, may decrease litter decomposition rates and N mineralization (Dyer et al. 1991; Pastor and Cohen 1997). Individual plants may also change their nutrient allocation, moving

carbohydrates from roots to shoots, and enhancing the decomposition rates of both shoots (Dyer et al. 1991; Holland et al. 1992) and roots (McNaughton and Chapin 1985; Holland and Detling 1990; Semmartin et al. 2004).

Trampling by animals can physically break down litter into smaller particles that are more readily decomposed (Sørensen et al. 2009) because of increased contact with soil microbes (Burke et al. 2011), which increases the decomposition of litter and its incorporation into soil (Schutt et al. 2014). Animal wastes (e.g., feces and urine) can also strongly influence soil C and nutrient pools, as well as microbial communities (Seagle and McNaughton 1992; Sankaran and Augustine 2004), in turn altering decomposition. Despite these changes, long-term grazing will eventually influence the ability of soil microbes to break down grazing-tolerant plant species regardless of their composition, and can lead to positive soil-microbe feedback on nutrient cycling processes over time (Bardgett and Wardle 2003).

The present study aims to assess the effects of long-term livestock grazing on litter decomposition in northern temperate grasslands. We conducted a litterbag study to quantify decomposition rates of litter, including the dominant grassland species that represent typical vegetation shifts due to grazing, and did so in communities with and without long-term grazing. The specific objectives of this study were to: 1) examine how long-term exposure to grazing may impact litter decomposition, 2) evaluate how litter decomposition differs in relation to different litter types, including grasses with divergent grazing response, and 3) assess whether rates of litter decomposition vary among different agroclimatic regions.

2.2 Materials and Methods

2.2.1 Study Area

This study was conducted in three agro-climatic subregions of Alberta, including the

Mixedgrass Prairie, Foothills Fescue and Central Parkland. These subregions vary widely in annual precipitation, annual temperature, and associated soil and vegetation types (Appendix A). In brief, the Mixedgrass Prairie subregion, located in the southeastern portion of the province, is the most xeric with mean annual precipitation of 333 mm yr^{-1}. Vegetation in the Mixedgrass Prairie is a mixture of drought tolerant C_3 and C_4 species, including *Hesperostipa comata*, *Bouteloua gracilis* and *Pascopyrum smithii*, on Brown or Dark Brown Chernozemic soils with about 4 % organic matter (OM; Naeth et al. 1991). The Foothills Fescue subregion is located on the eastern edge of the Rocky Mountains where temperatures are cooler and mean annual precipitation is 470 mm yr^{-1}. Native grasslands in this subregion are dominated by *Festuca campestris* and *Danthonia parryi*, and are on deep, well developed Black Chernozemic soils (OM averaging 11.62%; Naeth et al. 1991). The Central Parkland, located in north-central Alberta, is intermediate in environmental conditions, with a mean annual precipitation of 441 mm yr^{-1}. Vegetation in the Central Parkland includes a mix of *Populus tremuloides* forest interspersed with *Symphoricarpos occidentalis* shrublands and grasslands comprised of a mix of C_3 plants such as *Festuca hallii*, *Hesperostipa curtiseta*, *Pascopyrum smithii* and *Koeleria macrantha*. Grassland soils are generally Black or Eluviated Black Chernozems (8.14% OM; Naeth et al. 1991).

2.2.2 Study Species

We measured the decomposition of seven grass species commonly found across one or more of these study regions. Grazing is known to change species composition within these grasslands, and includes an increase in *Bouteloua gracilis* over other grasses in the Mixedgrass (Smoliak et al. 1972), and the replacement of native *Festuca* spp. and *Hesperostipa* spp. by introduced grasses such as *Poa pratensis* in both the Foothills Fescue (Willms et al.1985) and Parkland (Deutsch et al. 2010). The seven grass species examined

represented a range of grazing tolerance.

Litter of each grass species was gathered at a single location in September of 2013 shortly before senescence to standardize litter age and chemical composition, and thereby facilitate testing of the main treatment. Not all species were included at each site to maintain consistency with the geographic distribution of each species (Appendix C). Additionally, a local plant community litter source collected from each site and a laboratory grade (99% purity) cellulose paper (Fisherbrand® pure cellulose chromatography paper, Fisher Scientific Company, Ottawa, ON, Canada) were included as internal and external controls, respectively. Community litter mix samples were collected in April 2014 before the growing season. Details of each litter type are listed in Appendix C. All plant materials were immediately air dried at 60 °C for 72 hr prior to litterbag preparation to prevent decomposition while in storage. Ten subsamples of each grass species were randomly selected to determine initial moisture content to convert fresh litter mass to dry weight using a wet-dry regression (Harmon et al. 2000).

2.2.3 Experimental Design

Within each subregion, five study sites were randomly selected from the Alberta Environment and Parks (AEP) long-term cattle exclosure sites that were part of their Rangeland Reference Area program. Exclosures were all approximately 20 x 40 m in size and varied in age from 15 to 60 years. Litter decomposition was examined inside of these exclosures and in adjacent grassland where grazing occurs annually, thereby enabling assessment of decomposition within the same ecosite (i.e., on uniform soils, topography, etc.) but in areas of contrasting grazing history, for a total of 30 plant communities (i.e., study plots) in a paired-plot design.

2.2.4 Litterbags

We used litter bags (10 x 10 cm) made of fiberglass mesh (1 x 1 mm openings) to measure decomposition of plant material. A total of 2926 litterbags were filled with 2.00 ± 0.02 g of plant material and sealed with impulse sealers (n=12 per litter type per plant community). Each litterbag was individually numbered by attaching aluminum numbered tags for later identification and retrieval in the field. The details for litterbag use are provided in Appendix D. All samples were prepared in the lab prior to field deployment. After careful transport to the field to avoid the loss of litter from bags (which could bias results), bags were installed in each of the 30 plant communities. Finally, litterbags were anchored onto the soil surface in a 5 x 10 m area within each plant community using 3 inch sod staples at each site on the 25th of May, 2014.

2.2.5 Litterbag Collection and Processing

Two randomly selected litterbags of each litter type were retrieved from each plot after 0, 1, 3, 6, 12 and 18 months (May, June, August and October of 2014, and May and October of 2015). We immediately placed samples on dry ice to preserve the biochemical condition of microbial enzymes within samples at the time of collection (Hewins et al. 2016). Litterbags were then transported to the lab and stored at -20°C prior to analysis. To prepare samples for analysis, any visible live vegetation and soil particles adhering to the litterbags were removed. Litter samples were then removed from bags, weighed to determine mass loss, and a subsample assessed for moisture content, ash-free dry mass, and C and N concentration.

Subsamples used to determine moisture correction were dried at 60°C in an oven for 48 hr. The remaining dried sample was ground in a ball mill (Spec Sample Prep, Metuchen, NJ, USA) to 0.1 mm and assessed for ash-free content by placing it in a muffle oven for 6 hr at 550°C, and then reweighing when cool. Concentrations of C and N were determined using an

elemental analyzer (LECO® TruSpec Micro analyzer, LECO Corporation, MI, USA). Each sample was run in duplicate and known standard samples (Orchard leaves and Tobacco leaves, 502-055 and 502-082; LECO Corporation, St. Joseph, MI, USA) were included after every tenth sample. Ash free C and N mass were expressed as the percentage of C and N concentrations of decomposing litter. Litter C and N mass were calculated by multiplying the C and N concentration of litter (mg g^{-1}) by litter ash free dry mass remaining (in g) over the course of the study. In addition, initial lignin concentration was measured using the acetyl bromide method (Iiyama and Wallis 1990).

2.2.6 Statistical Analysis

All duplicate measurements within a plot and sampling time were first averaged. Decay constants (*k* values) were estimated by fitting a single pooled exponential decay function (Eqn. 1; Olson 1963) to the ash-free dry litter mass remaining against successive collection times. To fit the model, we used the following equation:

$$M_t = M_0 e^{-kt} \qquad \text{Equation 1}$$

Where :

M_t is the ash-free litter mass remaining at a specific time,

M_0 is the initial mass of litter,

e is the base of the natural logarithm, and

k is the decay constant.

Decay constants were estimated using R software (R Foundation for Statistical Computing, Vienna, Austria). To meet the requirements of parametric statistical tests, all data were checked for normality prior to analysis and log transformations were applied to mass loss data. For transformed data, all results and figures present original non-transformed data to facilitate interpretation. All duplicate measurements were averaged prior to statistical

analysis.

All response variables were subject to analysis of variance (ANOVA) using a generalized linear mixed model. Fixed effects included grazing treatment, subregion and litter type, as well as all interactions. Study site (n=5) was nested within subregion (Mixedgrass, Parkland and Foothills Fescue) as a random effect. Significance of effects was assessed at $\alpha \leq 0.05$ and *post-hoc* mean comparisons were conducted where applicable using a Tukey's test at $\alpha \leq 0.05$. Due to the unbalanced presence of individual litter types among study sites, it was impractical to test for interactions of litter type with subregion using a single analysis. Thus, the analysis was partitioned into separate ANOVAs to address our objectives.

To evaluate how subregion and grazing alter litter decomposition, the decay constant (k) and final mass remaining (%) of the cellulose and community mix litter types were examined separately using a two-way ANOVA. Similarly, a three-way ANOVA was performed using litter from *K.macrantha* and *P. smithii* to test if those litter types common to all 15 study sites had any effect on decomposition in a broader context.

To simplify the analysis and enable a more direct comparison of litter type and grazing effects with each subregion, ANOVAs were run for each subregion to test the effect of grazing treatment and litter type (i.e., all those litter types present within a subregion) as fixed effects. This process simplified the analysis and allowed for more direct comparison of litter type and grazing effects within each subregion, the latter of which had already been tested in the initial ANOVAs.

Finally, decay constants (*k* values) for litter C were estimated to quantify the rate of litter C decay using Eqn. 1. The relationship between litter decay (k_{lit}) and litter C decay constant (k_{carbon}) (hereafter k_{carbon}/k_{lit} ratio) was examined using simple linear regression for each litter type. This analysis could assist in explaining how rapidly C was lost during decomposition. Curves in the figures were fitted using the Dynamic Fitting tool in Sigmaplot v.12 (Systat

Software Inc., San Jose, CA). In addition, the initial lignin concentration, C:N ratio and lignin:N ratio were regressed to k_{lit} to assess the relationship between initial chemical content and decomposition rates.

2.3 Results

2.3.1 Litter Quality and Mass Loss

Initial litter quality varied among litter types for each of the following: lignin concentration, C and N concentration, and the initial ratios of lignin to N, and C to N ($P < 0.001$). Comparative initial litter quality attributes measured from each litter type indicated that *P. pratensis* and *B. gracilis* typically had the greatest N concentration, while the community mix litter and *B. gracilis* had the greatest lignin concentration (Table 2.1).

Decomposition of all litter types followed a negative exponential trend over the 18-month study period ($R^2 \geq 0.73$). We also used a double and triple pool exponential to fit litter mass data over time (Farrar et al. 2012), with no improvement to observed model fitting. During the first three months (May to August, 2014), litter decomposed to approximately 60% of its original mass (Appendix E), with the exception of the cellulose paper and community litter, which remained at 97.4% ± 2.4 (SE) and 91.1% ± 2.4, respectively, of its original mass. Between 3 and 6 months in the field (by October, 2014), decomposition of plant litter slowed substantially. After 18 months, the mass remaining of each litter type was 29 to 45%, depending on litter type (Appendix E). In general, we detected two rapid decomposition stages, one during each summer period (May to August, 2014 and May to October 2015), and a slow decomposition period during winter (August 2014 to May 2015).

2.3.2 Grazing and Subregion Effects on Decomposition

Grazing had no significant effect on the k_{lit} of the two standardized controls, cellulose

and the community mix ($P > 0.05$). Grazing did have a significant effect ($P < 0.05$) on the final mass remaining of the community mix litter (Grazed $_{community\ mix}$ 35.6% ± 2.5; Non-grazed $_{community\ mix}$ 45.8% ± 2.3), but not that of cellulose (Appendix F). The decay constant and final mass remaining of cellulose also varied among subregions (k: $F_{2, 27} = 3.58$, $P = 0.05$; final mass: $F_{2, 50} = 5.41$, $P = 0.011$), with no such response in the community mix litter. Measured decay constants of cellulose in the Foothills subregion were greater than that in the Parkland, and final mass remaining of cellulose was lower in the Foothills than in the other two subregions (Appendix F).

The three-way ANOVA conducted on the two plant species common to all sites (*P. smithii* and *K. macrantha*) indicated that grazing had marginal effects on k values and the ash-free dry mass remaining ($P \leq 0.10$) after 18 months (Appendix G), with no interactions of grazing by subregion. However, there were differences between the two grass species ($P < 0.001$) in both of these metrics; *P. smithii* decomposed more slowly than *K. macrantha* ($k_{P. smithii}$: 0.67 ± 0.02 vs $k_{K. macrantha}$: 0.82 ±0.04), and consequently had more mass remaining after 18 months (*P. smithii*: 38.8% ± 2.0 vs *K. macrantha*: 29.7% ± 2.3).

2.3.3 Grazing and Litter Type Effects in Each Subregion

Within the Foothills subregion, both grazing and litter type affected decay rates and the final litter mass remaining (Table 2.2), but no interactions were detected. In general, grazing accelerated litter decay in the Foothills, leading to less litter mass remaining in grazed areas after 18 months (Figure 2.1 A).

Among litter types (i.e., individual plant species) overall patterns indicated that the decay constant of *P. pratensis* was greater than that found in the other three grasses ($P < 0.05$) within the Foothills Fescue, with no differences among *F. campestris*, *K. macrantha* and *P. smithii*. In contrast, the opposite trend was found in the final litter mass remaining amongst

these four species (Figure 2.2 A), with lower final litter mass remaining in *P. pratensis*, but only in comparison to *P. smithii*.

Within the Mixedgrass subregion, no effects of grazing were detected on decay rates or final litter mass (Table 2.2). However, different litter types again had different decay rates and mass remaining after 18 months. In this subregion, *B. gracilis* decomposed more rapidly than all other litter species as indicated by its decay constant, with the final mass of *B. gracilis* being lower than that of *P. smithii* (Figure 2.2 B; *B. gracilis*: 24.5% ± 2.5 vs *P. smithii*: 42.0% ± 2.6).

In the Parkland subregion, we again observed effects of litter type, but grazing effects were marginal ($0.05 < P < 0.10$, Table 2.2). Grazing increased decay rates (Grazed: 0.92 ± 0.04; Non-grazed: 0.86 ± 0.04), leading to less litter mass remaining within grazed areas (26.7% ± 1.2) compared to non-grazed areas (30.2% ± 1.6) at the end of the study (Figure 2.1 B). Among litter types, *P. pratensis* once again had greater decomposition rates compared to all other species (Figure 2.2 C). Remaining litter masses at the end of the study in the Parkland only differed between *P. smithii* and *F. hallii*, with the latter lower in final mass.

2.3.4 Decay Constant k for Litter C

We found no evidence of grazing, subregion or interaction effects on k_{carbon} C ($P > 0.05$). However, litter type significantly affected k_{carbon}, with the community mix having the greatest k_{carbon} relative to other litter types (1.862 ± 0.065 for the community mix *vs* ≤ 1.465 for other grass species; $P < 0.001$). In general, no effect of grazing on k_{carbon}/k_{lit} ratio was detected (Figure 2.3).

Finally, there were strong positive linear relationships between k_{lit} and k_{carbon} across all litter types (Figure 2.4), and in all cases followed a ratio near 1:1 except within the community mix (Figure 2.4 H). The statistical analysis suggested that intercepts of both *B.*

gracilis and the community mix differed from the origin ($P < 0.05$), and were indicative of slowed and preferential C decomposition, respectively (Table 2.3). Additionally, intercepts of *P. smithii* and *F. campestris* were marginally different from 0 ($P < 0.10$; Table 2.3), and indicative of increased and decreased C breakdown, respectively. Finally, we found that decay constant *k* values were negatively correlated with initial lignin concentration ($r = -0.93$; $P < 0.001$, C:N ratio ($r = -0.82$; $P < 0.01$) and lignin:N ratio ($r = -0.87$; $P < 0.01$) (Figure 2.5).

2.4 Discussion

2.4.1 Grazing Impacts on Decomposition

Long-term exposure to livestock grazing appeared to increase the decomposition of litter, particularly that of grasses, and was most notable within the more mesic conditions of the Foothills Fescue and Parkland subregions. Similarly, temporal patterns of mass loss directly reflected seasonal changes in the opportunity for litter decomposition, occurring largely during the growing season when temperatures were above freezing (May through October), particularly the spring and early summer, which would favor periods of high plant growth and microbial activity (Aerts 1997). Among the four litter sources examined at all study sites, only cellulose exhibited variable decomposition regionally, with results paralleling that of the grasses tested regionally, exhibiting more rapid decomposition in the Foothills.

The greater decomposition of cellulose, and grasses under grazing, within the wetter and cooler environment of the Foothills Fescue likely reflects the greater soil organic matter and fertility found there, and corresponding increase in microbial activity at this location (Chapter 3). Microbes are more active in more mesic and warmer conditions, and produce extracellular enzymes essential for litter decomposition (DeAngelis et al. 2010). Previous studies have suggested that microbes respond to environmental change more rapidly when conditions

favor their growth, for example, when moisture becomes less limiting (Lundquist et al. 1999; Placella and Firestone 2013). In contrast, the dry soil conditions within the Mixedgrass, together with the lower soil organic matter levels (e.g., Foothills Fescue: 9.02% *vs* Mixedgrass: 5.25%), and presumably associated microbial activity, may play a more dominant role relative to grazing in controlling rates of litter decomposition.

Previous studies have found similar litter decomposition rates between grazed and non-grazed areas (Shariff et al. 1994; Menezes et al. 2001). More rapid decomposition under grazing found here could arise from changes within *in-situ* environmental conditions, such as increased incident radiation (Gallo et al. 2006), enhanced moisture in the soil due to the ongoing removal of vegetation and reduced transpiration (Deutsch et al. 2010), and the increased turnover of nutrients in soil under grazing (McNaughton et al. 1997). In a parallel investigation, Hewins et al. (2015) found reductions in extracellular activity associated with N and P cycling within grasslands exposed to grazing (i.e., relative to those non-grazed), and suggests microbial activity may be less limited in soil under grazed grasslands, which in turn, would enhance decomposition.

Grazing-associated reductions in aboveground live plant biomass and litter, and concomitant increases in the area of bare soil (Naeth et al., 1991), may also increase litter breakdown by enhancing the likelihood of litter samples being in more direct contact with the conditions (i.e., moisture, nutrients, microbes) necessary to support decomposition (Jones et al. 2009). Furthermore, grazing and defoliation are known to increase soil temperature, including within grasslands of the current study area (Bremer et al. 2001; Deutsch et al. 2010), and warmer soils can directly stimulate microbial activity and thereby decomposition (Yates et al. 2000). Increased plant and litter cover, and greater shading, together with reduced proximity to mineral soil, appear to collectively have inhibited litter decomposition within non-grazed areas of the present study.

Finally, our assessment of litter decomposition showed that relative rates of carbon decline (as exhibited by k_{carbon} *vs* k_{lit}) within the various litter types did not vary in relation to grazing. These results indicate that the presence of long-term grazing did not alter the relative loss of carbon from litter, and therefore may be unlikely to affect C transformation, or soil organic carbon formation.

2.4.2 Litter Type Impacts on Decomposition

Litter decomposition is regulated by several factors, including local climate, microbial community composition, as well as inherent litter quality (Couteaux et al. 1995). In this study, although subregions had markedly different soil (e.g., pH, OM and moisture), vegetation and associated climatic conditions, we detected no differences in the decomposition of litter derived from the community mix among subregions. In this situation, we suggest that the heterogeneity of the community litter presents a more chemically complex substrate, which requires a greater diversity of microbes and associated enzymes to degrade. Conversely, the single species grass samples may present a more uniform substrate wherein a relatively select few species of microbes may efficiently mineralize C and nutrients. Despite the wide ranging environmental conditions present in our study, these microbial communities face a similar challenge when attempting to decompose a more heterogeneous resource.

Nevertheless, litter source played a substantial role in governing litter mass loss in the current study. These differences were present both overall, as well as within each individual subregion. Overall rates of mass loss were lowest in the cellulose, intermediate in the mixed community litter, and greatest within the various grass species examined, and is likely a direct reflection of the chemical recalcitrance of each litter source to decomposition. For example, the absence of simple carbohydrates, oils and proteins, all of which are more readily leached and mineralized (Fioretto et al. 2005), likely slowed decomposition of the cellulose

standard. In contrast, the relatively rapid litter mass loss in various grasses, particularly during the first three months in the field, suggests that much of their biomass may have been comprised of simpler carbohydrates (i.e., sugars), hemi-cellulose, or other readily degradable compounds. We also note that as our litter samples were collected in the fall of 2013, these grass samples had already advanced in phenology through to dormancy. This in turn, could have altered (i.e., slowed) subsequent decomposition, at least relative to that of actively growing grass samples, which are known to be much higher in quality (Kazakou et al. 2009).

Among the two grass litter sources tested at all sites, decomposition of *K. macrantha* was greater, though marginally, than that of *P. smithii*. While these species had relatively similar composition of N and C at the start of the study (Table 1), *P. smithii* did have nearly 2% greater lignin, likely a reflection of its tendency to have more elevated stems, and this may have translated into the lower mass loss. As the grazing response of these species is known to differ, with grazing typically favoring *K. macrantha* over *P. smithii* (Dormaar et al. 1994), this finding suggests grazing may alter litter dynamics within grasslands where they co-occur, presumably in conjunction with changes in plant chemistry (Semmartin et al. 2004; Henry et al. 2005; Sherer-Lorenzen 2008).

Within our regional comparisons of grass species, we observed a clear pattern for *P. pratensis* to decompose faster relative to all other species in the moister (Foothills Fescue and Parkland) subregions studied. In contrast, *B. gracilis* decomposed more rapidly within the Mixedgrass Prairie. Given the lack of any grazing by litter type effects on decomposition, our results suggest inherent differences in the chemical composition of different litter sources are more likely to explain these grassland ecosystem C responses, rather than changes associated with the grazed environment (e.g., microclimate) itself. Despite this, grazing is known to influence the relative abundance of different dominant grasses within these grasslands (Smoliak et al. 1972; Willms et al. 1985; Vujnovic et al. 2002), and could thereby markedly

alter the composition of C inputs and subsequent turnover. This conclusion is similar to that of Yates et al. (2000), where changes in plant species composition arising from grazing had a relatively greater effect on litter decomposition than the direct impacts of grazing.

Greater decomposition of *P. pratensis* is notable given that this species is increasing under grazing in many regions of western Canada (Looman 1969; Tyser 1992; Bork et al. 2012), as well as in portions of the northern Mixedgrass of the United States (Biondini et al. 1998). While known to be a relatively productive grass (Willms et al. 1996), *P. pratensis* is typically high in N (Sullivan and Garber 1941), as evidenced here by its low C:N ratio (Table 1), which in turn, may account for its more rapid decomposition. This outcome agrees with previous studies in that litter decomposition occurs more rapidly in litter sources with a lower C:N ratio (or higher initial N concentration). The latter are found in more labile materials (Hättenschwiler and Vitousek 2000; Güsewell and Gessner 2009) and decompose markedly faster than recalcitrant litter sources, in part due to a higher abundance of the bacterial community (Wardle et al. 2004; Rooney et al. 2006; Paterson et al. 2008).

Decomposition is a complex process wherein microbes simultaneously require nutrients (e.g., N) and energy (C) to grow, maintain their activities, and reproduce (Oertli 1993). Litter N is the primary factor constraining litter breakdown during the early phase of decomposition (Gallardo and Merino 1993; Berg 2000; Hobbie et al. 2012). As a result, litter with greater initial N concentration (i.e. lower C:N ratio) generally has more rapid decomposition rates than litter with a lower N concentration, though the demand for litter N will increase as decomposition continues (Ross et al. 2002). Our results across all litter types demonstrated that decomposition rates were negatively correlated with litter lignin:N ratios. While high in C, lignin is a complex compound that is recalcitrant and difficult for microbes to decompose, and is therefore one of the key factors controlling the latter stages of decomposition (Johansson et al. 1995). Decomposition can be expected to be particularly slow for plant

species with a high lignin:N ratio (Taylor et al. 1989), such as found in litter derived from the later-seral grasses *P. smithii* and *F. campestris* in the current study.

The relatively greater decomposition rate of *B. gracilis* within the Mixedgrass was unexpected, as this species is a warm-season plant with higher fiber and lower digestibility, including of plant N (Pittermann and Sage 2000), and has been associated with reduced turnover in this species (Gill et al. 2002), thereby leading to greater soil C (Henderson et al. 2004). One possibility is that being the only warm-season grass tested, at the time of litter collection in late fall, this species may have still been in a relatively vegetative stage of growth, which would have altered litter composition of this species towards higher N. This in turn, would have favored more rapid decomposition, particularly in comparison to the other cool-season grasses, which by fall would have been several months into dormancy.

The extent of decomposition within the community mix was lower than that of the pure grass samples, and mass losses early on in the mix were more similar to that of the cellulose control. This greater recalcitrance may reflect differences in chemical composition, such as the greater initial lignin content. Community mixes represented a diversity of plant species, including forbs, the latter of which would have been relegated to lignified residual stems at the time of removal from the field due to their extensive senescence. Community litter would also have undergone additional decay following the growing season over the late fall and winter prior to collection in spring 2014, thereby accounting for the lower quality and slower decomposition at the start of the litterbag trial.

Despite the slow decomposition of community litter, we found more rapid C turnover (i.e., higher litter C to litter mass decay constant ratio) in the community mix litter sources compared to that from single grass species. Past studies have found that different chemical and physical properties of species in the community can affect decomposition (Quested et al. 2002; Liu et al. 2007; Perez-Suarez et al. 2012). For example, the community mix likely

consisted of numerous forb species, which are known to contain less complex C compounds than grasses (Henry et al. 2005) and more N (Bork et al. 2012). Previous studies from the study region here indicate that forbs may comprise as much as 20% of total biomass in the Foothills Fescue (Bork et al. 2012), 31% in the Parkland (Deustch et al. 2010) and 16% in the Mixedgrass Prairie (Bork and Irving 2015).

The presence of litter from forbs could ultimately account for the higher k_{carbon} in mixed litter sources. As moderate grazing increases the composition of forbs (Hayes and Holl 2003; Kimball and Schiffman 2003), this shift could, in theory, accelerate rates of C turnover, by altering soil microbial community composition and richness (Chapman and Newman 2010; De Marco et al. 2011). A more diverse (heterogeneous) soil microbial community could enhance the decomposition of mixed litter by supporting higher microbial abundance and activity, even when the litter source had a greater amount of both recalcitrant and N-enriched compounds (Scherer-Lorenzen 2008). Although we do not have direct evidence for changes in microbial community composition in the current study, assessment of extracellular enzyme activity (EEA) could help us to better understand the process and mechanisms associated with mixed litter decomposition (Chapter 3).

In comparison to the community litter, our results revealed most grasses had similar rates of C turnover relative to mass loss, with a few exceptions. *B. gracilis* had a k_{carbon}/k_{lit} ratio lower than 1, suggesting the litter of this species may become C enriched over time with advancing decomposition. In this situation, reduced C turnover rather than enhanced C inputs may explain the consistent positive relationships observed between *B. gracilis* abundance and soil C with grazing (Henderson et al. 2004), the latter of which favors this species (Smoliak et al. 1972; Sims and Coupland 1979; Kelly and Burke 1997). Notably, this may occur despite the relatively rapid overall decomposition rates noted earlier for this species. In contrast to *B. gracilis*, both *P. pratensis* and *P. smithii* tended to have C turnover greater than that of overall

litter mass. This suggests these species may be less conducive to building ecosystem C over time (assuming a similar biomass input level), provided the fate of this C is decomposition and CO_2 release to the atmosphere rather than via leaching or other transformations into the soil.

2.5 Conclusions

Litter decomposition in relation to long-term grazing has not been broadly studied in northern temperate grassland ecosystems, including how this process may vary among dominant grass species. I conclude that grazing generally increased litter decomposition, particularly in wetter subregions. Litter type also mediated litter decomposition, with selected single grass species (e.g., *P. pratensis*, *B. gracilis*) and the community mix demonstrating the slow decomposition. In general, differences in litter decomposition and associated changes in C concentration were complex and a function of land use activity (e.g., livestock grazing), as well as environmental conditions (e.g., soil and climate). These findings have important implications for understanding litter decomposition and C accumulation within grassland ecosystems. Future studies focusing on litter decomposition in grasslands would benefit from exploring the mechanisms of decomposition, by examining extracellular enzyme activities (Chapter 3) and nutrient transfer with isotope labels, to enable more precise predictions of litter decomposition under regional variations and land use-induced microclimate changes.

Table 2.1. Mean (±SE) initial concentration of lignin, carbon (C) and nitrogen (N), and the initial lignin to N ratio and C to N ratio for each litter species.

Species	Initial concentration (%)			Initial concentration ratio	
	lignin	C	N	lignin:N	C:N
P. smithii	9.91±0.52c[1]	46.16±0.23b	0.81±0.08ab	12.24±0.21c	56.83±1.33c
B. gracilis	11.69±0.282d	47.10±0.31c	1.07±0.01c	10.92±0.51c	43.89±1.28b
F. campestris	9.18±0.31b	47.52±0.38c	1.00±0.01bc	9.18±0.31b	52.25±0.91bc
F. hallii	7.36±0.32a	46.92±0.40b	0.99±0.05bc	7.43±0.41a	47.48±1.52b
H. comata	7.49±0.66a	47.14±0.36c	0.83±0.02b	9.02±0.33b	56.65±1.39c
K. macrantha	8.08±0.42a	45.48±0.54a	0.74±0.03a	10.91±0.48c	61.07±1.42c
P. pratensis	8.99±0.65b	44.62±0.64a	1.17±0.02d	7.69±0.32a	38.16±2.01a
Community mix	12.16±0.87d	46.40±0.28b	0.89±0.08b	13.66±0.61d	56.52±1.07c

[1] Within a column, means with different letters differ, $P < 0.05$.

Table 2.2. Two-way ANOVA results showing the effects of grazing, litter type, and their interaction on decay constant *k* and the final litter mass remaining, assessed by subregion. Bold values indicate significant effects ($P < 0.05$).

Source	*k* value								
	Foothills			Mixedgrass			Parkland		
	F	Df	*P*	F	Df	*P*	F	Df	*P*
Grazing (G)	6.29	1,38	**0.017**	1.60	1,38	0.22	3.19	1,48	0.08
Litter Type (LT)	8.60	3,36	**<0.001**	8.05	3,36	**<0.001**	21.68	4,46	**<0.001**
G:LT	0.08	3,36	0.90	0.24	3,34	0.87	0.26	4,46	0.90
Source	Final mass remaining								
	Foothills			Mixedgrass			Parkland		
	F	Df	*P*	F	Df	*P*	F	Df	*P*
Grazing (G)	19.72	1,66	**<0.001**	0.04	1,72	0.83	5.10	1,96	0.056
Litter Type (LT)	45.08	3,64	**<0.001**	38.58	3,70	**<0.001**	110.42	4,93	**<0.001**
G:LT	1.31	3,64	0.27	0.33	3,70	0.89	0.39	4,93	0.88

Table 2.3. Statistical tests comparing the k_{carbon} and k_{lit} intercept to the 0 origin. Significant coefficients are shown in bold ($P < 0.05$).

Litter Type	intercept	t value	*P*-value
P. smithii	0.035	1.836	0.08
B. gracilis	-0.047	-2.36	**0.046**
F. campestris	-0.097	-2.002	0.08
F. hallii	0.003	0.094	0.93
H. comata	-0.005	-0.238	0.81
K. macrantha	-0.027	-1.078	0.29
P. pratensis	0.021	0.479	0.64
Sitemix	0.233	5.741	**<0.001**

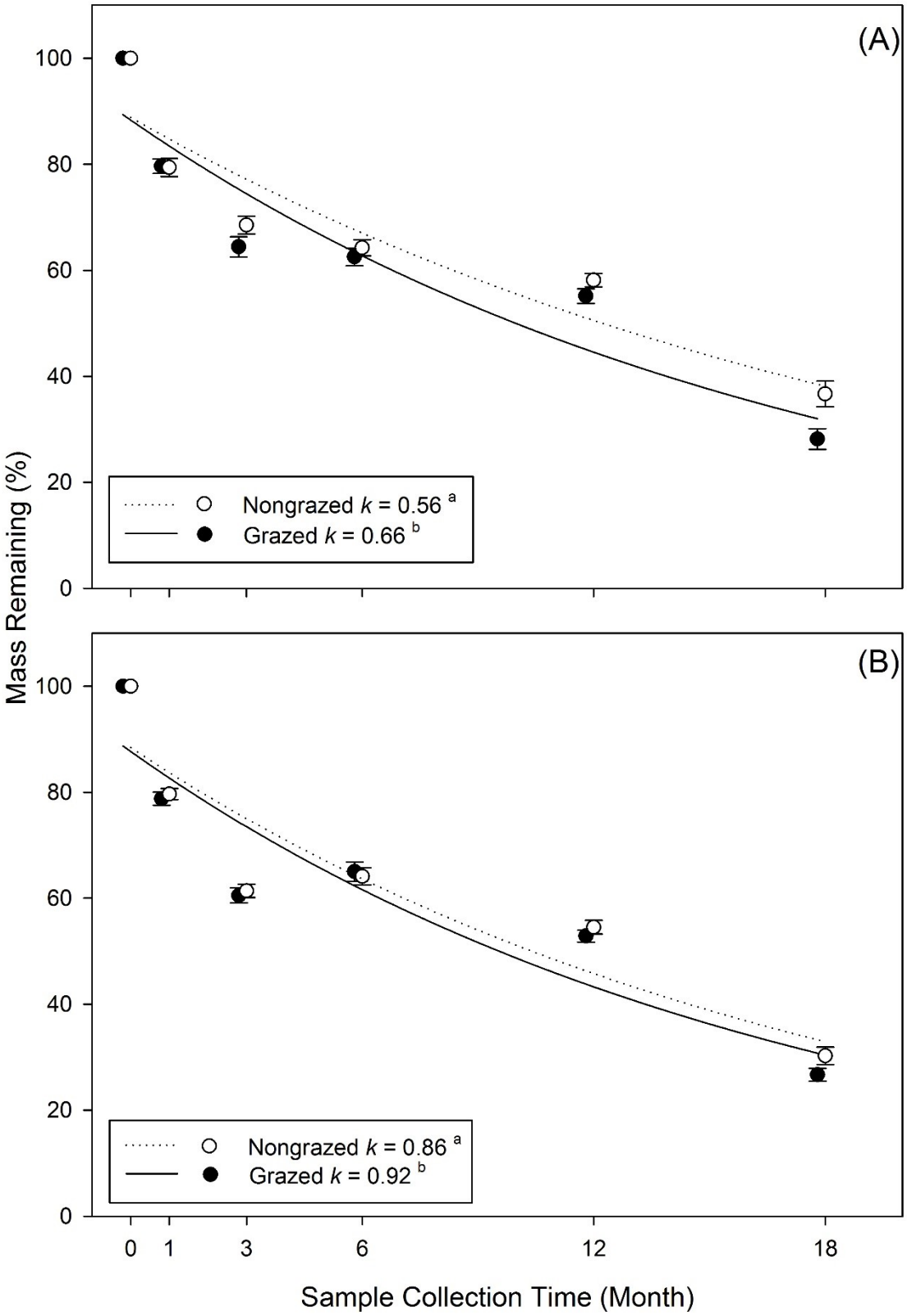

Figure 2.1. Mean (±SE) ash-free dry mass remaining (%) of each grazing treatment in the (A) Foothills and (B) Parkland subregions. Decay curves were fitted for each dataset using single-pool exponential decay function (Eqn. 1). Mean *k* values of different grazing treatments with different letters differ ($P < 0.05$).

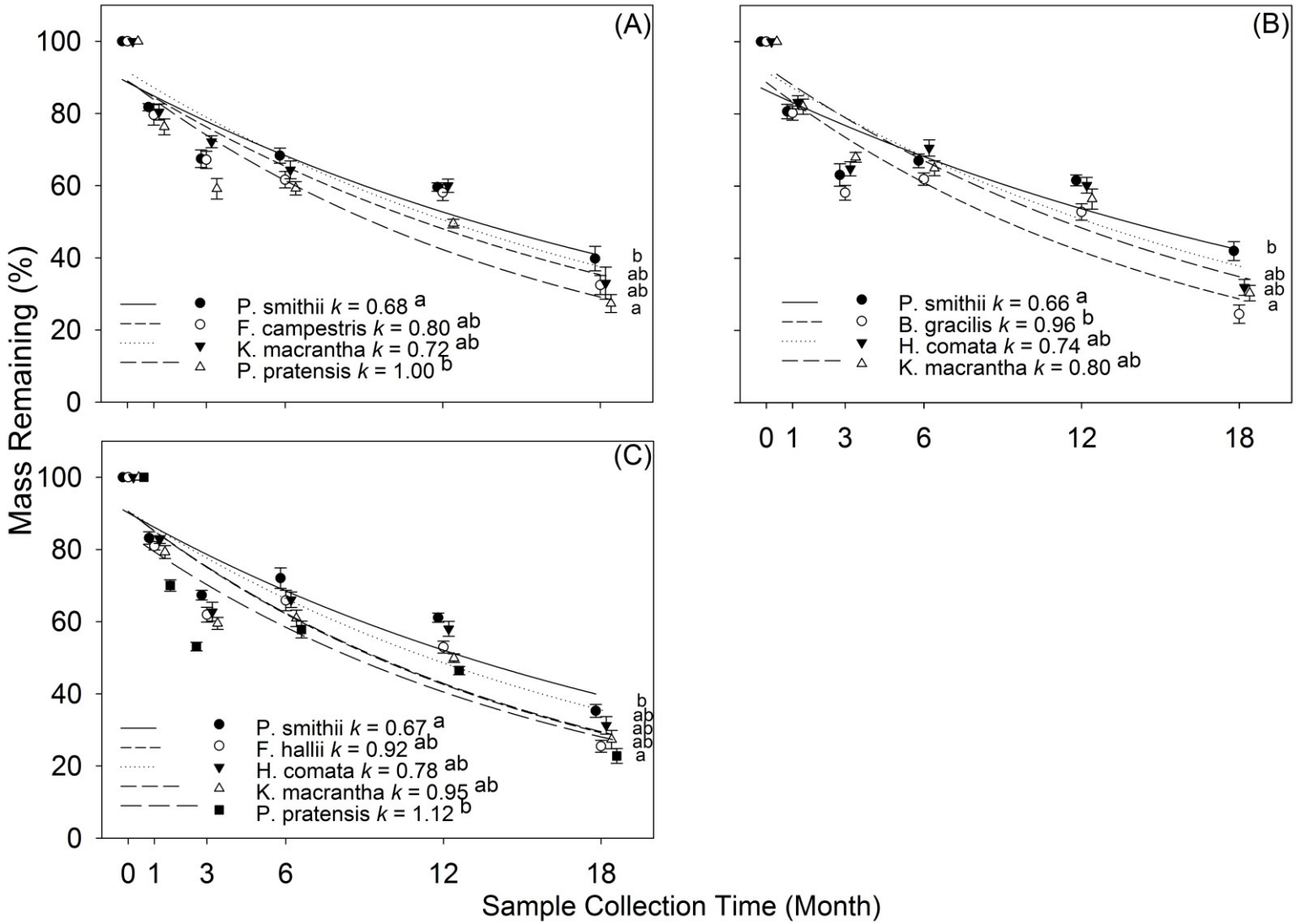

Figure 2.2. Ash-free mean (±SE) dry mass of litter remaining (%) of each species with each of the (A) Foothills, (B) Mixedgrass and (C) Parkland subregion. Mean *k* values of litter types with different letters differ ($P < 0.05$).

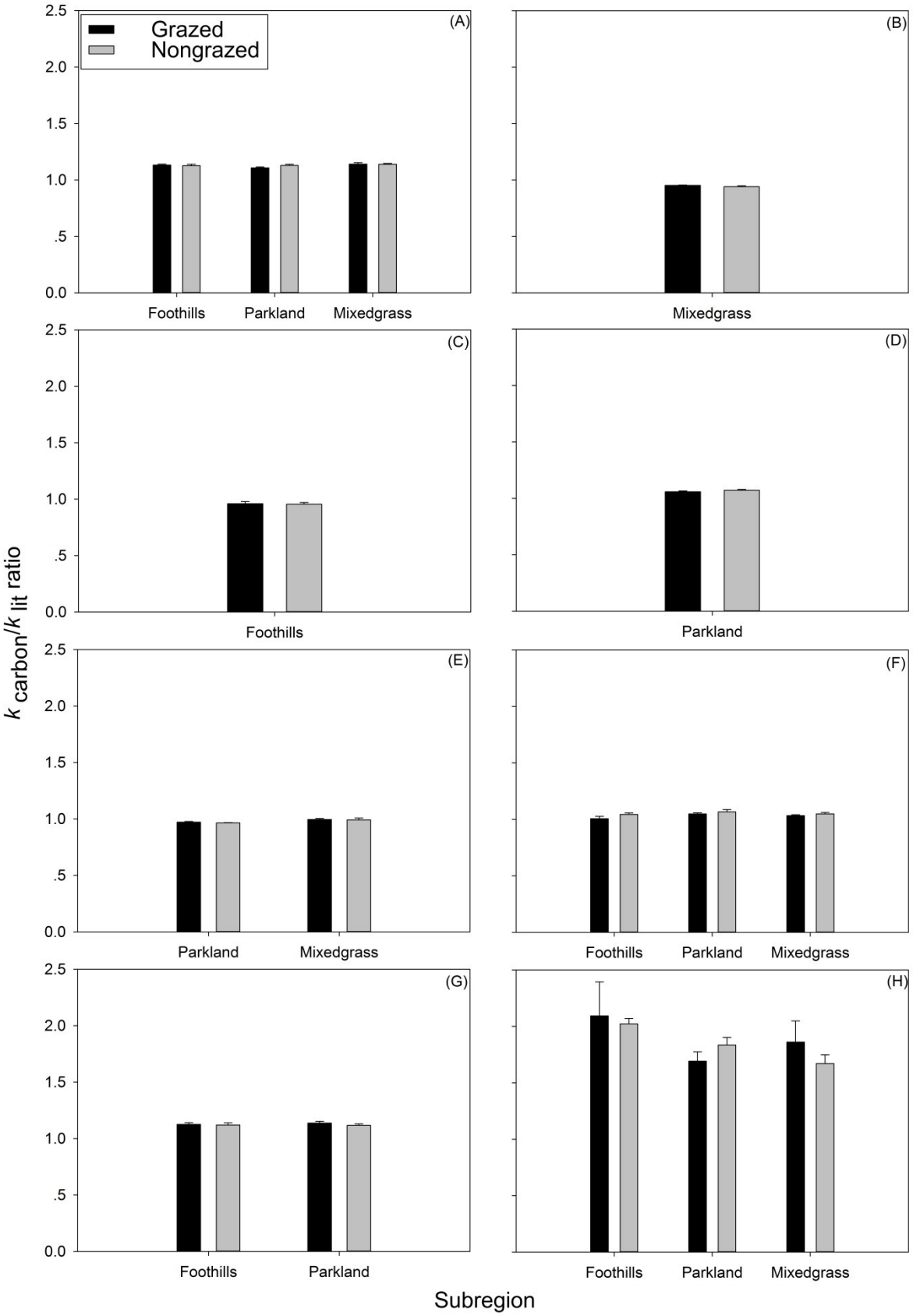

Figure 2.3. Mean (±SE) k_{carbon}/k_{lit} ratios for each of (A) *P. smithii*, (B) *B. gracilis*, (C) *F. campestris*, (D) *F. hallii*, (E) *H. comata*, (F) *K. macrantha*, (G) *P. pratensis*, and (H) the community litter mix, in relation to grazing and/or subregion.

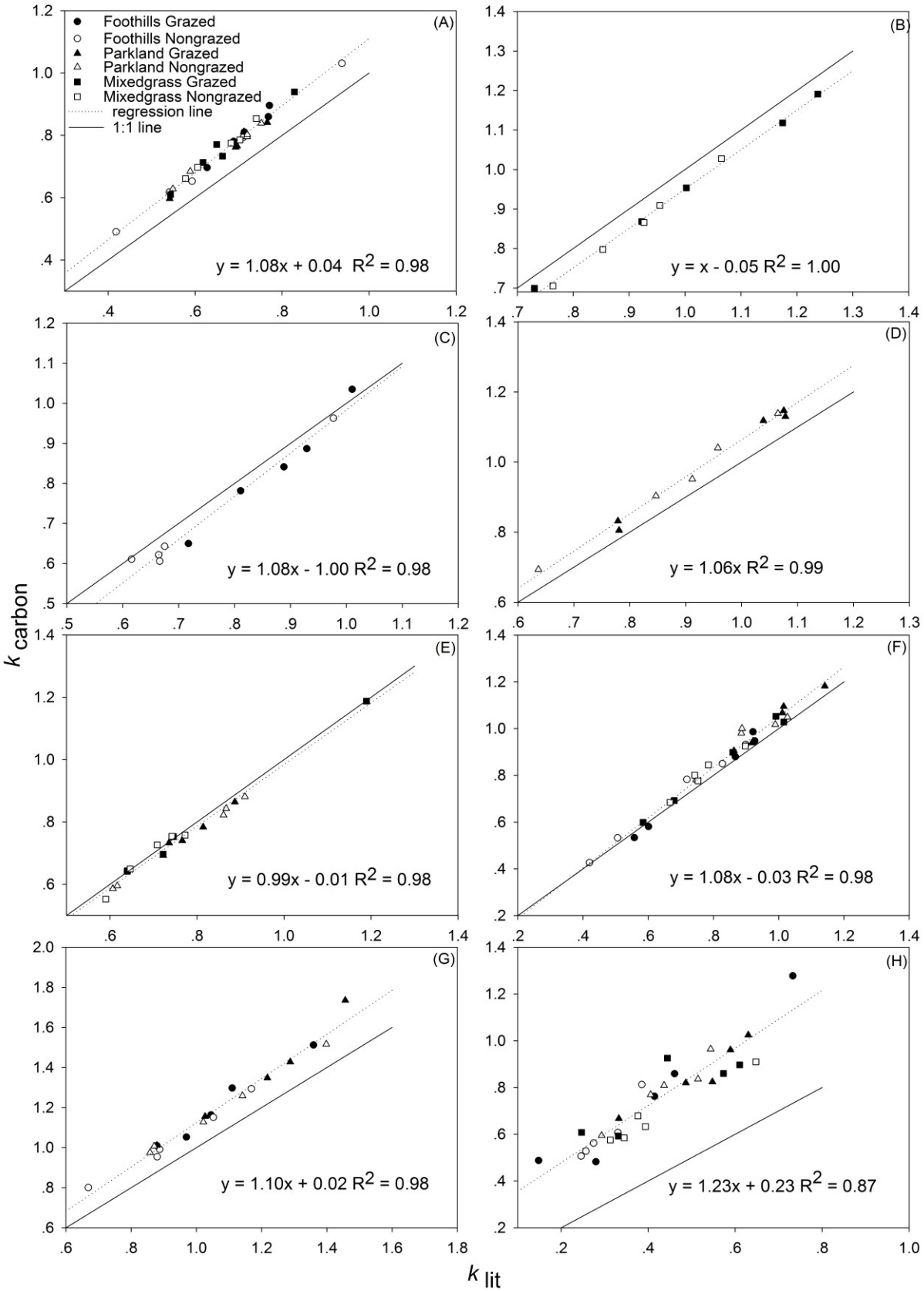

Figure 2.4. Relationship between k_{carbon} and k_{lit} of (A) *P. smithii*, (B) *B. gracilis*, (C) *F. campestris*, (D) *F. hallii*, (E) *H. comata*, (F) *K. macrantha*, (G) *P. pratensis*, and (H) the community litter mix within each subregion. Data are combined across grazing treatments. Regression lines are dashed and 1:1 line is solid.

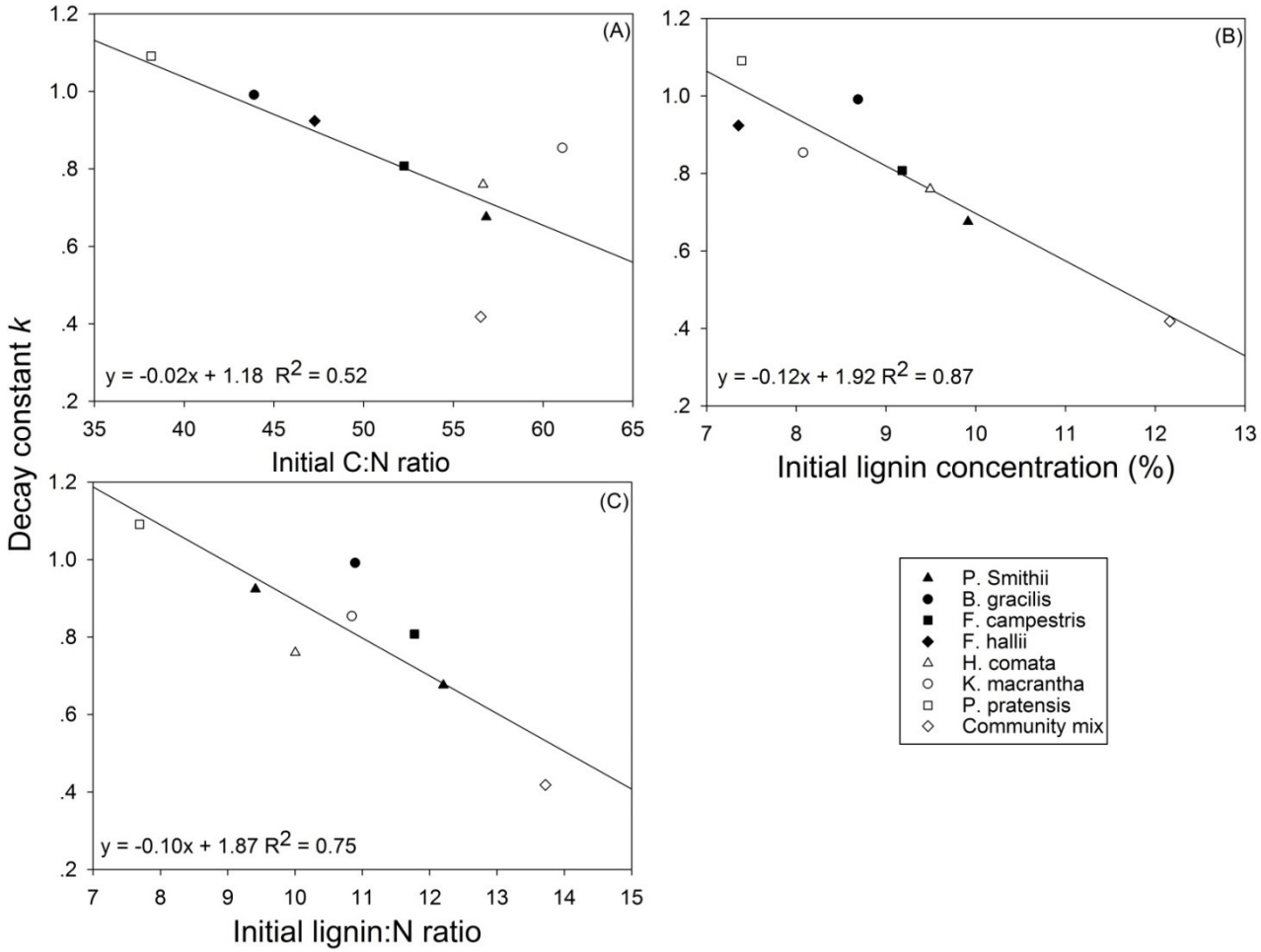

Figure 2.5. Relationship between decay constant k_{lit} of different litter types and (A) initial C:N ratio, (B) initial lignin (%) concentration, and (C) lignin:N ratios of litter. Regressions are best fit lines across all litter sources.

Chapter 3. Grazing Effects on Soil and Litter Extracellular Enzyme Activity across Northern Temperate Grasslands

3.1 Introduction

Grasslands are distributed around the globe and cover approximately 40% of the Earth's terrestrial surface (Holechek et al. 1994). These ecosystems are critical for providing many ecological goods and services (EG&G) such as maintaining biodiversity and wildlife habitat, providing livestock forage, and purifying water and air (Havstad et al. 2007). Additionally, they play a large role in storing carbon (C). Grassland C pools hold 770 Gt of C, which is nearly three times that of the atmospheric C pool (Lal 2004; Oelkers and Cole 2008; Havstad et al. 2007). A major component of soil C is organic matter. Two primary sources contribute to soil organic C: (1) accumulated humus from dead plants, microbes and animals through decomposition, and (2) plant and microbe exudates (i.e., extracellular enzymes, simple and complex sugars) released into soil during plant growth. Therefore, understanding the mechanisms governing soil C could help identify management practices that increase grassland C storage and reduce greenhouse gas emissions.

Livestock grazing affects nutrient cycling in grasslands, as well as C cycling, by altering environmental conditions (i.e., moisture and temperature; Hobbes 1996; Yates et al. 2000; Asner et al. 2004) and plant community composition (McNaughton et al. 1988; Bardgett and Wardle 2003; Leriche et al. 2003). Studies investigating vegetation composition shifts under livestock grazing over time show that grazing favors defoliation tolerant species and decreases the relative abundance of defoliation intolerant species (McNaughton and Augustine 1998; Adler et al. 2001). Inherent litter quality (i.e., low nitrogen (N) concentration) or morph-physiological differences (i.e., C_3 vs. C_4) among grazing-tolerant species may also contribute to reduced levels of mineralized N to sustain microbes (van der Valk 1986; Dyer et

al. 1991). Additionally, animal wastes (e.g., feces and urine) can increase the input of soil C and nutrients, which could affect the soil microbial community and associated enzyme activity (Singh and Rai 2004).

Extracellular enzymes, released from plant roots and soil biota, are an integral component of biogeochemical cycling that are important for catalyzing litter breakdown, and ultimately affect biogeochemical cycling, which in turn, may enhance microbial activity (Sinsabaugh et al. 1991; Dick et al. 1994; Romani et al. 2006). Extracellular enzyme activity (EEA) is a strong indicator of biogeochemical cycling within the ecosystem (Moorhead and Sinsabaugh 2000), and can therefore be used to directly quantify organic matter transformation rates (Bell et al. 2013).

Relationships between environmental factors (i.e., moisture and temperature) and EEAs have previously been examined (Bell and Henry 2011; Geisseler et al. 2011). Changes in EEAs result from a complicated combination of biological, chemical and physical processes in the environment (Tabatabai 1994; Sinsabaugh 2002). For example, using a meta-analysis of data from 40 ecosystems, Sinsabaugh et al. (2008) found that soil EEAs were primarily affected by soil organic matter (SOM), pH and climate. In addition, temperature rather than moisture was the primary controller of EEAs at the global scale (German et al. 2012), while moisture was a more influential factor on EEAs at the regional level (Brockett et al. 2012).

While the influence of environmental factors on EEAs has been extensively studied, the effects of livestock grazing on EEAs is poorly understood, despite this relationship being critical for understanding nutrient cycling in grassland ecosystems. Past studies are inconsistent with respect to the effect of grazing on soil EEAs. For example, a decrease in dehydrogenase, β-glucosidase and acid phosphatase within soil was found as livestock stocking rates increased in shrublands of Tunisia, possibly due to land degradation and diminution of organic matter inputs under intensive grazing (Fterich et al. 2012). Another

study in Manitoba, Canada, found that arylsulfatase and phosphatase activities in soil were not affected by grazing system and stocking rates, but acid phosphatase activity was reduced under heavy stocking rates (Banerjee et al. 2000). Using an assessment of soil EEAs in response to the presence and absence of long-term grazing across multiple subregions in Alberta, Canada, Hewins et al. (2015) observed that grazing had weak effects on several hydrolytic C, N, and phosphorus (P) cycling enzymes, including cellobiosidase, phosphatase and N-acetyl-β glucosaminidase, while all EEAs were strongly affected by regional climate (Hewins et al. 2015).

While most of these investigations demonstrate the effects of grazing on soil EEAs, an incomplete understanding of livestock grazing effects on litter EEAs, including the relationship between litter EEAs and soil EEA, has limited our knowledge of grassland biogeochemical cycling since extracellular enzymes are key driver of litter decomposition and the regulation of C and nutrient turnover (Allison and Vitousek 2005). As grazing is a primary land use in remaining grasslands, further studies are needed investigating litter and soil EEAs to better understand the mechanisms by which livestock effect grassland microbial communities, biogeochemical cycling, and ultimately the provision of EG&S.

We conducted an 18-month litterbag study across a broad agroclimatic gradient in northern temperate grasslands of Alberta, Canada, in order to capture a wide range of variability in EEAs that mediate C, N and P cycling during litter decomposition. The specific objectives of this study were to:

1. Determine if livestock grazing affects EEAs in both litter and soil layers in northern temperate grasslands,
2. Assess if regional differences in abiotic and biotic environments (i.e., pH, plant community, temperature and moisture) play a role in observed EEAs, and
3. Investigate whether EEAs differ among litter types, including grasses with

contrasting responses to grazing, and thereby understand how biogeochemical cycling may change with livestock grazing and associated changes in community composition.

3.2 Materials and Methods

3.2.1 Study Area and Experimental Design

We assessed EEAs at 15 paired sites, distributed throughout three primary grassland agroclimatic subregions of southern and central Alberta, including the Mixedgrass Prairie, Aspen Parkland and Foothills Fescue (5 in each subregion; Appendix A). In general, the Mixedgrass Prairie is relatively xeric with mean annual precipitation of 333 mm yr^{-1}, and is comprised of a mixture of drought tolerant C_3 and C_4 species, including *Hesperostipa comata*, *Bouteloua gracilis* and *Pascopyrum smithii*. The Foothills Fescue subregion is generally cooler with higher mean annual precipitation of 470 mm yr^{-1} and is dominated by productive bunchgrasses such as *Festuca campestris* and *Danthonia parryi*. The Aspen Parkland has intermediate environmental conditions, with a mean annual precipitation of 441 mm yr^{-1}. Dominant vegetation in this region includes a mix of *Populus tremuloides* forest with interspersed *Symphoricarpos occidentalis* shrublands and native grasslands. The latter are dominated by a mix of primarily C_3 vegetation such as *Festuca hallii*, *Hesperostipa curtiseta*, *Pascopyrum smithii* and *Koeleria macrantha*.

Litter and soil EEAs were examined inside and outside each of five rangeland reference areas (RRAs) where the plant communities were representative of the typical species assemblages found in each subregion (n=15 total). Each RRA is comprised of a fenced site, approximately 20 x 40 m in size, which serves as a permanent grassland ecological monitoring site managed by Alberta Environment and Parks (AEP). Areas inside RRAs represent long-term grazing exclusion (15 to 60 yrs old), which enabled comparison of grazed

and non-grazed grassland communities on the same ecosite. Grasslands outside each exclosure are exposed to light to moderate intensities of cattle grazing between summer and fall (June 1st – October 15th). Although exclosures were not intended to exclude wild ungulates (e.g., antelope, deer, moose and elk), observed use of vegetation within exclosures was very low, presumably because wildlife avoid entering exclosures of this size (Gross and Knight 2000).

3.2.2 Litter Species

In this study, we quantified the EEAs within decomposing samples of seven grass species commonly found across one or more of the three study subregions. Litter samples were harvested from the field in September, 2013 near the start of senescence, with each species collected from a single site in order to standardize the litter source for comparison across subregions and grazing treatments (Appendix C). We did not include all species at each site to assure consistency of species tests with the actual geographic distribution of each species (Appendix C). In addition, a local plant community litter mix from each site (collected in April, 2014 before the growing season began) and a laboratory grade (99% purity) cellulose paper (Fisherbrand® pure cellulose chromatography paper, Fisher Scientific Company, Ottawa, ON, Canada) were included as internal and external controls for each community. All litter materials were air-dried at 60 °C for 72 hr prior to deployment in the field to prevent further enzyme activity and decomposition while in transport and storage.

3.2.3 Litterbag Preparation

Litter samples (2.00 ± 0.02 g) were sealed into litterbags (10 x 10 cm) made of fiberglass mesh (1 x 1 mm openings; New York Wire Co., Hanover, PA, USA). A total of 2380 litterbags were made (i.e., 30 communities x 6/7 litter types x 6 sampling times x 2

duplicates; Appendix C). An aluminum numbered tag was attached to each litterbag for later identification and retrieval in the field. Detailed procedures for litterbag preparation are listed in Appendix D. All samples were prepared in the lab prior to deployment. Litterbags were carefully transported to the field and anchored onto the soil surface in a 5 x 10 m area within each community using 3 inch sod staples on the 25th of May, 2014.

3.2.4 Sample Collection and Processing

Two litterbags of each litter type were randomly selected and removed from each community after 0, 1, 3, 6, 12 and 18 months (May, June, August and October of 2014, and May and October of 2015). All samples were immediately placed on dry-ice to prevent further decomposition and associated microbial enzyme activity (Hewins et al. 2016). Litterbags were transported to the lab and stored at -20°C until further processed. Litter samples were removed from bags and any visible fresh vegetation and soil particles adhering to the bags removed.

At the time of litterbag retrievals, 10 soil cores were also taken (3.25 cm diameter) at random locations within each of the 30 plant communities using a JMC Backsaver soil core (JMC Soil Samplers, Newton, IA, USA) in June, August and October of 2014, and again in May and October of 2015. Soil cores were promptly separated into two depth classes (surface LFH/mulch layer and underlying 0-5 cm of mineral soil), bulked across cores within a community, and then transferred to plastic bags and stored on dry ice for transport to the University of Alberta.

Soil samples were stored at -20°C prior to analysis. To prepare soil samples, rocks and coarse fragments were removed with a 2 mm sieve, and roots removed using tweezers from both the LFH and 0-5 cm soil layer. Soil pH was determined for all 0-5 cm mineral samples with a Fisher Scientific Accumet benchtop pH meter (Fisher Scientific Co., Pittsburgh, PA,

USA). Soil C and N concentration were determined for both the LFH and mineral soil using an elemental analyzer (LECO Corporation, St. Joseph, MI, USA). Each sample was run in duplicate and calibrated using soil standards of known C and N concentration to ensure accuracy (502-308; LECO Corporation, St. Joseph, MI, USA).

3.2.5 Extracellular Enzyme Assays (EEA)

Extracellular enzyme assays were used to assess biological activity in litter and soil samples. Methylumbelliferone (MUB)-linked substrates were used to determine hydrolytic activity, while spectrophotometric methods were used to quantify oxidative enzyme activity (Saiya-Cork et al. 2002). The five hydrolytic enzymes assayed were β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), acid phosphatase (Phos), β-xylosidase (Xylo) and β-Cellobiosidase (Cello). The oxidative substrates used was L-3, 4-dihydrophenylalanine (L-DOPA) to quantify phenol oxidase (PPO) and peroxidase (Pero) activity.

To quantify enzyme assays, 0.5 g of litter or soil were buffered to 50 mM in an acetate solution made using sodium acetate anhydrous. The buffer pH was based on the actual pH of the LFH layer (± 0.1) and adjusted using acetic acid (certified ACS-Pur, glacial, Fisher Scientific, Pittsburgh, PA, USA). A ratio of 0.5 g of litter sample to 400 µM of substrate (Sigma-Aldrich, St. Louis, MO, USA) was used after the optimal substrate concentration test, using a range of 100, 200 and 400 µM substrate (German et al. 2011). A 0.3% H_2O_2 solution was used in a peroxidase assay by mixing deionized water to 30% H_2O_2 (certified ACS grade, Fisher Scientific, Pittsburgh, PA, USA).

Hydrolytic enzyme activity was measured using 96-well Costar black polystyrene microplates (Corning Inc., Corning, NY, USA) and oxidative assays were performed in 96-well clear flat-bottom polystyrene microplates (Corning Inc., Corning, NY, USA). Microplates were incubated in the dark at room temperature (23 °C) for 4h and 18h for

hydrolytic enzymes and oxidative enzymes, respectively. Plates were read using a SpectraMax M3 microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA). Hydrolytic plates were read with a 365 nm excitation and 450 nm emission filter setting, while oxidative plates measured absorbance at a 460 nm wavelength (Saiya-Cork et al. 2002). All enzyme activities were calculated in μmol per gram of litter or soil per hour using the following standard equations (DeForest 2009).

$$\text{Hydrolytic Activity } (\mu\text{mol g}^{-1}\text{ h}^{-1}) = \frac{NFU \times 500\ ml}{Emission\ Coef. \times 0.2\ ml \times Time\ (h) \times Litter\ (g)}$$

Where:

$$\text{NFU = net fluorescence units} = \left(\frac{Sample\ assay - control}{Quench\ coefficient}\right) - Neg.control$$

$$\text{Oxidative Activity } (\mu\text{mol g}^{-1}\text{ h}^{-1}) = \left(\frac{NAU}{L-DOPA\ Ext.Coeff}\right) \times \left(\frac{0.1563}{hrs}\right)$$

Where:

NAU = net absorbance units = Assay – (Sample + Substrate)

L-DOPA Ext. Coeff = 7.9

3.2.6 Statistical Analysis

Results from duplicate EEA runs on litter or soil samples were averaged prior to analysis. Statistical analyses were conducted using R (R Foundation for Statistical Computing, Vienna, Austria). All data including EEAs were checked for normality and log transformed before analysis in order to reduce skewness; however, results and figures are presented as original non-transformed data to facilitate interpretation. Cumulative enzyme activities (hereafter cEEA) were then approximated for each litter type and soil samples from each plant community by integrating enzyme activities over the 18 month period that samples spent in the field (Sinsabaugh et al. 2002; Allison and Vitousek 2004). A cubic spline approximation was used to integrate cumulative enzyme activities to ensure accuracy (Mohanty et al. 2013).

All response variables were subsequently analyzed using generalized linear mixed models with analysis of variance (ANOVA). Fixed effects included grazing treatment, subregion and litter type, as well as all interactions. Study site (n=5) was nested within each subregion as a random factor. Significance of effects was assessed at $P \leq 0.10$. Additionally, *post-hoc* mean comparisons were conducted using a Tukey's test ($\alpha = 5\%$). As the geographic distribution of species varied among regions, the presence of individual litter types among study sites was unbalanced. As a result, it was impractical to test the interactive effects among all response variables with a single analysis, and instead, separate ANOVAs were conducted to address our research questions, as described below.

To assess how grazing, subregion and litter types alter enzyme activity in a broader context, a three-way ANOVA model was applied to each enzyme on the *P. smithii*, *K. macrantha*, cellulose and community mix litter types, which were common to all 15 study sites. Grazing, subregion and litter types were fixed factors in this analysis, while sites within each subregion were random factors.

To assess grazing effects on the more diverse range of litter types, separate ANOVAs were run for each subregion using a mixed model, with grazing and litter type as fixed factors, while sites within subregion were random. This process focused the analysis to a more direct comparison of litter type and grazing effects within each subregion, the latter of which had already been directly tested previously.

To examine effects of grazing and subregion on soil EEAs, ANOVAs were run with subregion and grazing treatment as fixed effects, and site as a random factor for each enzyme. This analysis was conducted separately on each of the LFH and 0-5 cm soil layers.

Last, levels of both litter and soil cEEA were ordinated using non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance metric across all study sites. NMDS enables exploration of relationships between EEAs and environmental factors,

including grazing treatment, soil pH, C, N, C:N ratio, SOM, mean annual precipitation (MAP), mean annual temperature (MAT) as well as annual heat/moisture index (AH:M), which is a combined measure of temperature and humidity ((MAT+10)/(MAP/1000)). All environmental data were generated using ClimateAB v3.21 (available at http://tinyurl.com/ClimateAB), based on methodology described by Mbogga et al. (2010) and Alberta Enviroment (2005). All environmental factors were fitted in the ordination plot as vectors using the *envift* function in the R-package vegan (Oksanen et al. 2013).

3.3 Results

3.3.1 Grazing and Subregion Effects on Common Litter cEEAs

No PPO or Pero activity was detected in 69% and 71% of litter samples respectively, therefore these two oxidative enzymes were excluded from analysis. The three-way ANOVA performed on the litter types common to all study sites (*P. smithii*, *K. macrantha*, cellulose and community mix) indicated that grazing affected three cEEAs, including NAG, Phos, and Xylo ($P \leq 0.04$; see Appendix J), with a marginal effect of grazing on Cello ($P = 0.07$). Grazing increased Xylo, and to a lesser extent Cello, while inhibiting the activity of Phos and NAG over the course of the 18-month study (Figure 3.1). No grazing effect was found on BG.

All cEEAs differed significantly according to litter type ($P < 0.001$). Within each of the five enzymes, observed cEEAs were consistently lower within litterbags containing cellulose (Table 3.1), while the community mix samples had greater activity of BG and Cello compared to *P. smithii*. Levels of cEEAs of *K. macrantha* were similar to that of the community mix for all EEAs, but exceeded that evident in *P. smithii* for Phos and Xylo (Table 3.1).

The ANOVA results also indicated that cEEAs varied among subregions ($P < 0.046$), with the exception of Phos, for which no significance was detected ($F_{2, 117} = 1.33$; $P = 0.15$).

Litter samples incubated in the Parkland subregion generally had the lowest cEEAs of all four enzymes, while samples placed in the Foothills had greater cEEAs of BG compared to either of the other subregions (Figure 3.2). Interaction effects were also observed between grazing treatment and subregions on BG and Cello cEEA (Appendix J), for which a marginal significance was detected (BG: $F_{2, 117} = 3.57$, $P = 0.07$; Cello: $F_{2, 117} = 1.73$, $P = 0.06$). Closer examination revealed that grazing increase BG and Cello activity in the Foothills, while decreasing these cEEAs in the Parkland (Figure 3.3).

3.3.2 Grazing Effects on Litter cEEAs in Each Subregion

Within the Foothills subregion, litter type modulated all cEEAs with the exception of Xylo (Table 3.2). Among all litter types in this subregion, *P. pratensis* had the greatest cEEA for each of BG, NAG and Cello (Table 3.3). The level of cEEA for Phos was also higher in *P. pratensis* than in *P. smithii*. No differences were detected among the cEEAs of *F. campestris*, *K. macrantha* and *P. smithii* for any enzyme (Table 3.3).

Grazing had a marginal effect on Xylo cEEA in the Foothills subregion (Table 3.2), with grazing increasing Xylo activity (Grazed: 26.0 ± 3.9 μmol g^{-1} vs. Non-grazed: 17.9 ± 2.2 μmol g^{-1}). Additionally, interactions were detected between grazing and litter type for Phos and NAG (Table 3.2). Closer inspection showed that the absence of grazing decreased cumulative Phos activity on *P. smithii* to levels below the activity found in litterbags containing *P. pratensis* (Figure 3.4 A). A similar pattern was apparent with NAG, although the absence of grazing reduced NAG activity directly within *P. smithii*, while this same treatment increased NAG activity in *P. pratensis* (Figure 3.4 B).

Within the Mixedgrass subregion, no effects of grazing were evident on any of the cEEAs (Table 3.2). However, cEEAs did vary for each of the five enzymes among litter types over the course of study (Table 3.2). In general, *B. gracilis* had the highest cumulative BG

activity compared to that within other litter species in this subregion, and cEEAs of *B. gracilis* were higher than *P. smithii* for Phos, Xylo, NAG and Cello (Table 3.3). Activity of BG within *H. comata* remained intermediate between *B. gracilis* and *P. smithii* in the Mixedgrass.

In the Parkland, we observed patterns similar to the Mixedgrass subregion with respect to frequent litter type effects (all but NAG), but not grazing (Table 3.2). Overall, *P. smithii* had the lowest cEEA of Phos and Xylo compared to the other four litter species, while *P. pratensis* tended to have greater cEEAs of BG and Cello in comparison to *P. smithii* (Table 3.3).

3.3.3 Grazing and Subregion Effects on Soil cEEAs

Our results generally indicated that long-term grazing did not influence the cEEAs in either soil layer (LFH, $P \geq 0.11$; 0-5 cm mineral soil, $P \geq 0.38$). Moreover, no interaction effects of grazing and subregion were found on cEEAs over the course of study (LFH, $P \geq 0.13$; 0-5 cm mineral soil, $P \geq 0.18$). However, cEEA of BG was affected by subregion ($F_{1, 58} = 4.34$, $P = 0.04$). The Mixedgrass subregion had lower ($P = 0.03$) cumulative BG activity (22.3 ± 3.6 μmol g^{-1}) compared to the Parkland (29.3 ± 4.3 μmol g^{-1}), while the greatest cumulative BG activity ($P = 0.07$) was found in the Foothills subregion (42.5 ± 6.2 μmol g^{-1}). Cumulative Xylo activity was also marginally affected by subregions ($F_{1, 58} = 2.57$, $P = 0.10$). Cumulative Xylo activity was 8.1 ± 1.6 μmol g^{-1}, 3.5 ± 0.6 μmol g^{-1} and 4.2 ± 1.3 μmol g^{-1} for the Foothills, Mixedgrass and Parkland subregions, respectively.

3.3.4 Environmental Effects on cEEAs

Within the 0-5 cm soil layer, we detected relationships between cEEAs and MAT, as well as soil pH (Table 3.4). Based on our results, PPO activity was inversely correlated with

pH (Figure 3.5). NAG was also positively correlated with the presence of grazing. Additionally, Cello, Phos and NAG were all more active in areas with higher MAT (Figure 3.5).

Within the LFH soil layer, cEEAs were associated within MAP, annual heat/moisture index (AH:M) and SOM (Table 3.4). Based on the associated ordination (Figure 3.5), Cello activity was positively correlated with MAP and SOM. The opposite pattern was evident for AH:M however, where Cello activity declined under greater AH:M (i.e., within more moisture stressed environments). NAG was highly correlated with grazing.

Assessment of cEEAs within the litter samples indicated the former were associated with MAT and environmental pH (Table 3.5). Examination of the corresponding ordination suggested that BG and Xylo activities were negatively correlated with increasing MAT and litter pH (Figure 3.6), while NAG activity tended to be positively associated with these environmental metrics, particularly litter pH.

3.4 Discussion

3.4.1 Grazing Effects on EEAs

In this 18-month field study, we detected long-term grazing effects on litter EEAs of four common litter sources, although these effects varied widely. Grazing increased the activities of two enzymes responsible for C cycling (i.e., increased Xylo and Cello), and decreased those associated with organic-nutrient mineralization (i.e., decreased Phos and NAG). Regional climate variation affected Xylo activity, an effect that was most apparent with increases in the mesic and cool environment of the Foothills Fescue. Additionally, our results showed that EEA levels in different soil depths were affected by different environmental conditions, but not grazing. For example, EEAs in the surface LFH layer were more constrained by moisture (i.e., higher MAP and AH:M), while greater temperatures (i.e.,

MAT) limited EEAs in mineral soil.

Grazing increased C mineralizing EEAs (Xylo and Cello) on four common litter types, which may be associated with enhanced C turnover rate and higher C demand. This finding could reflect grazing-induced vegetation and microbe shifts, and changes in soil biophysical parameters (e.g., pH) that subsequently regulate organic C (Klemmedson 1956; Derner et al. 1997). It is known that grazing can increase C turnover rates and lead to greater soil organic C (Conant et al. 2001). Higher levels of SOM can enhance microbial activity by supplying more substrates for microbes in soil and litter, which in turn, stimulate the synthesis of microbial enzymes (Burke et al. 2011; Datta et al. 2013; Schutt et al. 2014). A number of studies have demonstrated SOM abundance varies across our study subregions, with SOM levels ranging from 5% in the Mixedgrass to more than 9% in the Foothills (Dormaar et al. 1977; Naeth et al. 1991). Mineralization of organic C is catalyzed by enzymes that are produced mainly by microbes, and to some extent plants (Treseder and Vitousek 2001), and our findings of high C cycling enzymes in the Foothills is in agreement with the potential for greater microbial activities within this richer SOM environment.

When testing grazing effects in more detail within each subregion, this pattern was only found in the Foothills Fescue on Xylo, suggesting the wetter and cooler environment found there may lead to Xylo-responding microbes (e.g., *Bacteroidetes*; Pepe-Ranney et al. 2016) more active in producing extracellular enzymes critical for litter breakdown (DeAngelis et al. 2010). As grazing is well known to reduce litter (Naeth et al. 1991), this may increase the demand for EEAs associated with improving microbial access to C, and thereby accelerate cycling of C. Past studies have found that when environmental conditions become less constrained for microbial growth, such as under higher moisture, microbes recover more rapidly from environmental stress and disturbance (Lundquist et al. 1999; Placella and Firestone 2013). In contrast, microbial activity may be reduced in hotter or drier environment

conditions (Blume et al. 2002). In general, when conditions are less favorable for microbes (e.g., drier soil and higher temperature), environmental constraints may have a more pronounced role in regulating EEAs compared to localized disturbances such as grazing.

In our study, we found that the activity of both N- and P- liberating enzymes decreased with grazing across subregions, possibly due to the extra mineralized nutrient input (e.g., NH_3) present within livestock urine and feces, and which does not require further breakdown (Lucas et al. 1975). Animal wastes deposited under ongoing grazing activities, can strongly affect soil inputs and nutrient pools, which in turn, change microbial community composition (Seagle and McNaughton 1992; Sankaran and Augustine 2004). Previous studies have found that 80 to 90% of nutrients consumed by livestock are returned to the soil (Haynes and Williams 1993; Wachendorf et al. 2008), and depending on animal use patterns, can lead to nutrient redistribution in the landscape, as well as localized changes in nutrient cycling, including increases in N and P availability (Moleele and Perkins 1998; Shand et al. 2000). Consequently, the direct (e.g., animal waste) and indirect (e.g., herbivore-induced vegetation shifts, discussed in section 3.4.2) effects of livestock grazing can change EEAs indicative of microbial activity, and therefore biogeochemical cycles associated with C and soil macronutrients. Alterations to the latter may impact the potential for plant growth and ecosystem C accumulation (Holland and Detling 1990).

Greater observed EEAs in the Mixedgrass relative to the Parkland were unexpected, as the Parkland is generally more mesic and contains more diverse vegetation than the Mixedgrass (Lyseng 2016). Notably, Hewins et al. (2015) found similar patterns from EEAs within soil samples, with the Foothills and Mixedgrass having greater EEAs compared to the Parkland. Multivariate analysis suggested that within the LFH layer, SOM, mean annual precipitation and annual heat/moisture index were important predictors of EEAs. Therefore, Hewins et al. (2015) suggested that relatively lower SOM in the Parkland could contribute to

lower EEAs in their study. In addition, vegetation in the Mixedgrass is generally comprised of a mix of drought tolerant C_3 (*P. smithii*, *H. comata*, and *K. macrantha*) and C_4 grass species (*B.gracilis*), and develops a thin or intermittent layer of litter, with select plant species (e.g., *B. gracilis*) having a shallow root system (Coupland and Johnson 1965). In contrast, Parkland grasslands are typically covered with well-developed litter layers (Deutsch et al. 2010) and may have a greater abundance of rhizomatous grasses (e.g., *F. hallii* and *P. pratensis*). As grazing limits litter accumulation (Naeth et al. 1991), this in turn, may expose soil biota to increased direct radiation, and greater temperature and moisture (Knapp and Seastedt 1986), all of which can alter microbial community activities (Cleveland et al. 2014). Also, different grass species may lead to varying SOM accumulation. For example, SOC accumulated more rapidly beneath short-statured *B. gracilis* compared to taller-statured patches of *S. scoparium* within long-term grazed sites (Derner et al. 1997), likely resulting from varying root and shoot organic matter inputs (Vasquez de Aldana et al. 1996). Therefore, we postulate that the increased abundance of litter and rhizomatous grasses in more mesic grassland may provide sufficient C, especially labile C and nutrients to microbes, whereas in the xeric environment of the Mixedgrass, low SOM may necessitate increased EEA for microbes to access C and nutrients from mineral soil.

We did not detect grazing effects on EEAs in either LFH or mineral soils, which is consistent with the findings of Hewins et al. (2015). In the present study, enzymes were mediated by SOM availability, as SOM is a key source of C and other nutrients (i.e., N and P) for microbes. Therefore, limited grazing effects on all enzymes could indicate a relatively stable condition of SOM relative to this land use. While Alberta only has a history of livestock grazing for about 130 years, wildlife grazing extends back much further than this (10,000 years; e.g., Morgan 1980). Moreover, many ecological processes, including C accumulation and stabilization, may take long periods of time. Therefore, it is possible that

soil organic matter in soils across our study sites may already be resistant to grazing effects, and therefore provide a relatively stable pool of C and nutrients for microbes.

3.4.2 Litter Type Effects on EEAs

Levels of EEA were strongly affected by litter type, and these trends were apparent among and within subregions. These results are in agreement with previous studies showing litter chemical quality governs the release of enzyme in the environment (Allison and Vitousek 2004). Among the four common litter types placed in all three subregions, the greatest EEA was generally found in the more diverse community mix litter samples, while cellulose paper had the lowest EEAs. At the chemical level, these two sample types were highly divergent, with the external standard (paper) consisting of 99% cellulose (i.e., was homogeneous), while the community mix contained N and a wide array of chemical compounds (i.e., was chemically heterogeneous), including simple carbohydrates (e.g., saccharides), hemi-cellulose and lignin. The inherent heterogeneity of the community mix may lead to a greater abundance and diversity of microbes, and thus a higher variety of enzymes to liberate nutrients (Chapman and Newman 2009). The enzyme BG is released by a variety of organisms including bacteria, fungi and plants, and higher overall activity of BG in the community mix litter may be indicative of greater diversity within the microbial community (Esen 1993). Also of note is that the community litter was collected in early spring 2014, rather than fall 2013, and could therefore have started decomposing over the late fall and winter prior to collection. This initial decomposition may have led to the loss of soluble compounds, thereby contributing to a need for more EEAs to facilitate advanced decomposition of this litter source.

Within each regional comparison of EEAs among different litter types, our results indicated that *P. pratensis* had higher BG, NAG and Cello activities than all other species in

the Foothills, and *P. smithii* had the lowest Phos and Xylo activities in the Parkland subregion. In the Mixedgrass subregion, *B. gracilis* had the lowest BG activities. Due to the limited grazing effects on most litter sources within each subregion, we postulate that the quality (i.e., inherent chemical composition) of different litter types may play a more dominant role in mediating observed EEAs. Although plant species composition can vary markedly among subregions due to different precipitation, temperature or soil type, grazing also has the ability to alter plant community in a manner that increases the relative abundance and composition of grazing tolerant species (i.e., increasers) at the expense of those plants susceptible to grazing. Furthermore, these different plant species associated with divergent grazing may vary in inherent chemical composition, such as lignin and N content (Smoliak et al. 1972; Willms et al. 1985; Alder et al. 2001; Vujnovic et al. 2002). As a result, our results show that not only are regional changes in vegetation important in altering EEAs, but that grazing-induced changes in species composition, particularly the replacement of grazing-sensitive grasses with those more tolerant (i.e., *B. gracilis* in the Mixedgrass, and *P. pratensis* in the Parkland and Foothills) can also indirectly regulate EEAs by altering plant communities. Similar results were observed by Olivera et al. (2014), where grazing increased the concentration of soluble phenolics and the phenolics:N ratio; the greater resulting recalcitrance of litter may limit substrate availability for microorganisms, thereby reducing microbial enzyme activity, in turn reducing C and nutrient cycling (Olivera et al. 2014).

Greater EEAs within *P. pratensis* could be an important indicator of how introduced plant species alter C and nutrient cycling in grasslands containing this species. It is well-known that *P. pratensis* has been increasing on grasslands in the Northern Great Plains (Bahm et al. 2011; DeKeyser et al. 2013; White et al. 2013), including western Canadian grasslands (Looman 1969; Willms et al. 1985; Bork et al. 2012) and northern Mixedgrass Prairies of the United States (Biondini et al. 1998) over the past three decades. Our current

results indicate invasion by *P. pratensis* may alter fundamental ecological processes, including accelerating nutrient and C cycling, thereby changing how the resultant plant communities provide ecological goods and services such as forage production and C storage. For example, an increase in *P. pratensis* growth over time under more intense defoliation, particularly during summer (Tannas, 2011), could have a marked impact on C and nutrient cycling, and even help account for the increase in root and shoot biomass observed under long-term grazing in mesic grasslands of the Foothills in southwestern Alberta (Lyseng 2016). Therefore, it is plausible that widespread invasion of *P. pratensis* in grasslands has played a dominant role in driving EEAs, C cycling, and ultimately C storage, in response to long-term grazing.

Studies have also found that microbial communities are modified due to plant community shifts from native to non-native species (Batten et al. 2006 and 2008; Salles and Mallon 2014), in turn leading to alterations in ecological processes (i.e., nutrient cycling; Grant et al. 2009). For instance, *P. pratensis* is known to have high N concentrations (Sullivan and Garber 1941) and low lignin (Wedin et al. 1995; see also Chapter 2), and therefore litter derived from *P. pratensis* may be favored by microbes as a nutrient-rich resource, particularly in comparison to high lignin:N ratio species in our study, such as *P. smithii* and *F. campestris*. Lyseng (2016) found grazing-induced increases in diversity within the Parkland and Foothills Fescue, and losing plant diversity from a heterogeneous community to dominance by *P. pratensis* could alter the peak pulse of litter inputs (i.e., substrate availability for microbes) from throughout the growing season towards spring and early summer. Microbes could also produce more enzymes in support of litter degradation to access sufficient nutrients for growth.

The greater comparative EEAs of *B. gracilis* than *P. smithii* in the Mixedgrass subregion were unexpected, as the warm-season (C_4) species *B. gracilis* is known to be high in lignin

and low in N concentration (Pittermann and Sage 2000; see also Chapter 2). Nitrogen is an essential nutrient for all life, and would be positively correlated with microbial activity and soil organic matter (Farrell et al. 2014; Lange et al. 2015). Our results suggest that initial litter sample collection time may have affected enzyme activity. As the only C_4 species tested in our study, *B. gracilis* may have been in a relatively vegetative stage of growth at collection relative to other cool-season grass species. As such, less recalcitrant compounds with higher N may have favored microbes to colonize this litter source. However, if this is the case, the greater EEAs and decomposition rates (Chapter 2) of *B. gracilis* would have a profound effect on C storage in grassland. Climates have gradually changed in the past 200 hundred years, and the increased amount of CO_2 has led to an elevation of temperature (Forster et al. 2007). Monz et al. (1994) found that elevated CO_2 and temperature, together with decreased precipitation, reduced the abundance of *P. smithii* and increased the colonization of *B. gracilis*, as this species is highly resistant to drought (Monz et al. 1994). Moreover, *B. gracilis* is resistant to grazing (Milchunas et al. 1989) and is becoming dominant in North American grasslands (Porras-Alfaro et al. 2008). Therefore, the turnover rate of C could increase with climate-induced and grazing-induced species shifts towards this species. Overall, plant community composition changes have a marked ability to alter C and nutrient cycling processes, within grasslands.

3.5 Conclusions

This study revealed both generalized and regional specific effects of livestock grazing on EEAs representative of biogeochemical cycling processes, primarily in plant litter rather than soil. Results from this study demonstrated that long-term grazing leading to changes in vegetation and the local micro-environment were associated with both negative and positive effects on litter enzyme activities. Livestock grazing generally increased the activities of C-

mineralizing enzymes (i.e., Xylo and Cello), whereas it decreased N- (NAG) and P- (Phos) liberating enzymes. When testing the effects of grazing on litter within each subregion, only litter common to the Foothills Fescue responded to grazing. The Parkland subregion had the lowest enzyme activities overall, while peak activities were found in the Foothills Fescue. Litter EEAs also varied markedly among plant species, with the introduced plant *P. pratensis* in particular increasing enzyme activities in the Parkland and Foothills Fescue, thereby illustrating that the microbial community has been modified by ongoing shifts in grassland composition under grazing. Future analysis of the microbial community composition on sites could help further understand the effects of grazing on C and nutrient cycling, including C accumulation.

Table 3.1. Mean (±SE) cumulative enzyme activities (cEEAs) over an 18 month period for litter types common to all study sites. Cumulative activity was calculated by integrating enzyme activity over time; results are presented in units of micro-moles of substrate per g of litter sample (μmol g^{-1}). Phos: acid phosphatase, Xylo: β-xylosidase, BG: β-glucosidase, NAG: N-Acetyl-glucosaminidase and Cello: β-Cellobiosidase.

Litter Type	Phos	Xylo	BG	NAG	Cello
Cellulose	3.2 ± 0.6a	2.5 ± 4.8a	19.3 ± 1.7a	7.7 ± 1.1a	7.8 ± 0.9a
P. smithii	18.6 ± 1.9b[1]	13.0 ± 2.2b	92.8 ± 9.3b	52.0 ± 5.1b	39.3 ± 4.4b
K. macrantha	29.1 ± 3.2c	22.4 ± 2.6c	112.0 ± 10.0bc	70.2 ± 7.8b	47.1 ± 5.3bc
Community mix	36.8 ± 4.1c	22.6 ± 2.9c	139.0 ± 13.4c	66.6 ± 7.5b	61.7 ± 6.6c

[1] Within a column, means with different letters differ, $P < 0.05$.

Table 3.2. Results of the ANOVA tests for the effect of specific litter types, grazing treatments and their interactions, on the cumulative 18 month activity of each of five extracellular enzymes, within each of three subregions. Bold indicates effects that are significant ($P < 0.10$).

Source	Phos			Xylo			BG			NAG			Cello		
	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*
----------Foothills----------															
Litter type (LT)	4.25	3,56	**0.01**	1.64	3,56	0.20	10.43	3,56	**<0.001**	4.11	3,56	**0.02**	3.82	3,56	**0.02**
Grazing (G)	0.42	1,58	0.52	4.04	1,58	**0.054**	1.14	1,58	0.30	0.03	1,58	0.85	2.24	1,58	0.15
LT*G	2.87	3,56	**0.054**	0.80	3,56	0.51	2.13	3,56	0.12	2.81	3,56	**0.058**	0.93	3,56	0.44
----------Mixedgrass----------															
LT	2.96	3,56	**0.049**	3.11	3,56	**0.04**	3.23	3,56	**0.03**	2.98	3,56	**0.05**	4.30	3,56	**0.01**
G	0.22	1,58	0.64	0.01	1,58	0.92	0.22	1,58	0.64	0.82	1,58	0.37	0.24	1,58	0.63
LT*G	0.42	3,56	0.74	0.27	3,56	0.85	0.15	3,56	0.93	0.32	3,56	0.81	0.16	3,56	0.92
----------Parkland----------															
LT	4.99	4,65	**0.002**	6.32	4,65	**<0.001**	7.11	4,65	**<0.001**	2.07	4,65	0.11	6.82	4,65	**<0.001**
G	2.48	1,68	0.12	1.32	1,68	0.26	2.71	1,68	0.11	2.41	1,68	0.13	2.65	1,68	0.11
LT*G	1.04	4,65	0.40	0.87	4,65	0.48	0.94	4,65	0.45	0.43	4,65	0.78	0.19	4,65	0.94

Table 3.3. Mean (±SE) aggregate activity over an 18 month period for each of five EEAs (μmol g^{-1} h^{-1}) associated with different litter types (i.e., plant species) in each of three subregions in Alberta, Canada.

Litter type	Phos	Xylo	BG	NAG	Cello
			----------Foothills----------		
P. smithii	23.5±4.2a[1]	17.9±5.9	119.9±18.9a	65.9±10.7a	48.7±9.8a
F. campestris	28.3±3.5ab	18.7±5.2	148.5±16.0a	87.4±10.3a	68.5±10.0a
K. macrantha	28.1±4.7ab	21.9±3.5	129.6±14.7a	75.0±11.2a	54.9±10.2a
P. pratensis	40.7±4.8b	29.3±3.2	232.8±20.4b	113.8±15.7b	96.7±14.5b
			----------Mixedgrass----------		
P. smithii	16.9±2.0a	14.6±1.8a	91.1±11.2a	52.3±6.0a	42.1±6.0a
B. gracilis	45.1±9.8b	38.3±8.5b	223.3±24.0c	128.2±28.1b	82.7±10.3b
H. comata	29.2±4.6ab	30.6±4.2ab	174.6±21.2b	92.5±17.6ab	73.1±10.5ab
K. macrantha	30.9±6.8ab	23.2±5.2ab	118.5±20.4ab	71.6±13.9ab	46.5±9.1ab
			----------Parkland----------		
P. smithii	15.5±3.0a	6.5±1.4a	67.6±14.0a	37.9±7.7	27.2±5.2a
F. hallii	40.2±5.9b	21.5±3.7b	136.9±20.3bc	76.4±15.3	67.4±9.5bc
H. comata	34.7±5.5b	21.1±3.5b	108.8±18.6abc	83.5±21.5	64.6±12.1bc
K. macrantha	28.4±5.3b	22.2±5.0b	88.0±15.5ab	64.0±16.2	39.9±8.3ab
P. pratensis	40.1±6.8b	20.3±4.3b	157.9±27.6c	84.5±17.7	70.2±12.1c

[1] Within a column and subregion means with different letters differ, $P < 0.05$.

Table 3.4. Non-metric multidimensional scaling (NMDS) results showing relationships between soil cEEAs and grazing, mean annual precipitation (MAP), mean annual temperature (MAT), annual heat moisture index (AH:M), soil pH, carbon (C), nitrogen (N), C to N ratio (C:N) and soil organic matter (SOM). Significant environmental variables are in bold ($P < 0.10$).

Variable	r^2	*P*
	----------0-5 cm----------	
Grazing	0.09	0.28
MAP	0.05	0.54
MAT	0.24	**0.02**
AH:M	0.16	**0.09**
pH	0.31	**0.01**
C	0.07	0.40
N	0.07	0.37
C:N	0.02	0.75
SOM	0.01	0.93
	----------LFH----------	
Grazing	0.02	0.80
MAP	0.22	**0.04**
MAT	0.03	0.69
AH:M	0.20	**0.05**
pH	0.05	0.53
C	0.04	0.59
N	0.03	0.62
C:N	0.03	0.68
SOM	0.33	**0.004**

Table 3.5. Non-metric multidimensional scaling (NMDS) results showing relationships between litter cEEAs and grazing, mean annual precipitation (MAP), mean annual temperature (MAT), annual heat moisture index (AH:M), environmental pH, and soil organic matter (SOM). Significant environmental variables are in bold ($P < 0.05$).

Variable	r^2	*P*
Grazing	0.04	0.10
MAP	0.002	0.91
MAT	0.09	**0.005**
AH:M	0.03	0.23
pH	0.16	**<0.001**
SOM	0.01	0.64

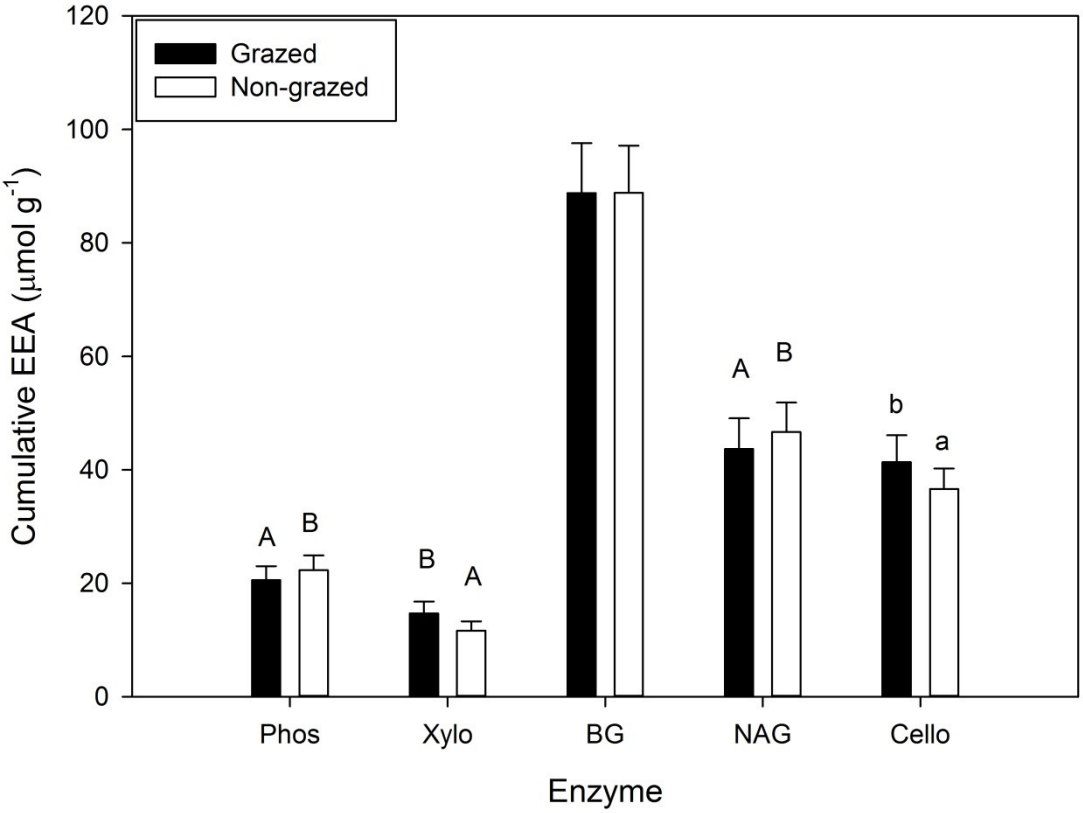

Figure 3.1. The effect of grazing treatment on mean (±SE) cEEA of acid phosphatase (Phos), β-xylosidase (Xylo), β-glucosidase (BG), N-Acetyl-glucosaminidase (NAG), and β-Cellobiosidase (Cello) of four common litter types. Error bars represent standard error. Upper case letters represent differences between grazing treatments at $P < 0.05$ and lower cases represent differences between grazing treatments at $P = 0.07$.

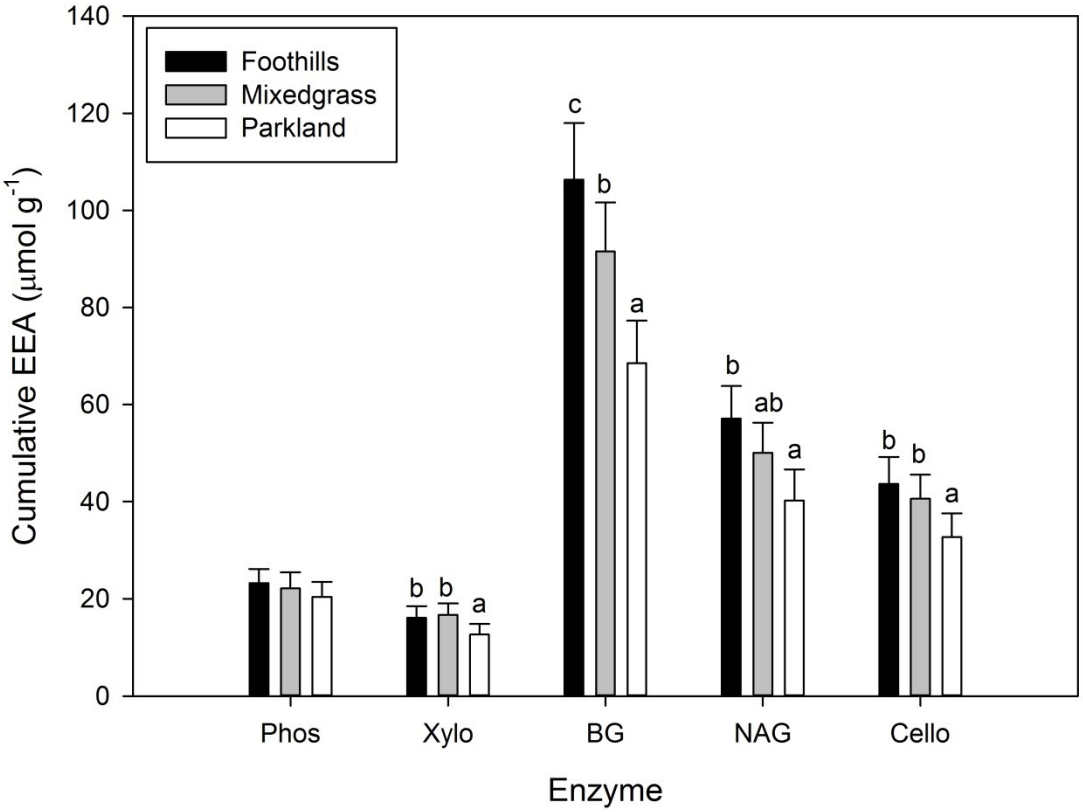

Figure 3.2. The effect of subregion on mean (±SE) cEEAs of acid phosphatase (Phos), β-xylosidase (Xylo), β-glucosidase (BG), N-Acetyl-glucosaminidase (NAG), and β-Cellobiosidase (Cello) of four litter types common to all study sites. Error bars represent standard error. Different letters represent differences among subregion means within each EEA ($P < 0.05$).

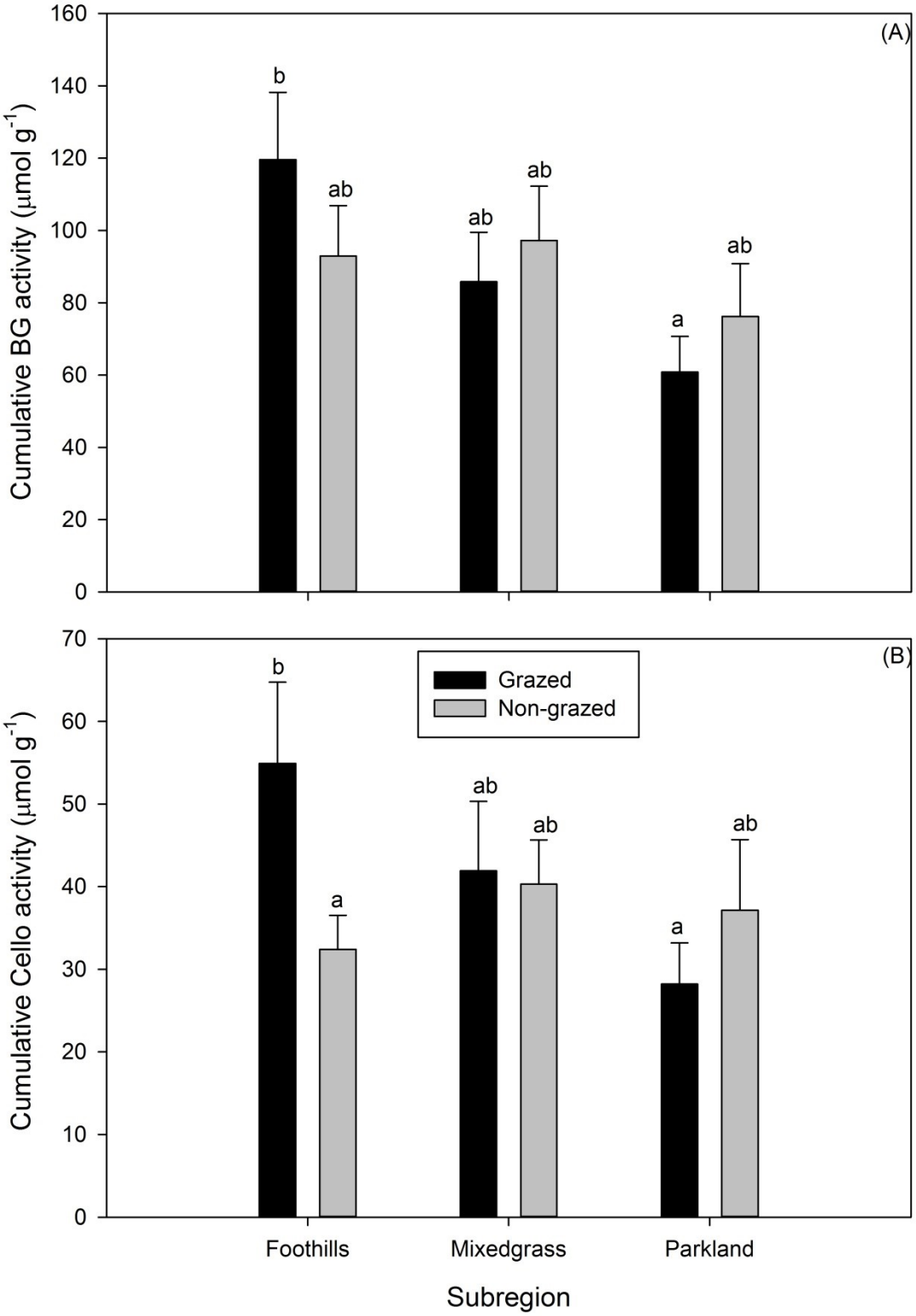

Figure 3.3. The interaction effects between subregion and grazing on mean (±SE) cumulative (A) β-glucosidase (BG) and (B) β-Cellobiosidase (Cello) activity of four litter types common to all study sites. Different letters represent significance between means ($P < 0.05$).

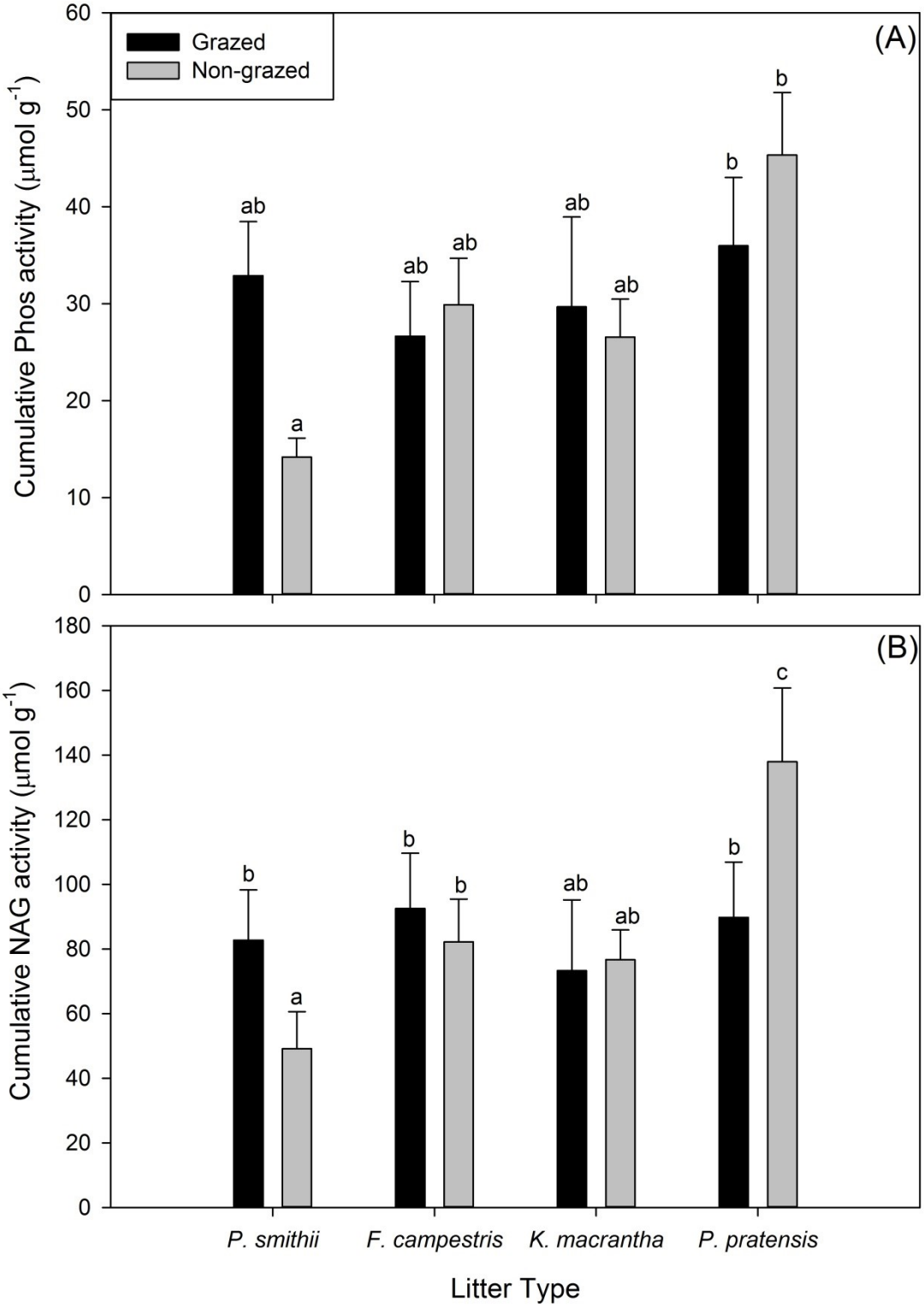

Figure 3.4. Mean (±SE) cumulative EEAs of (A) acid phosphatase (Phos), and (B) N-Acetyl-glucosaminidase (NAG) for each of *P. smithii*, *F. campestris*, *K. macrantha* and *P. pratensis* in the Foothills subregion during the 18-month study. Different letters represent significant differences among means ($P < 0.05$).

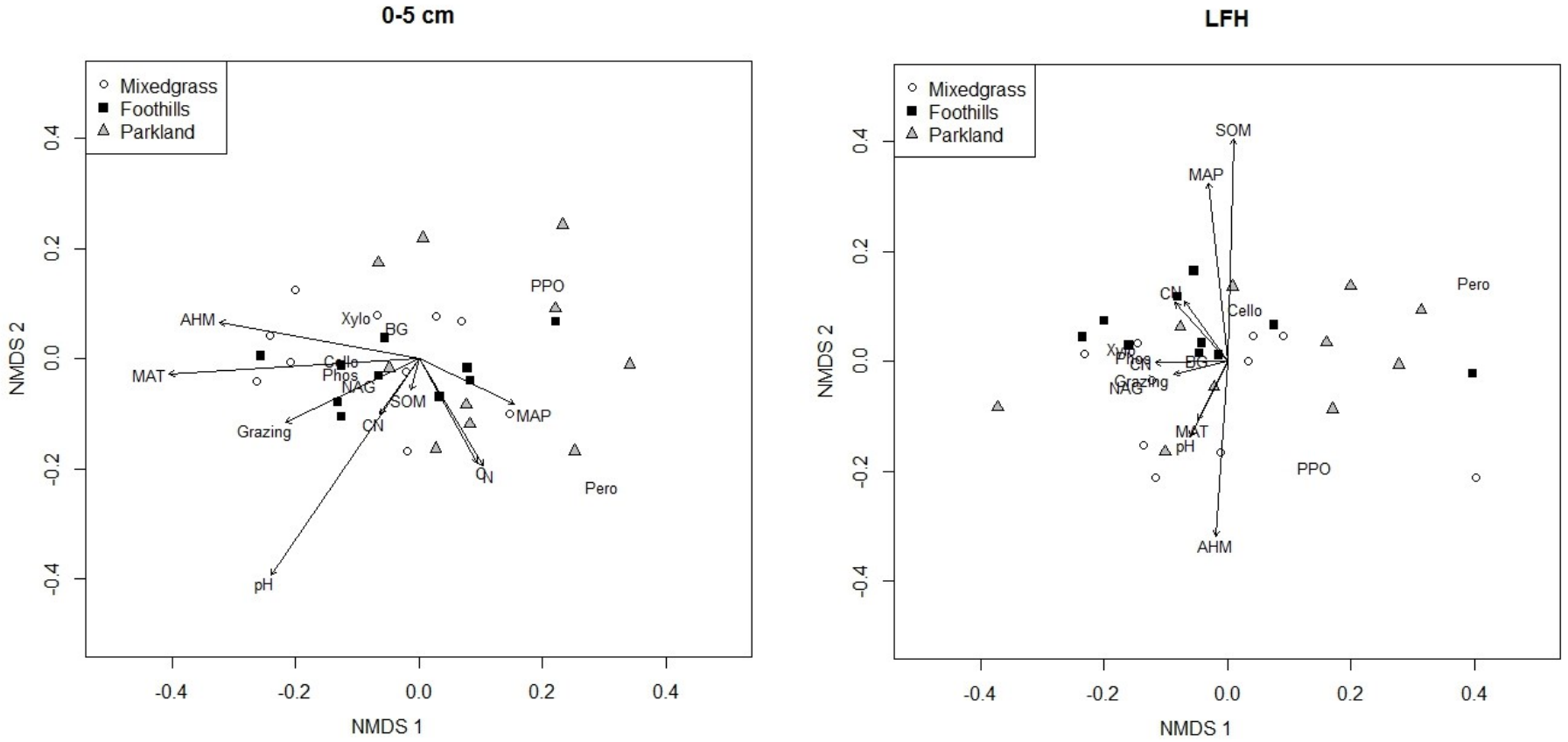

Figure 3.5. Non-metric multidimensional scaling (NMDS) ordinations showing patterns of cEEAs in the the 0-5 cm mineral layer (left) and LFH mulch layer (right) of soil. Vector lengths indicate the strength of relationships between environmental factors and the activity of enzymes.

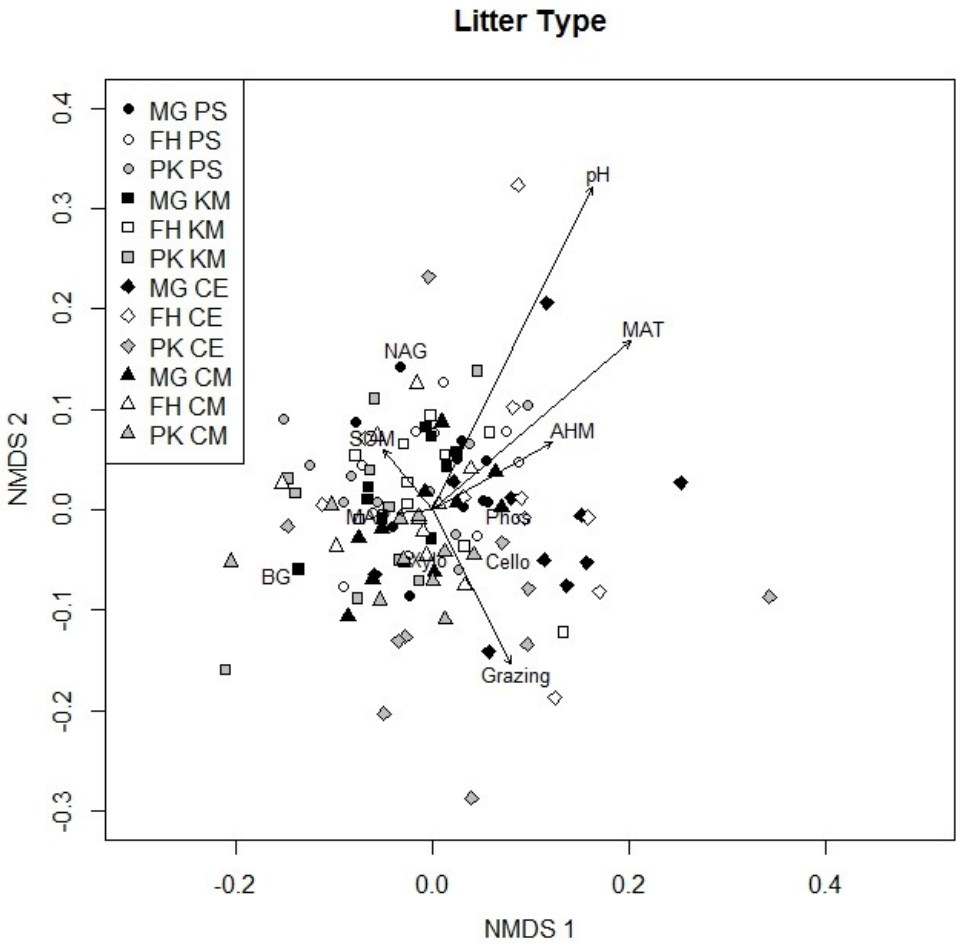

Figure 3.6. Non-metric multidimensional scaling (NMDS) ordination showing patterns of cEEAs for each of four litter types common to all study sites. Vector lengths indicate the strength of relationships between environmental factors and the activity of enzymes. MG: Mixedgrass, PK: Parkland, FH: Foothills; PS: *P. smithii*, KM: *K. macrantha*, CE: Cellulose and CM: Community mix.

Chapter 4. Synthesis

4.1 Introduction

Grasslands cover approximately 40% of the earth's terrestrial surface and are critically important as wildlife habitat, for livestock forage, and support a large human population (Holechek et al. 1994). Ecological stresses on grassland ecosystems have been increased as global food demand continues to rise (Eckert et al., 1995). Grasslands provide a large pool of C storage, which could mitigate the rise in atmospheric CO_2 (Schlesinger 1990). Litter decomposition is one of the key factors that regulate biogeochemical cycling (Bradford et al. 2016) and is of great importance in the grassland carbon (C) cycle, thereby influencing the size and stability of organic C stores (Schimel and Schaeffer 2012) as well as local nutrient cycling in the ecosystem (Aber et al. 1990; Berg 2000). While biological C sequestration is an important role of grasslands, little is known of the fundamental mechanisms regulating litter decomposition and C cycling within them, particularly in relation to ongoing land uses such as grazing.

Most litter consists of complex compounds that require specialized extracellular enzymes to catalyze the breakdown process (Allison and Vitousek 2004). The relationship between extracellular enzymes and decomposition may be indirect and complex, since there are other biotic and abiotic factors, such as temperature, moisture and microbial community characteristics that alter enzyme activities (Sinsabaugh 1994).

In this study, I examined the effects of long-term livestock grazing on the decomposition of different plant species in three agroclimatic subregions of the Northern Great Plains. In Chapter 2, I reported litter decomposition rates of these different species. In Chapter 3, I examined extracellular enzyme activity within both litter and soil samples. The main goal of this study was to test the hypothesis that livestock grazing, and regional differences in climate, soil and vegetation, alters litter decomposition and associated indicators of C and nutrient (N

and P) cycling, in order to address questions on where, when and how livestock grazing affects C cycling in grassland ecosystems. In this chapter, I outline the key findings from this study and highlight the implications of land use management for C storage and nutrient cycling of grasslands. Finally, I end by discussing the importance of proper grazing management in providing the full complement of ecological goods and services in grasslands, including C storage.

4.2 Summary of Key Results

The relationship between grazing and litter decomposition rates among three contrasting northern temperate grassland environments was examined in Chapter 2, with associated EEA on these substrates assessed in Chapter 3. Results showed that grazing affected both the litter decomposition rates and extracellular enzyme activities, though the effect differed among subregions. Grazing generally increased litter decomposition rates in the moister environment of the Foothills Fescue, but did not alter it in the other two subregions, potentially due to microbial communities being more sensitive to disturbance (i.e., grazing) in cooler and moister areas (Placella and Firestone 2013). It is well understood that temperature and moisture can affect microbial communities by changing their composition and physiological patterns (Jenkinson et al. 1991; Rodrigo et al. 1997; Kirschbaum 2000a). Additional changes can occur in microbial community structure, such as the fungal:bacterial ratio, and ultimately alter the aggregate function of microbial communities (Bapiri et al. 2010; Yuste et al. 2010; Wallenstein and Hall 2011). It is likely that local climate variations among subregions alter microbial communities, which lead to differences in decompositioin rate responses to grazing. In our study, we did not examine the taxonomy of microbial communities (e.g., genetic sequencing), although differences in litter decomposition rates and extracellular enzyme activities (Chapter 3) suggest that microbial community responses to grazing effects differed

among subregions.

Results of this study also showed that litter types played a critical role in regulating decomposition rates (Chapter 2) as well as extracellular enzyme activities (Chapter 3). For example, high rates of EEAs on *P. pratensis* led to relatively rapid and larger mass loss in litter from this species, while *Festuca* species demonstrated slower decomposition and intermediate EEAs. This is likely due to low C:N ratio and more labile materials in *P. pratensis*, which contribute to faster decomposition and greater EEAs. *P. pratensis* is well-adapted to disturbances and can recover from stresses, and it can readily invade native grassland after disturbances (i.e., grazing) with its aggressive rhizomes and widespread seed (Moser et al. 1968). Therefore, invasion by *P. pratensis* is likely to shift ecological processes, including C turnover, thereby altering net C inputs to the ecosystem over time.

Another key finding was that community mix litter samples had greater decomposition rates and enzyme activities compared to all other species among subregions, and the community litter also lost more C relative to its mass loss. This could demonstrate that mixtures of litter are more likely to lead to decomposition, and the combination of the microbial community and mix of plant species may lead to the preferential breakdown of C. Additionally, the presence of numerous forbs in the community mix, which are high in N and have less complex C (i.e., lignin), also contribute to the higher decomposition than other species through changes in the microenvironment. This is mainly because grasses have higher C:N ratio (Wardle et al. 1997; Hector et al. 2000), making them which are less favorable to microbes.

These findings contribute towards the growing body of evidence that plant community compositional differences are the principal cause of variation in litter decomposition and enzyme activities (Cornwell et al. 2008; Freschet et al. 2012b). In this study, different litter types not only decomposed at different rates due to the direct effects of variable inherent litter

chemical composition, but also as a result of the indirect effects of interactions between livestock grazing and agroclimatic conditions.

4.3 Future Direction

In the context of ongoing land use (e.g., livestock grazing), rising CO_2 levels, and concerns over climate change, my research provides a better understanding of grassland nutrient cycling and the role that grazing and environment play in the cycling of C. To further develop our understanding, it would be valuable to investigate further how temporal changes in substrates and microbial community composition may influence decomposition, as well as establish how these biochemical changes could shape litter decomposition and enzyme activity, both in dominant forage grasses, as well as in litter mixtures, over the short-term (i.e., weeks) and long-term (i.e., decades).

In addition, improving our knowledge of the relationship between enzyme activity and litter decomposition could help us to better understand the mechanisms of nutrient cycling in grassland, which in turn, may provide enhanced opportunities to identify those factors constraining plant growth, and therefore forage production and C storage. Analyzing extracellular enzymes for litter decomposition has several advantages. Enzymes are directly involved in the decomposition of organic matter and are highly relevant to litter decomposition. Moreover, enzyme activities are affected by local environmental conditions, such as temperature, pH and moisture. Additionally, substrate quality of organic matter also influences enzyme activity. Collectively, all these factors can be used as indicators of environmental conditions and organic matter availability (Sinsabaugh 1994). Consequently, exploring the more complex relationships present among litter types, EEAs and decomposition using advanced analytical tools such as structural equation models (SEM; Lamb 2008) could shed further light on understanding the effects of grazing on microbial

community composition and function in grasslands.

Another important consideration underlying decomposition and enzyme activity not covered herein is the role of the microbial community composition and its function. Different microbes have different roles in the environment, and therefore may have different responses to disturbance and enzyme capabilities (Berg and McClaugherty 2008). Identifying the microbial functional community with next generation sequencing would enable us to more fully understand the response of microbes to environmental changes. Future research that incorporates these aspects would provide the understanding required to improve predictions of where, when and how grassland C is likely to change, including in response to the direct and indirect effects of land use and climate change.

Lastly, future research could also consider different intensities of grazing and grazing system comparisons. This would provide better comparisons among the grazing system effects and help to identify management practices more beneficial for C storage.

4.4 Conclusion

Changes in land use such as grazing and climate are important in managing the C storage of grasslands, by influencing abiotic and biotic factors, as well as their interactions, that drive biogeochemical cycling processes. Findings from this study provide evidence of the role of grazing, indirect by mediating grassland composition, in influencing grassland C and nutrient cycling, such as increased decomposition rates (Chapter 2) and extracellular enzyme activity (Chapter 3). Whilst C cycling processes in grasslands are strongly influenced by biotic controls, these controls will interact with climatic conditions to regulate decomposition.

The results of this research provide information that there is a basis to improve land management and nutrient cycling in grasslands. Combining the results of this study with the

history of land use, changes in plant community composition due to anthropogenic activity (e.g., grazing) can be incorporated into grassland management to, enhance the basis for mutual understanding, cooperation and sustainable use of grassland ecosystems. Moreover, a better understanding of increasing nutrient cycling in grassland could benefit its forage productivity and subsequent livestock productivity. Increased C storage via better land management strategies also benefit all of society through a reduction of greenhouse gas emissions, and may benefit landowners if economic incentives are implemented to increase C storage.

Bibliography

Aber, J. D., Goodale, C. L., Ollinger, S. V., Smith, M. L., Magill, A. H., Martin, M. E., Hallett, R. and Stoddard, J.D. 2003. Is nitrogen deposition altering the nitrogen status of Northeastern forests? Bioscience, 53:375–389.

Achuff, P. L. 1994. Natural regions, subregions and natural history themes of Alberta. Report prepared for park services, Alberta Environmental Protection, Edmonton, Alberta. Pp. 72.

Adair, E. C., Parton, W. J., del Grosso, S.J., Silver, W. L., Harmon, M. E., Hall, S. A., Burke, I.C. and Hart, S. C. 2008. Simple three-pool model accurately describes patterns of long-term litter decomposition in diverse climates. Global Change Biology, 14: 2636–2660.

Adams, B. W., Carlson, J., Milner, D., Hood, T., Cairns, B. and Herzog, P. 2004. Beneficial grazing management practices for Sage-Grouse (*Centrocercus urophasianus*) and ecology of Silver Sagebrush (*Artemisia cana*) in Southeastern Alberta. Lethbridge, AB, Canada: Public Lands and Forests Division, Alberta Sustainable Resource Development, Publication No. T/049. Pp. 60.

Adler, P., Raff, D. and Lauenroth, W. 2001. The effect of grazing on the spatial heterogeneity of vegetation. Oecologia, 128: 465–479.

Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. Oikos, 79: 439-449.

Aerts, R. and De Caluwe, H. 1997. Nutritional and plant-mediated controls on leaf litter decomposition of Carex species. Ecology, 78: 244-260.

AESRD. 2013. Environment and Sustainable Resource Development Annual Report 2012–2013. (http://aep.alberta.ca/about-us/corporate-documents/documents/ESRD-AnnualReport-2012-13.pdf).

Aguiar, M. R., Paruelo, J. M., Sala, O. E. and Lauenroth, W. K. 1996. Ecosystem consequences of plant functional types changes in a semiarid grassland. Journal of Vegetation Science, 7: 381–390.

Ajwa, H.A. and Tabatabai, M.A. 1994. Decomposition of different organic materials in soils. Biology and Fertility of Soils, 18: 175–182.

Alberta Environment. 2005. Alberta Climate Model (ACM) to provide climate estimates (1961- 1990) for any location in Alberta from it geographic coordinates. Alberta Environment Publ. No. T/749. ISBN No. 0-7785-3939-3 (Printed Edition), ISBN. 0-7785-3941-5 (On-line Edition).

Alberta Soil Information Centre (ASIC). 2001. AGRASID 3.0: Agricultural Region of Alberta Soil Inventory Database (Version 3.0). Edited by Brierley, J. A., Martin, T. C. and Spies, D. J. Agriculture and Agri-Food Canada, and Alberta Agriculture, Food and Rural Development, Conservation and Development Branch. Edmonton, Alberta. Available: http://www.agric.gov.ab.ca/asic.

Allison, S. D. 2005. Cheaters, diffusion, and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecology Letters, 8: 626–635.

Allison, S. D. and Treseder, K. K. 2011. Climate change feedbacks to microbial decomposition in boreal soils. Fungal Ecology, 4: 362–374.

Allison, S. D. and Vitousek, P. M. 2004. Rapid nutrient cycling in leaf litter from invasive species in Hawai'i. Oecologia, 141: 612–619.

Allison, S. D. and Vitousek, P. M. 2004. Extracellular enzyme activities and carbon chemistry as drivers of tropical plant litter decomposition. Biotropica, 36: 285–296.

Allison, S. D. and Vitousek, P. M. 2005. Responses of extracellular enzymes to simple and

complex nutrient inputs. Soil Biology and Biochemistry, 37: 937–944.
Allison, S. D., Gartner, T., Holland, K., Weintraub, M. and Sinsabaugh, R. L. 2007. Soil enzymes: linking proteomics and ecological process. In: Manual of Environmental Microbiology. ASM Press, 3rd Edition, Washington D. C., pp. 704–711.
Allison, S. D., Wallenstein M. D. and Bradford M. A. 2010. Soil carbon response to warming dependent on microbial physiology. Nature Geoscience, 3: 336–340.
Amador, J. A., Glucksman, A. M., Lyons, J. B. and Gorres, J. H. 1997. Spatial distribution of soil phosphatase activity within a riparian forest. Soil Science, 162: 808–825.
Amin, B., Chabbert, B., Moorhead, D. and Bertrand, I. 2013. Impact offine litter chemistry on lignocellulolytic enzyme efficiencyduring decomposition of maize leaf and root in soil. Bio-geochemistry, 117: 169–183.
Anderson, J. M. and Hetherington, S. L. 1999 Temperature, nitrogen availability and mixture effects on the decomposition of heather (*Calluna vulgaris (L.*) Hull) and bracken (*Pteridium aquilinum (L.*) Kuhn) litters. Functional Ecology, 13(Suppl. 1): 116–124.
Asner, G. P., Elmore, A. J., Olander, L. P., Martin, R. E. and Harris, A. T. 2004. Grazing systems, ecosystem responses, and global change. Annual Review of Environment and Resources, 29: 261–299.
Augustine, D. J. and McNaughton, S. J. 1998. Ungulate effects on functional species composition of plant communities: herbivore selectivity and plant tolerance. Journal of Wildlife Management, 62: 1164–1182.
Bahm, M. A., Barnes, T. G. and Jensen, K. C. 2011. Restoring native plant communities in smooth brome (*Bromus inermis*)-dominated grasslands. Invasive Plant Science and Management. 4: 239–250.
Bailey, A. W., Schellenberg, M. P., McCartney, D. and Bailey, P. 2010. Politics, policies, settlers, and consequences for Canadian prairie grasslands: a range management prospective. Rangelands, 32: 17–25.
Bandick, A. K. and Dick, R. P. 1999. Field management effects on soil enzyme activities. Soil Biology and Biochemistry, 31: 1471–1479.
Banerjee, M. R., Burton, D. L., McCaughey, W. P. and Grant, C. A. 2000. Influence of pasture management on soil biological quality. Journal of Range Management, 53: 127–133.
Bapiri, A., Bååth, E. and Rousk, J. 2010. Drying-rewetting cycles affect fungal and bacterial growth differently in an arable soil. Microbial Ecology, 60: 419–428.
Bardgett, R. D. and Wardle, D. A. 2003. Herbivore mediated linkages between above-ground and below-ground communities. Ecology, 84: 2258–2268.
Bardgett, R. D., Jones, A. C., Jones, D. L., Kemmitt, S. J., Cook, R. and Hobbs, P. J. 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biology and Biochemistry, 33: 1653–1664.
Bardgett, R. D., Wardle, D. A. and Yeates, G. W. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry, 30: 1867–1878.
Bardgett, R. D., Wardle, D. A. and Yeates, G. W. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biology and Biochemistry, 30: 1867–1878.
Bardgett, R.D. and Wardle, D.A. 2003. Herbivore mediated linkages between aboveground and belowground communities. Ecology, 84: 2258–2268.
Batten, K. M., Scow, K. M. and Espeland, E. K. 2008. Soil microbial community associated with an invasive grass differentially impacts native plant performance. Microbial Ecology. 55:220–228.
Batten, K.M., Scow, K. M., Davies, K. F. and Harrison, S. P. 2006. Two invasive plants alter

soil microbial community composition in serpentine grasslands. Biological Invasions, 8: 217–230.
Bauchop, T. and Elsden, S. R. 1960. The growth of micro-organisms in relation to their energy supply. Journal of General Microbiology, 23: 457–469.
Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K. and Wallenstein, M. D. 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experiments, 81: 50961.
Bell, T. H. and Henry, H. A. L. 2011. Fine scale variability in soil extra-cellular enzyme activity is insensitive to rain events and temperature in a mesic system. Pedobiology, 2: 141–146.
Berg, B. 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management, 133: 13–22.
Berg, B. and Ekbohm, G. 1991. Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a Scots pine forest. VII. Canadian Journal of Botany, 69: 1449–1456.
Berg, B. and McClaugherty, C. 2008. Plant litter – Decomposition, Humus formation, Carbon sequestration. Springer Verlag. Heidelberg. Berlin. p. 338.
Berg, B., Laskowski, R. 2006. Litter decomposition: a guide to carbon and nutrient turnover. Advances in Ecological Research 38. Elsevier. Pp. 421.
Binnema, T. 2006. “With Tears, Shrieks, and Howlings of Despair”: The Smallpox Epidemic of 1781-1782. In Alberta Formed Alberta Transformed, ed. by Michael Payne, Donald Wetherell, and Catherine Cavanaugh, Pp. 111–132. University of Alberta Press, Edmonton.
Biondini, M. E., Patton, B. D. and Nyren, P. E. 1998. Grazing intensity and ecosystem processes in a northern mixed-grass prairie, USA. Ecological Applications, 8: 469–479.
Blume, E., Bischoff, M., Reichert, J., Moorman, T., Konopka, A. and Turco, R. 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology, 592: 1–11.
Bork, E. W. and Irving, B. D. 2015. Seasonal availability of cool- and warm-season herbage in the northern mixed prairie. Rangelands, 37: 178–185.
Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R. and Wood, S. A. 2016. Understanding the dominant controls on litter decomposition. Journal of Ecology, 104: 229–238.
Bremer, D. J., Auen, L. M., Ham, J. M. and Owensby C. E. 2001. Evapotranspiration in a prairie ecosystem: effects of grazing by cattle. Agronomy Journal, 93: 338–348.
Brockett, B. F. T., Prescott, C. E. and Grayston, S. J. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry. 44:9–20.
Brzostek, E. R. and Finzi, A. C. 2011. Substrate supply, fine roots and temperature control proteolytic enzyme activity in temperate forest soils. Ecology, 92: 892–902.
Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S. and Thomas, T. 2011. Bacterial community assembly based on functional genes rather than species. Proceedings of the National Academy of Sciences, 108: 14288–14293.
Burke, R. M. and Cairney, J. W. G. 2002. Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza, 12: 249–255.
Burns, R. G. 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biology and Biochemistry, 14: 423–427.
Caldwell, B. A. 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia, 49: 637–644.

Caldwell, B. A., Griffiths, R. P. and Sollins, P. 1999. Soil enzyme response to vegetation disturbance in two lowland Costa Rican soils. Soil Biology and Biochemistry, 31: 1603–1608.
Canfield, D. E., Glazer, A. N. and Falkowski, P. G. 2010. The evolution and future of Earth's nitrogen cycle. Science, 330: 192–196.
Castelli O. Fent L., McEwan D., Adams B., Ott R. and McNeil. R. 2005. Grassland Vegetation Inventory (GVI) Final Report. GVI Committee, Lethbridge, Alberta: 1–67.
Chapin, F.S., III , P.A. Matson , and H.A. Mooney . 2002. Principles of terrestrial ecosystem ecology. Springer, New York, NY.
Chapman, J. A., King, J. S., Pregitzer, K. S. and Zak, D. R. 2005. Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on decomposition of fine roots. Tree Physiology, 25: 1501–1510.
Chapman, K., Whittaker, J.B. and Heal, O.H. 1988. Metabolic and faunal activity in litters of three mixtures compared to pure stands. Agriculture Ecosystems and Environment, 24: 33–40.
Chapman, S. K. and Newman, G. S. 2009. Biodiversity at the plant-soil interface: microbial abundance and community structure respond to litter mixing. Oecologia, 162: 763–769.
Chapman, S. K. and Newman, G. S. 2010. Biodiversity at the plant-soil interface: microbial abundance and community structure respond to litter mixing. Oecologia, 162: 763–769.
Clarholm, M. 1993. Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. Biology and Fertility of Soils, 16: 287–292.
Cleveland, C. C., Reed, S. C., Keller, A. B., Nemergut, D. R., O'Neil, S. P., Ostertag, R. and Vitousek, P. M. 2014. Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. Oecologia, 174: 283–294.
Conant, R. T., Paustian, K. and Elliott E. T. 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications, 11: 343–355.
Cornelissen, J. 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. Journal of Ecology, 84: 573–582.
Cornelissen, J. and Thompson, K. 1997. Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytologist, 135: 109–114.
Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., Hobbie, S. E., Hoorens, B., Kurokawa, H., Pérez-Harguindeguy, N., Quested, H. M., Santiago, L. S., Wardle, D. A., Wright, I. J., Aerts, R., Allison, S. D., Van Bodegom, P., Brovkin, V., Chatain, A., Callaghan, T. V., Díaz, S., Garnier, E., Gurvich, D. E., Kazakou, E., Klein, J. A., Read, J., Reich, P. B., Soudzilovskaia, N. A., Vaieretti, M. V. and Westoby, M. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters, 11: 1065–1071.
Cou'teaux, M.M., P. Bottner, and B. Berg. 1995. Litter decomposition, climate and litter quality. Trends in Ecology and Evolution, 10: 63–66.
Coughenour, M. B. 1991. Spatial components of plant-herbivore interactions in pastoral, ranching and native ungulate ecosystems. Journal of Rangeland Management, 44: 530–542.
Coulson, J. C. and Butterfield, J. 1978. An investigation of the biotic factors determining the rates of plant decomposition on Blanket Bog. Journal of Ecology, 66: 631–650.
Coupland, R. T. and Johnson, R. E. 1965. Rooting characteristics of native grassland species in Saskatchewan, Journal of Ecology, 53: 475–507.
Coupland, R.T., Brayshaw, T. 1953. The fescue grassland in Saskatchewan. Ecological

Society of America, 34: 386–405.
Cusack, D. F., Chou, W. W., Yang, W. H., Harmon, M. E., Silver, W. L. and Team, T. L. 2009. Controls on long-term root and leaf litter decomposition in neotropical forests. Global Change Biology, 15: 1339–1355.
Datta, S., Christena, L. R. and Rajaram, Y. R. S. 2013. Enzyme immobilization: an overview on techniques and support materials. Biotech, 3: 1–9.
Daubenmire, R. and Prusso, D. C. 1963. Studies of decomposition rates of tree litter. Ecology, 44: 589–592.
Davidson, E. A., Samanta, S., Caramori, S. S. and Savage, K. 2012. The dual Arrhenius and Michaelis-Menten (DAMM) kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. Global Change Biology, 18: 371–384.
Davidson, E.A., Trumbore S.E. and Amundson R. 2000. Biogeochemistry: soil warming and organic carbon content. Nature, 408: 789–790.
De Marco, A., Meola A., Maisto G. Maria G. and De Santo A.V. 2011. Non-additive effects of litter mixtures on decomposition of leaf litters in a Mediterranean maquis. Plant and Soil, 344: 305 – 17.
DeAngelis, K. M., Silver, W. L., Thompson, A. W. and Firestone M. K. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology, 12: 3137–3149.
DeForest, J. L. 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biology and Biochemistry, 41: 1180–1186.
DeKeyser, E. S., Meehan, M., Clambey, G. and Krabbenhoft, K. 2013. Cool season invasive grasses in northern Great Plains natural areas. Natural Areas Journal, 33(1): 81–90.
Delgado, C., Narrod, C. and Tiongco, M. 2008. Determinants and implications of the growing scale of livestock farms in four fast-growing developing countries. Research Report 157. International Food Policy Research Institute (IFPRI), Washington D.C.
Derner, J. D., Briske, D. D. and Boutton, T. W. 1997. Does grazing mediate soil carbon and nitrogen accumulation beneath C_4, perennial grasses along an environmental gradient? Plant and Soil, 191: 147–156.
Deutsch, E. S., Bork, E. W. and Willms, W. D. 2010. Separation of grassland litter and ecosite influences on seasonal soil moisture and plant growth dynamics. Plant Ecology, 209: 135–145.
Deutsch, E. S., Bork, E. W. and Willms, W. D. 2010. Soil moisture and plant growth responses to litter and defoliation impacts in Parkland grasslands. Agriculture, Ecosystems and Environment, 135: 1–9.
Deutsch, E. S., Bork, E. W., Cahill, J. F. and Chang, S. X. 2011. Short-term plant community responses to warming and defoliation in a northern temperate grassland. ISRN Ecology.
Dick, R. P. 1997. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst, C. E., Doube, B. M. and Gupta, V. V. S. R. (eds) Biological indicators of soil health. CABI. Wellingford. Pp. 121–156.
Dick, R. P., Sandor, J. A. and Eash, N. S. 1994. Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. Agriculture, Ecosystem and Environment, 50: 123–131.
Dick, W. A., Cheng, L. and Wang, P. 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biology and Biochemistry, 32: 1915–1919.
Dilly, O. and Nannipieri, P. 1998. Intracellular and extracellular enzyme activity in soil with reference to elemental cycling. Zeitschrift Fur Pflanzenernahrung Und Bodenkunde, 161: 243–248.

Dormaar, J. F. and Willms W. D. 1990. Effect of grazing and cultivation on some chemical properties of soils in the mixed prairie. Journal of Range Management, 43: 456–460.
Dormaar, J. F., Adams, B. W. and Willms W. D. 1994. Effect of grazing and abandoned cultivation on a *Stipa-Bouteloua* community. Journal of Range Management, 47: 28–32.
Dormaar, J.F, Johnston, A., Smoliak, S. 1984. Seasonal changes in carbon content, and dehydrogenase, phosphatase, and urease activities in mixed prairie and fescue grassland Ah horizons. Journal of Range Management, 37: 31–35.
Dormaar, J.F., Willms, W.D. 1998. Effect of forty-four years of grazing on fescue grassland soils. Journal of Range Management, 51: 122–126
Dormmr, J.F., Johnston, A. and Smolink, S. 1977. Seasonal variation in chemical characteristics of soil organic matter of grazed and ungrazed mixed prairie and fescue grassland. Journal of Range Management, 30: 195–198.
Downing, D., Pettapiece, W. 2006. Natural regions and subregions of Alberta. Government of Alberta. Publication Number: T/852.
Dyck, I. G. 1983. The Prehistory of Southern Saskatchewan. In Tracking Ancient Hunters: Prehistoric Archaeology in Saskatchewan, edited by Epp, H. T. and Dyck, I. Saskatchewan Archaeological Society, Regina. Pp. 63–139.
Dyer, M. I., Acra, M. A., Wang, G. M., Coleman, D. C., Freckman, D. W., McNaughton, S. J. and Strain, B. R. 1991. Source-sink carbon relations in two *Panicum coloratum* ecotypes in response to herbivory. Ecology, 72: 1472–1483.
Eckert, J. B., Baker, B. B and Hanson, J. D. 1995. The impact of global warming on local incomes from range livestock systems. Agricultural Systems, 48: 87–100.
Eivazi, F. and Tabatabai, M. A. 1977. Phosphatases in soils. Soil Biology and Biochemistry, 9: 167–172.
Eriksson, K. E. L., Blanchette, R. A. and Ander, P. 1990. Microbial and enzymaticv degradation of wood and wood components. Springer-Verlag Berlin Heidelberg, New York.
Esen, A. 1993. β-Glucosidases, overview. In: Esen, A. (Ed.), b-glucosidase: biochemistry and molecular biology. American Chemical Society, Washington, DC, pp. 1–13.
Esen, A. 1993. β-glucosidases: overview. In: Esen, A. (ed) β-glucosidases and molecular biology. American Chemical Society, Washington, DC, Pp. 9–17.
FAO, 2015. FAO statistical pocketbook 2015. World Food and Agriculture. Rome. 2015.
Farrar, J., Boddy, E., Hill, P. W. and Jones, D. L. 2012. Discrete functional pools of soil organic matter in a UK grassland soil are differentially affected by temperature and priming. Soil Biology and Biochemistry, 49: 52–60.
Farrell, M., Macdonald, L. M., Hill, P. W., Wanniarachchi, S. D., Farrar, J., Bardgett, R. D. and Jones, D.L. 2014. Amino acid dynamics across a grassland altitudinal gradient. Soil Biology and Biochemistry, 76: 179–182.
Fenner, N., Freeman, C. and Reynolds, B. 2005. Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling. Soil Biology and Biochemistry, 37: 1277–1287.
Fioretto, A., Nardo, C. D., Papa, S. and Fuggi, A. 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biology and Biochemistry, 37: 1083–1091.
Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M. and Van Dorland, R. 2007: Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

Frank, A. B., Tanaka, D. L., Hofmann, L. and Follett, R. F. 1995. Soil carbon and nitrogen of northern great plains grasslands as influenced by long-term grazing. Journal of Range Management, 48: 470–474.
Freschet, G, T., Aerts, R. and Cornelissen, J. H. C. 2012b. A plant economics spectrum of litter decomposability. Functional Ecology, 26: 56–65.
Fterich, A., Mahdhi, M., Lafuente, A., Pajuelo, E., Caviedes, M. A., Rodriguez-Llorente, I. D. and Mars, M. 2012. Taxonomic and symbiotic diversity of bacteria isolated from nodules of *Acacia tortilis* subsp. *raddiana* in arid soils of Tunisia. Canadian Journal of Microbiology, 58: 738–751.
Gallardo, A. and Merino, J. 1993. Leaf decomposition in two Mediterranean ecosystems of Southwest Spain: influence of substrate quality. Ecology, 74: 152–161
Gallo, M. E., Sinsabaugh, R. L. and Cabaniss, S. E. 2006. The role of ultraviolet radiation in litter decomposition in arid ecosystems. Applied Soil Ecology, 34: 83–91.
Garibaldi, L. A., Semmartin M. and Chaneton E. J. 2007. Grazing-induced changes in plant composition affect litter quality and nutrient cycling in flooding Pampa grasslands. Oecologia, 151: 650–662.
Gartner, T. B. and Cardon, Z. G. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos, 104: 230–46.
Geisseler, D., Horwath, W. R. and Scow, K. M. 2011. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia, 2: 71–78.
German, D. P., Marcelo, K. R. B., Stone, M. M. and Allison, S. D. 2012. The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. Global Change Biology, 18: 1468–1479.
German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L. and Allison, S. D. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry, 43: 1387–1397.
German, M. A., Dai, N., Matsevitz, T., Hanael, R., Petreikov, M., Bernstein, N., Ioffe, M., Shahak, Y., Schaffer, A. A. and Granot, D. 2003. Suppression of fructokinase encoded by LeFRK2 in tomato stem inhibits growth and causes wilting of young leaves. The Plant Journal, 34: 837–846.
Gholz, H. L., Wedin, D. A., Smitherman, S. M., Harmon, M. E. and Parton, W. J. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology, 6: 751–765.
Ghose, T. K. 1977. Bioconversion of cellulosic substances into energy chemicals and microbial protein (Symposium Proceedings, Indian Institute of Technology and Swiss Federal Institute of Technology). New Delhi : Aroon Purieat Thomson Press.
Gianfreda, L. 2015. Enzymes of importance to rhizosphere processes. Journal of Soil Science and Plant Nutrition. en: http://dx.doi.org/10.4067/S0718-95162015005000022.
Gill, R. A., Burke, I. C., Lauenroth, W. K. and Milchunas, D. G. 2002. Longevity and turnover of roots in the shortgrass steppe: influence of diameter and depth. Plant Ecology, 159: 241–251.
Goebel, T., Waters, M. and O'Rourke, R. 2008. The late pleistocene dispersal of modern humans in the Americas. Science, 319: 1497–1502.
Golley, F. B. 1965. Structure and function of an old-field broomsedge community. Ecological Monographs, 35: 113–131.
Government of Alberta. 2015–16 Annual Report. (http://www.finance.alberta.ca/publications/annual_repts/govt/2015-16/goa-2015-16-annual-report-complete.pdf).
Grant, T. A., Flanders-Wanner, B., Shaffer, T. L., Murphy, R. K. and Knutsen, G. A. 2009. An emerging crisis across Northern Prairie refuges: prevalence of invasive plants and a

plan for adaptive management. Ecological Restoration, 27: 58–63.
Gross, J. A. and Knight, J. E. 2000. Elk presence inside various-sized cattle exclosures. Journal of Range Management, 53: 287–290.
Güsewell, S. and Gessner, M. O. 2009. N:P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology, 23: 211–219.
Hamilton, J. G., DeLucia, E. H., George, K., Naidu, S. L., Finzi, A. C. and Schlesinger, W. H. 2002. Forest carbon Balance under elevated CO_2. Oecologia, 131: 250–260.
Hansen, R. A. and Coleman, D. C. 1998. Litter complexity and composition are determinants of the diversity and species composition of oribatid mites (*Acari : Oribatida*) in litterbags. Applied Soil Ecology, 9: 17–23.
Harborne, J. B. 1997. Role of phenolic secondary metabolites in plants and their degradation in nature. In: Cadish, G. and Giller, K. E. (eds) Driven by nature: plant litter quality and decomposition, CAB International, London, Pp. 67–74.
Harmon, M. E. 2009. Woody detritus mass and its contribution to carbon dynamics of old-growth forests: the temporal context. In old-growth forests: Function, fate and value, Ecological studies 207, ed. Wirth, C., Gleixner, G. and Heimann. M. Berlin: Springer.
Harmon, M. E., Krankina, O. N., Yatskov, M. and Matthews, E. 2000: Predicting broad-scale carbon stores of woody detritus from plot-level data. In Assessments Methods for Soil Carbon. Lal, R., Kimble, J. M., Follett, R. F. and Stewart, B. A. Eds. Lewis Publishers, 533–552.
Harrison, A. F. 1971. The inhibitory effect of oak leaf litter tannins on the growth of fungi, in relation to litter decomposition. Soil Biology and Biochemistry, 3: 167–172.
Hättenschwiler, S. and Vitousek, P. M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology and Evolution, 15: 238–243.
Havstad, K.M., Peters, D.P.C., Skaggs, R., Brown, J., Bestelmeyer, B., Fredrickson, E., Herrick, J. and Wright, J., 2007. Ecological services to and from rangelands of the United States. Ecological Economics, 64: 261–268.
Hayashi, H. Alia, Mustardy, L., Deshnium, P., Ida, M. and Murata, N.1997. Transformation of Arabidopsis thaliana with the codA gene for choline oxidase; accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. The Plant Journal, 12: 133–142.
Hayes, G.F. and Holl K.D. 2003. Cattle grazing impacts on annual forbs and vegetation composition of mesic grasslands in California. Conservation Biology, 17: 1694–1702.
Haynes, R. and Williams P. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy, 49: 119–199.
Heal, O., Anderson, J. and Swift, M. 1997. Plant litter quality and decomposition: an historical overview. *In*: Cadisch G, and Giller KE, editors. Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, UK. Pp. 3–32.
Hector, A., Beale, A. J., Minns, A., Otway, S. J. and Lawton, J. H. 2000. Consequences of the reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. Oikos, 90: 357–371.
Henderson, D. C., Ellert, B. H., Naeth, M. A. 2004. Utility of ^{13}C for ecosystem carbon turnover estimation in grazed mixed grass prairie. Geoderma, 119: 219–231.
Henderson, D., Ellert, B. and Naeth M. 2004. Grazing and soil carbon along a gradient of Alberta rangeland. Journal of Range Management, 57: 402–410.
Henry, H. A., Cleland, E. E., Field, C. B. and Vitousek, P. M. 2005. Interactive effects of elevated CO_2, N deposition and climate change on plant litter quality in a California annual grassland. Oecologia, 142: 465–473.
Hewins, D. B., Chuan, X., Bork, E. W. and Carlyle, C. N. 2016. Measuring the effect of

freezing on hydrolytic and oxidative extracellular enzyme activities associated with plant litter decomposition. Pedobiologia, 59: 5–6.
Hewins, D. B., Fatemi, F., Adams, B., Carlyle, C. N., Chang, S. X. and Bork, E. W. 2015. Grazing, regional climate and soil biophysical impacts on microbial enzyme activity in grassland soil of western Canada. Pedobiologia, 58: 201–209.
Hobbie, S. E. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. Ecological Monographs, 66: 503–522.
Hobbie, S.E., Oleksyn, J., Eissenstat, D. M. and Reich, P. B. 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia, 162: 505–513.
Hobbs, N. T. 1996. Modification of ecosystems by ungulates. The Journal of Wildlife Management, 60: 695–713.
Holechek, J. L., Hawkes, J. and Darden, T. D. 1994. Macro-economics and cattle ranching. Rangelands, 16: 118-123.
Holechek, J. L., Tembo, A., Daniel, A., Fusco, M. and Cardenas, M. 1994. Long term grazing influences on Chihuahuan Desert Rangeland. The Southern Naturalist, 39: 342–349.
Holland, E. A. and Coleman, D. C. 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology, 68: 425–433.
Holland, E. A. and Detling J. K. 1990. Plant response to herbivory and belowground nitrogen cycling. Ecology, 71: 1040–1049.
Holland, E. A., Patton, W. J., Detling, J. K., and Coppock, L. 1992. Physiological responses of plant populations to herbivory and their consequences for ecosystem nutrient flow. The American Naturalist, 140: 685–706.
Holland, E.A. and Detling, J.K. 1990. Plant response to herbivory and belowground nitrogen cycling. Ecology, 71: 1040–1049.
Iiyama, K. and Wallis A. F. A. 1990. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. Journal of the Science of Food and Agriculture, 51: 145–161.
IPCC, 2001: Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Houghton, J., Ding T.Y., Griggs, D. J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K. and Johnson, C.A. (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, Pp. 881.
Isobe, K. and Ohte, N. 2014. Ecological perspectives on microbes involved in N-cycling. Microbes and Environments, 29: 4–16.
Izaurralde, R.C., McGill, W.B., Robertson, J.A., Juma, N.G. and Thurston J.J. 2001. Carbon balance of the Breton classical plots over half a century. Soil Science Society of America Journal, 65: 431–441.
James, E. S., Russel, L. W. and Mitrick, A. 1991. Phosphate stress response in hydroponically grown maize. Plant and Soil, 132: 85–90.
Jaramillo, V. J. and Detling, J. K. 1988. Grazing history, defoliation, and competition: effects on shortgrass production and nitrogen accumulation. Ecology, 69: 1599–1608.
Jenkinson, D. S. 1977. Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from ^{14}C labeled rye-grass decomposing under field conditions. Journal of Soil Science, 28: 424–434.
Jenkinson, D. S., Adams, D. E. and Wild, A. 1991. Model estimates of CO_2 emissions from soil in response to global warming. Nature, 351: 304–306.
Johansson, M. B., Berg, B. and Meentemeyer, V. 1995. Litter mass-loss rates in late stages of decomposition in a climatic transect of pine forests. Long-term decomposition in a Scots pine forest. IX. Canadian Journal of Botany, 73: 1509–1521.

Jones, M.B. and Donnelly, A. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO_2. New Phytologist, 164: 423–439.
Jones, D. L., Kielland, K., Sinclair, F. L., Dahlgren, R. A., Newsham, K. K., Farrar, J. F. and Murphy, D. V. 2009. Soil organic nitrogen mineralization across a global latitudinal gradient. Global Biogeochemical Cycles, 23: GB1016
Joyce, L. A., Briske, D. D., Brown, J. R., Polley, H. W., McCarl, B. A. and Bailey, D. W. 2013. Climate change and north American rangelands: assessment of mitigation and adaptation strategies. Rangel Ecology and Management, 66: 512–528.
Juma, N. G. and Tabatabai, M. A. 1978. Distribution of phosphomonoesterases in soils. Soil Science, 126: 101–108.
Kamimura, Y. and Hayano, K. 2000. Properties of protease extracted from tea-field soil. Biology Fertility and Soils, 30: 351–355.
Kaneko, N. and Salamanca, E. F. 1999. Mixed leaf litter effects on decomposition rates and soil microarthropod communities in an oak-pine stand in Japan. Ecological Research, 14: 131–138.
Kanfer, J. N., Mumford, R. A., Raghavan, S. S. and Byrd, J. 1974. Purification of β-glucosidase activities from bovine spleen affinity chromatography. Analytical Biochemistry, 60: 200–205.
Kang, H. and Freeman, C. 2009. Soil enzyme analysis for leaf litter decomposition in global wetlands. Communications in Soil Science and Plant Analysis, 40: 3323–3334.
Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F. and Schuman G.E. 1997. Soil quality: a concept, definition, and framework for evaluation. Soil Science Society of America Journal, 61:4–10.
Katz, M. and Reese, E. T., 1968. Production of glucose by enzymatic hydrolysis of cellulose. Applied Microbiology, 16: 419–420.
Kazakou, E., Violle, C., Roumet, C., Pintor, C., Gimenez, O. and Garnier, E. 2009. Litter quality and decomposability of species from a Mediterranean succession depend on leaf traits but not on nitrogen supply. Annals of Botany, 104: 1151–1161.
Kelly, R. H. and Burke I. C. 1997. Heterogeneity of soil organic matter following death of individual plants in shortgrass steppe. Ecology, 78: 1256–1261.
Kimball, S. and Schiffman, P.M. 2003. Differing effects of cattle grazing on native and alien plants. Conservation Biology, 17: 1681–1693.
Kirk, T. K. 1983. Degradation and conversion of lignocelluloses. In: Smith, J. E., Berry, D.R. and Kristiansen, B. (eds) The filamentous fungi. IV. Fungal technology. Arnold, London, Pp. 266–295.
Kirschbaum, M. U. F. 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? Biogeochemistry, 48: 21–51.
Kiss, S., Dragan-Bularda, M. Radulescu, D. 1978. Soil polysaccharidases: activity and agricultural importance. In: Burns, R. G. (ed). Soil enzymes. Academic, London, Pp. 117–147.
Klemmedson, J. O. 1956. Interrelations of vegetation, soils and range conditions induced by grazing. Journal of Range Management, 9: 134–138.
Klemmedson, J. O. 1992. Decomposition and nutrient release from mixtures of Gambel oak and Ponderosa pine leaf litter. Forest Ecology and Management, 47: 349–361.
Klumpp, K. Tallec, T., Guix, N. and Soussana J. F. 2011. Long-term impacts of agricultural practices and climatic variability on carbon storage in a permanent pasture. Global Change Biology, 17: 3534–3545.
Knapp, A. K. and Seastedt, T. R. 1986. Detritus accumulation limits productivity of tallgrass prairie. BioScience, 36: 662–668.

Koch, O., Tscherko, D. and Kandeler, E. 2007. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils: temperature sensitivity in alpine soils. Global Biogeochemical Cycles, 21: GB4017.
Kourtev, P. S. Ehrenfeld, J. G. and Haggblom, M. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology, 83: 3152–3166.
Lal, R. 2002. Soil carbon dynamics in cropland and rangeland. Environmental Pollution, 116: 353–362.
Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. Science, 304: 1623.
Lamb, E. G. 2008. Direct and indirect control of grassland community structure by litter, resources and biomass. Ecology, 89: 216–225.
Lange, M., Eisenhauer, N., Sierra, C., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vazquez, P. G., Malik, A. A., Roy, J. Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E. and Gleixner, G. 2015. Plant diversity drives soil carbon storage by increased soil microbial activity. Nature Communications, 6: 6707.
Lavado, R. S. and Taboada, M. A. 1988. Soil water, salts, and sodium dynamics in a Natraquoll of Argentina. Catena, 15: 577–594.
Legg, T. M., Zheng, Y., Simone, B., Radloff, K. A., Mladenov, N., González, A., Nemergut, D. R. (2012). Carbon, metals, and grain size correlate with bacterial community structure in sediments of a high arsenic aquifer. Frontiers in Microbiology, 3: 82. http://doi.org/10.3389/fmicb.2012.00082.
Leriche, H., Le Roux, X., Desnoyers, F., Benest, D., Simioni, G. and Abbadie, L. 2003. Response of grass dry-matter- and nitrogen- yields to clipping in an African savanna : an experimental test of the herbivory optimization hypothesis. Ecological Applications, 13: 1346–1354.
Liu, P., Huang, J. H., Han, X.G. and Sun, O.J. 2009. Litter decomposition in semiarid grassland of Inner Mongolia, China. Rangeland Ecology and Management, 62: 305–13.
Logue, J. B., and Lindstrom, E. S. 2008. Biogeography of bacterioplankton in inland waters. Freshwater Reviews, 1: 99–114.
Looman, J. 1969. The fescue grasslands of western Canada. Plant Ecology, 19: 128–145.
Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J. I., Richaume, A. and Le Roux, X. 2005. Effect of grazing on microbial functional groups involved in soil N dynamics. Ecological Monographs, 75: 65–80.
Lucas, D. M., Fontenot, J. P. and Webb, K. E. 1975. Composition and digestibility of cattle fecal waste. Journal of Animal Science, 5: 1480–1486.
Lundquist, E. J., Jackson, L. E. and Scow, K. M. 1999. Wet-dry cycles affect dissolved organic carbon in two California agricultural soils, Soil Biology and Biochemistry, 31: 1031–1038.
Luxhøi, J., Magid, J., Tscherko, D. and Kandeler, E. 2002. Dynamics of invertase, xylanase and coupled quality indices of decomposing green and brown plant residues. Soil Biology and Biochemistry, 34: 501–508.
Lyseng, M. P. 2016. Impact of grazing on Alberta's northern temperate grasslands. M. Sc. Thesis. University of Alberta. Edmonton.
Mbogga, M. S., Hansen, C., Wang, T. and Hamann, A. 2010. A comprehensive set of interpolated climate data for Alberta. Government of Alberta, Publication Number: Ref. T/235. ISBN: 978-0-7785-9184-9 (on-line edition), 978-0-7785-9183-2 (print edition).
McArthur, J. V., Aho, J. M., Rader, R. B. and G. L. Mills. 1994. Interspecific leaf interactions

during decomposition in aquatic and floodplain ecosystems. Journal of the North American Benthological Society, 13: 57–67.
McGinnies, W. J. 1987. Effects of hay and straw mulches on the establishment of seeded grasses and legumes on rangeland and a coal strip mine. Journal of Range Management, 40: 119–121.
McLaren, A. D. 1975. Soil as a system of humus and clay immobilized enzymes. Chemica Scripta, 8: 97–99.
McNaughton, S. J. 1985. Ecology of a grazing ecosystem—the Serengeti. Ecological Monographs, 55: 259–294.
McNaughton, S. J. and Augustine, D. J. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. Journal of Wildlife Management, 62: 1165–1183.
McNaughton, S. J. and Chapin, F. S, 1985. Effects of phosphorous nutrition and defoliation on C_4 graminoids from the Serengeti Plains. Ecology, 66: 1617–1629.
McNaughton, S. J., Banyikwa, F. F. and McNaughton M. M.1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. Science, 278: 1798–1800.
McNaughton, S. J., Ruess, R. W. and Seagle S. W. 1988. Large mammals and process dynamics in African ecosystems. BioScience, 38: 794–800.
Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. Ecology, 59: 465–475.
Menezes, S. C., Elliott, E. T., Valentine, D. W. and Williams, S. A. 2001. Carbon and nitrogen dynamics in elk winter ranges. Journal of Range Manage, 54: 400–408.
Milchunas, D. G., Lauenroth, W. K., Chapman, P. L. and Kazempour, M. K. 1989. Effects of grazing, topography, and precipitation on the structure of a semiarid grassland. Vegetatio, 80: 11–23.
Mohanty, R. K., Jain, M. K. and Dhall, D. 2013. High accuracy cubic spline approximation for two dimensional quasi-linear elliptic boundary value problems. Applied Mathematical Modelling, 37: 155–171.
Moleele, N. M., Ringrose, S., Matheson, W. and Vanderpost, C. 2002. More woody plants? The status of bush encroachment in Botswana's grazing areas. Journal of Environmental Management, 64: 3–11.
Monz, C. A., Hunt, H. W., Reeves, F. B. and Elliott, E. T. 1994. The response of mycorrhizal colonization to elevated CO_2 and climate cange in *Pascopyrum smithii* and *Bouteloua gracilis*. Plant and Soil, 165: 75–80.
Moorhead, D. L. and Sinsabaugh, R. L. 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology. 14: 71–79.
Moorhead, D. L. and Sinsabaugh, R. L. 2006. A theoretical model of litter decay and microbial interaction. Ecology Monographs, 76: 151–174.
Moorhead, D. L., Lashermes G. and Sinsabaugh R. L. 2012. A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biology and Biochemistry, 53: 133–141.
Moorhead, D. L., Rinkes, Z. L., Sinsabaugh, R. L. and Weintraub, M. N. 2013. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. Front Microbiology, 4: 223.1–223.12.
Morgan, R. G. 1980. Bison movement patterns on the Canadian plains: an ecological analysis. Plains Anthropologist, 25: 143–160.
Moretto, A. S., Distel, R. A., Didoné, N. G. 2001. Decomposition and nutrient dynamic of leaf litter and roots from palatable and unpalatable grasses in a semi-arid grassland. Applied Soil Ecology, 18: 31–37.

Moser, L. E., Anderson, S. R. and Miller, R. W. 1968. Rhizome and tiller development of Kentucky bluegrass (*Poa pratensis L.*) as influenced by photoperiod, cold treatment variety. Agronomy Journal, 60: 632–635.
Mudge, S. R., Rae, A. L., Diatloff, E. and Smith, F. W. 2002. Expression analysis suggests novel roles for members of Pht1 family of phosphate transporters in Arabidopsis. The Plant Journal, 31: 341–353.
Naeth, M. A., Bailey, A. W., Pluth, D. J., Chanasky, D. S. and Hardin, R. T. 1991. Grazing impacts on litter and soil organic matter in Mixedprairie and Fescue grassland ecosystem of Alberta. Journal of Range Management, 44: 7–12.
Nakari-Setälä, T., Aro, N., Ilmén, M., Muñoz, G., Kalkkinen, N. and Penttilä, M. 1997. Differential expression of the vegetative and spore-bound hydrophobins of Trichoderma reesei: cloning and characterization of the hfb2 gene. European Journal of Biochemistry, 248: 415–423.
Niemi, R. M. and Vepsalainen, M. 2005. Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils. Journal of Microbiological Methods, 60: 195–205.
Odum, E. P. 1960. Organic production and turnover in old field succession. Ecology, 41: 34–49.
Oelkers, E. H. and Cole, D. R. 2008. Carbon dioxide sequestration: A solution to a global problem. Elements, 4(5): 305–310.
Oertli, B. 1993. Leaf litter processing and energy flow through macroinvertebrates in a woodland pond (Switzerland). Oecologia, 96: 466–477.
Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. and Wagner, H., 2013. Vegan: Community ecology package R package version 2, 0–10.
Olivera, N. L., Prieto, L., Carrera, A. L., Cisneros, H. S. and Bertiller, M. B. 2014. Do soil enzymes respond to long-term grazing in an arid ecosystem? Plant and Soil, 378: 35–48.
Olofsson, J., Moen, J. and Oksanen, L. 2002. Effects of herbivory on competition intensity in two arctic-alpine tundra communities with different productivity. Oikos, 96: 265–272.
Olson, J. S. 1963. Energy storage and the balance of producers and decomposers in ecological systems. Ecology, 44: 322–331.
Palm, C. A. and Rowland, A. P. 1997. Chemical characterization of plant quality for decomposition. In: Cadisch, G. and Giller, K.E., ed., Driven by nature: plant litter quality and decomposition. Wallingford, UK, CAB International, 379–392.
Parton, W., Silver, W. L., Burke, I. C., Grassens, L. , Harmon, M. E., Currie, W. S., King, J. Y., Adair, E. C., Brandt, L. A., Hart, S. C. and Fasth B. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science, 315: 361–364.
Pastor, J. and Cohen, Y. 1997. Herbivores, the functional diversity of plant species, and the cycling of nutrients in ecosystems. Theoretical Population Biology, 51: 165–79.
Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A. and Ord, B. 2008. Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: independent of the presence of roots and mycorrhizal fungi. Soil Biology and Biochemistry, 40: 1103–1113.
Pérez-Suárez. M., Arredondo-Moreno. J. T. and Huber-Sannwald, E. 2012. Early stage of single and mixed leaf-litter decomposition in semiarid forest pine-oak: the role of rainfall and microsite. Biogeochemistry, 108: 245–258.
Pittermann, J. and Sage, R.F. 2000. Photosynthetic performance at low temperature of *Bouteloua gracilis Lag.,* a high-altitute C4 grass from the Rocky Mountains, USA. Plant, Cell and Environment, 23: 811–823.

Placella, S.A. and Firestone, M.K. 2013. Transcriptional response of nitrifying communities to wetting of dry soil. Applied and Environmental Microbiology, 79: 3294–3302.
Placella, S.A. and Firestone, M.K. 2013. Transcriptional response of nitrifying communities to wetting of dry soil. Applied and Environmental Microbiology, 79: 3294–3302.
Polley, H. W., Briske, D. D., Morgan, J. A., Wolter, K., Bailey, D. W. and Brown. J. R. 2013. Climate change and North American rangelands: evidence, trends, and implications. Rangeland Ecology and Management, 66: 493–511.
Porras-Alfaro, A., Herrera, J., Sinsabaugh, R. L., Odenbach, K. J., Lowrey, T. and Natvig, D. O. 2008. Novel root fungal consortium associated with a dominant desert grass. Applied and Environmental Microbiology, 74: 2805–2813.
Prescott, C. E., Zabek, L. M., Staley, C. L. and Kabzems, R. 2000b. Decomposition of broadleaf and meedle litter in forests of British Columbia: influence of litter type, forest type, and litter mixtures. Canadian Journal of Forest Research, 30: 1742–1750.
Prescott, C.E. 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry, 101: 133–149.
Quested, H. M., Press, M. C., Callaghan, T. V. and Cornelissen, J. H. C. 2002. The hemiparasitic angiosperm *Bartsia alpina* has the potential to accelerate decomposition in sub-arctic communities. Oecologia, 130: 88–95.
Richmond, P. A. 1991. Occurrence and functions of native cellulose. In: Haigler, C. H. and Weimer, P. J. (eds) Biosynthesis and biodegradation of cellulose. Marcel Dekker, New York, Pp. 5–23.
Rietl, A. J., Overlander, M. E., Nyman, A. J. and Jackson, C. R. 2016. Microbial community composition and extracellular enzyme activities associated with *Juncus roemerianus* and *Spartina alterniflora* vegetated sediments in Louisiana saltmarshes. Microbial Ecology, 71: 290–303.
Rinkes, Z. L., Weintraub, M. N., DeForest, J. L., Grandy, A. S. and Moorhead D. L. 2014. Interactions between leaf litter quality, particle size, and microbial community during the earliest stage of decay. Biogeochemistry, 117: 153–168.
Risser, P. G., Karr, J. R. and Forman. R. T. 1984. Landscape ecology-directions and approaches. Illinois Natural History Survey Special Publication No. 2, Champaign, IL.
Robinson, C.H. 2002. Controls on decomposition and soil nitrogen availability at high latitudes. Plant and Soil, 242: 65–81.
Rodrigo, A., Recous, S., Neel, C. and Mary, B. 1997. Modeling temperature and moisture effects on C-N transformations in soils: comparison of nine models. Ecological Modeling, 102: 325–339.
Romani, A.M., Vazques, E. and Butturini, A. 2006. Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: biogeochemical link across the stream-riparian interface. Microbial Ecology, 52: 501–512.
Rooney, D., Kennedy, N., Deering, L., Gleeson, D. and Clipson, N. 2006. Effect of sheep urine deposition on the bacterial community structure in an acidic upland grassland soil. Applied and Environmental Microbiology, 72: 7231–7237.
Ross, L. C., Woodin, S. J., Hester, A. J., Thompson, D. B. A. and Birks, H. J. B. 2012. Biotic homogenization of upland vegetation: patterns and drivers at multiple spatial scales over five decades. Journal of Vegetation Science, 23: 755–770.
Ruess, R. W. and McNaughton, S. J. 1987. Grazing and the dynamics of nutrient and energy regulated microbial processes in the Serengeti grasslands. Oikos, 49: 101–110.
Saiya-Cork K. R. Sinsabaugh R. L. and Zak D.R. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an acer saccharum forest soil. Soil

Biology and Biochemistry, 34: 1309–1315.
Sala, O. E., Biondini, M. E. and Lauenroth, W. K. 1988. Bias in estimates of primary production: An analytical solution. Ecological Modelling, 44: 43–55.
Salah, Y. M. S. and Scholes, M. C. 2010. Effect of temperature and litter quality on decomposition rate of *Pinus patula* needle litter. Procedia Environmental Sciences, 6: 180–193.
Salamanca, F., Kaneko, N. and Katagiri, S. 1998. Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. Ecological Engineering, 10: 53–73.
Salamanca, F., Kaneko, N. and Katagiri, S. 2003. Rainfall manipulation effects on litter decomposition and the microbial biomass of the forest floor. Applied Soil Ecology, 22: 271–281.
Salles, J. F. and Mallon, C. A. 2014. Invasive plant species set up their own niche. New Phytologist, 204: 435–437.
Sankaran, M. and Augustine, D.J. 2004. Large herbivores suppress decomposer abundance in a semiarid grazing ecosystem. Ecology, 85: 1052–1061.
Scherer-Lorenzen, M. 2008. Functional diversity affects decomposition processes in experimental grasslands. Functional Ecology, 22: 547–555.
Schimel, J. P. and Weintraub, M. N. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry, 35: 549–563.
Schlesinger, W. H. 1990. Evidence from carbon sequence studies for low carbon-storage potential of soils. Nature, 348: 232–234.
Schlesinger, W. H. and Raich, J. W. 1992. The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B: Chemical and Physical Meteorology, 44: 81–99.
Schlesinger, W. H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrell, W.M., Virginia, R.A. and Whitford, W.G. 1990. Biological feedbacks in global desertification. Science, 247: 1043–1048.
Schlesinger, W. H., Tartowski, S. L. and Schmidt, S. M. 2006. Nutrient cycling within an arid ecosystem. In: Havstad, K. M., Huenneke, L. F. and Schlesinger, W. H. (eds) Structure and function of a Chihuahuan desert ecosystem. The Jornada Basin Long-Term ecological research site, Oxford, USA, Pp. 133–149.
Schmidt, E. L. and Ruschmeyer, O. R. 1958. Cellulose decomposition in soil burial beds. I. Soil properties in relation to cellulose degradation. Applied Microbiology, 6: 108–114.
Schuman, G. E. and Reeder, J.D. 2002. Influence of livestock grazing on C sequestration in semi-arid mixed-grass and short-grass rangelands. Environmental Pollution, 116: 457–463.
Schuman, G. E., Ingram, L. J., Stahl, P. D. Derner, J.D. Vance, G.F. and Morgan, J.A. 2009. Influence of management on soil organic carbon dynamics in northern mixedgrass rangeland. Soil Carbon Sequestration and the Greenhouse Effect, Pp. 169–180.
Schuman, G. E., Janzen, H. H. and Herrick, J. E. 2002. Soil carbon dynamics and potential carbon sequestration by rangelands. Environmental Pollution, 116: 391–396.
Schuman, G. E., Reeder, J. D., Manley, J. T., Hart, R. H. and Manley, W. A. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed-grass rangeland. Ecological Applications, 9: 65–71.
Schütt, M., Borken, W, Spott, O., Stange, C. F. and Matzner, E. 2014. Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biology and Biochemistry, 69: 320–327.
Scurlock, J.M., Hall, D.O. 1998. The global carbon sink: A grasslands perspective. Global

Change Biology, 4: 229–233.
Seagle, S. W., and McNaughton. S. J. 1992. Spatial variation in forage nutrient concentrations and the distribution of Serengeti grazing ungulates. Landscape Ecology, 7: 229–241.
Semmartin, M., Aguitar, M. R., Distel, R., Moretto, A. S. and Ghersa, C. M. 2004. Litter quality and nutrient cycling affected by grazing-induced species replacements along a precipitation gradient. Oikos, 107: 148–160.
Shand, C. A., Williams, B. L., Smith, S. and Young, M. E. 2000. Temporal changes in C, P and N concentrations in soil solution following application of synthetic sheep urine to a soil under grass. Plant and Soil, 222: 1–13.
Shariff, A. R., Biondini, M. E. and Grygiel, C. E. 1994. Grazing intensity effects on litter decomposition and soil nitrogen mineralization. Journal of Range Manage, 47: 444–449.
Silver, W. L and Miya, R. K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia, 129: 407–419.
Sims, P. L. and Coupland R. T. 1979. Produces, Chapter 5 I:n Coupland, R. T. (ed.). Grassland ecosystems of the world: IBP 18. Cambridge University Press. New York, NY.
Singh, S. K. and Rai, J. P. 2004. Soil microbial population and enzyme activity related to grazing pressure in alpine meadows of Nanda Devi Biosphere Reserve. Journal of Environmental Biology. 25: 103–107.
Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry, 42: 391–404.
Sinsabaugh, R. L., Antibus, R. K. and Linkins, A. E. 1991. An enzymatic approach to the analysis of microbial activity during plant litter decomposition. Agriculture, Ecosystems and Environment, 34: 43–54.
Sinsabaugh, R. L., Carreiro, M. M. and Repert, D.A. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry, 60: 1–24.
Sinsabaugh, R. L., Hill, B. H. and Shah, J. J. F. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature. 462: 795–798.
Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C. L., Contosta, A. R., Cusack, D., Frey, S., Gallo, M. E., Gartner, T. B., Hobbie, S. E., Holland, K., Keeler, B. L., Powers, J. S., Stursova, M., Takacs-Vesbach, C., Waldrop, M., Wallenstein, M., Zak, D. R. and Zeglin, L. H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters, 11: 1252–1264.
Sinsabaugh, R.L., Antibus, R. K., Linkins, A. E., McClaugherty, C. A., Rayburn, L., Repert, D. and Weiland, T. 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology, 74: 1586–1593.
Sinsabaugh, R.S. 1994. Enzymic analysis of microbial pattern and process. Biology and Fertility of Soils, 17: 69–74.
Sinton, H. M. M. 1980. Effect of burning and mowing on *Festuca hallii* (Vasey) Piper, *Festuca scabrella* Torr. M. Sc. Thesis. University of Alberta. Edmonton.
Skujins, J. 1978. History of abiontic soil enzyme research. In: Soil Enzymes (ed. Burns, R.G.). Academic Press, New York. Pp. 1–50.
Smoliak, S., Dormaar, J.F. and Johnston, A. 1972. Long-term grazing effects on *Stipa-Bouteloua* prairie soils. Journal of Range Management, 25: 246–250.
Sørensen, L. H., Mikola, J., Kytöviita, M. and Olofsson, J. Trampling and spatial heterogeneity explain decomposer abundances in a sub-Arctic grassland subjected to simulated reindeer grazing. Ecosystems, 12: 830–842.
Spiers, G. A. and McGill, W. B. 1979. Effect of phosphorus addition and energy supply on

acid phosphatase production and activity in soils. Soil Biology and Biochemistry, 11: 3–8.
Steinweg, J. M., Dukes, J. S. and Wallenstein, M. D. 2012. Modeling the effects of temperature and moisture on soil enzyme activity: linking laboratory assays to continuous field data. Soil Biology and Biochemistry, 55: 85–92.
Steinweg, J. M., Dukes, J. S., Paul, E. A. and Wallenstein, M. D. 2013. Microbial responses to multi-factor climate change: effects on soil enzymes. Frontiers in Microbiology, 4: 146–157.
Sullivan, J. T. and Garber, R. J. 1941. The nitrogen content of *Poa pratensis*: its range and relation to flowering date. Journal of American Society of Agronomy, 33: 933–937.
Swift, M. J., Heal, O. W. and Anderson, J. M. 1979. Decomposition in terrestrial ecosystems. Balckwell Scientific Publications. London. UK.
Sylvia, D.M., P.G. Hartel, J. Furhmann and D. Zuberer. 2005. Principles and applications of soil microbiology. 2nd Ed. Prentice Hall Inc. Upper Saddle River. New Jersey.
Tabatabai, M. A. 1994. Soil enzymes. In: Weaver, R.W., Angle, S., Bottomley, P. (Eds.), Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America. Madison. pp. 775–833.
Tadano, T., Ozawa, K., Sakai, H., Osaki, M. and Matsui, H. 1993. Secretion of acid phosphatase by the roots of crop plants under phosphorusdeficient conditions and some properties of the enzyme secreted by lupin roots. Plant and Soil, 155: 95–98.
Talbot, J. M., Yelle, D. J., Nowick, J. and Treseder, K. K. 2012. Litter decay rates are determined by lignin chemistry. Biogeochemistry, 108: 279–295.
Tanentzap, A. J. and Coomes, D. A. 2011. Carbon storage in terrestrial ecosystems: Do browsing and grazing herbivores matter. Biological Reviews Cambridge Philosophical Society, 87: 72–94.
Tannas, S. C. 2011. Mechanisms regulating *Poa pratensis* L. and *Festuca campestris* Rybd. within the Foothills Fescue grasslands of southern Alberta. Ph. D. dissertation. University of Alberta.
Taylor, B.R., Parkinson, D. and Parsons, W. F. J. 1989. Nitrogen and lignin content as predictors of litter decay rates. A microcosm test. Ecology, 70: 97-104.
Theuerl, S. and Buscot, F. 2010. Laccases: Toward disentangling their diversity and functions in relation to soil organic matter cycling. Biology and Fertility of Soils, 46: 215–225.
Thornton P. K. 2010. Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society, 365: 2853–2867.
Tracy, B.F. and Frank, D. A. 1998. Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park. Oecologia, 114: 556–562.
Treseder, K. K. and Vitousek, P. M. 2001. Effects of soil nutrient availability on investment in Acquisition of N and P in Hawaiian rain forests. Ecology, 82: 946–954.
Trofymow, J.A., Moore, T.R., Titus, B., Prescott, C., Morrison, I., Siltanen, M., Smith, S., Fyles, J., Wein, R., Camiré, C., Duschene, L., Kozak, L., Kranabetter, M., and Visser, W. 2002. Rates of litter decomposition over 6 years in Canadian forests: influence of litter quality and climate. Canadian Journal of Forest Research, 32: 789–804.
Tyser, R.W. 1992. Vegetation associated with two alien plant species in a fescue grassland in Glacier National Park, Montana. Great Basin Naturalist, 52: 189–193.
van der Valk, A.G. 1986. The impact of litter and annual plants on recruitment from the seed bank of a lacustrine wetland. Aquatic Botany, 24: 13–26.
Vasquez de Aldana, B. R., Geerts, R. H. E. M. and Berendse F. 1996. Nitrogen losses fro perennial grass species. Oecologia, 106: 137–143.
Versaw, W. K. and Harrison, M. J. 2002. A chloroplast phosphate transporter, PHT2;1,

influences allocation of phosphate within the plant and phosphate-starvation responses. The Plant Cell, 141: 1751–1766.
Vitousek, P. M., and Howarth, R. W. 1991. Nitrogen limitation on land and in the sea: how it can occur. Biogeochemistry, 13: 87–115.
Vujnovic, K., Wein, R.W.and Dale, M.R.T. 2002. Predicting plant species diversity in response to disturbance magnitude in grassland remnants of central Alberta. Canadian Journal of Botany, 80: 504–511.
Wachendorf, C., Lampe, C., Taube, F. and Dittert, K. 2008. Nitrous oxide emissions and dynamics of soil nitrogen under N-15-labeled cow urine and dung patches on a sandy grassland soil. Journal of Plant Nutrition and Soil Science, 171: 171–180.
Waldrop, M. P., Balser, T. C. and Firestone, M. K. 2000. Linking microbial community composition to function in a tropical soil. Soil Biology and Biochemistry, 32: 1837–1846.
Wallenstein, M. and Hall, E. K. 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning Biogeochemistry, 109: 35–47.
Wallenstein, M. D., McMahon, S. K. and Schimel, J. P. 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology, 15: 1631–1639.
Wallenstein, M., Allison, S.D., Ernakovich, J., Steinweg, J.M. and Sinsabaugh, R. 2011. Controls on the temperature sensitivity of soil enzymes: a key driver of in situ enzyme activity rates. Soil Enzymology, 22: 245–258.
Wang, G., Post, W. M., Mayes, M. A., Frerichs, J. T. and Sindhu J. 2012. Parameter estimation for models of ligninolytic and cellulolytic enzyme kinetics. Soil Biology and Biochemistry, 48: 28–38.
Wang, X., VandenBygaart, A. J. and McConkey, B. C. 2014. Land management history of Canadian grasslands and the impact on soil carbon storage. Rangeland Ecology and Management, 67: 333–343.
Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setala, H, Van der Putten W. and Wall, D. H. 2004. Ecological linkage between aboveground and belowground biota. Science, 304: 1629–1633.
Wardle, D. A., Bonner, K. I. and Barker, G. M. 2002. Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Functional Ecology, 16: 585–595.
Wardle, D. A., Bonner, K. I. and Nicholson, K. S. 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos, 79: 247–258.
Wardle, D.A. 2002. Communities and Ecosystems: Linking the Aboveground and Belowground Components. Princeton University Press, Princeton, NJ.
Waring, B. 2013. Exploring relationships between enzyme activities and leaf litter decomposition in a wet tropical forest. Soil Biology and Biochemistry, 64: 89–95.
Wedin, D. A., Tieszen, L. L., Dewey, B. and Pastor, J. 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. Ecology, 76: 1383–1392.
White F. 1983. The Vegetation of Africa, a descriptive memoir to accompany the UNIESCO/AETF AT/UNSO Vegetation Map of Africa (3 Plates, Northwestern Africa, Northeastern Africa, and Southern Africa.,1:5,000,000). Paris: UNESCO.
White, A. R. 1982. Visualization of cellulases and cellulose degradation. In: Brown, R. M. (ed) Cellulose and other natural polymer systems: biogenesis, structure, and degradation. Plenum. New York. Pp. 489–509.
White, S. R., Tannas, S., Bao, T., Bennett, J. A., Bork, E. W. and Cahill, J. F. 2013. Using

structural equation modeling to test the passenger, driver and opportunist concepts in a *Poa pratensis* invasion. Oikos, 122: 377–384.
Willms, W. D., Adams, B. and McKenzie, R. 2011. Overview: anthropogenic changes of Canadian grasslands. In: Floate, K. D. Arthropods of Canadian grasslands. Volume 2: Inhabitants of a changing landscape. Lethbridge, Alberta, Canada: Biological Survey of Canada. Pp. 1–22.
Willms, W. D., Dormaar, J. F. and Adams, B.W. 1996. Seasonal changes of herbage biomass on the fescue prairie. Journal of Range Management, 49: 100–104.
Willms, W. D., J. F. Dormaar, and G. B. Schaalje. 1988. Stability of grazed patches on rough fescue grasslands. Journal of Range Management, 41: 503–508.
Willms, W. D., Smoliak, S. and Dormaar, J. F. 1985. Effect of stocking rate on a rough fescue grassland vegetation. Journal of Range Management, 38: 220–225.
Willms, W.D., Smoliak, S. and Bailey, A.W. 1986. Herbage production following litter removal on Alberta native grasslands. Journal of Range Management, 39: 536–539.
Willoughby, M. G. 1997. Rangeland reference areas: Castle River range condition and trend from 1953–1995. Alberta Environmental Protection, Land and Forest Service. Edmonton. AB. Pp. 22.
Woodwell, G. M. 1994. Biotic feedbacks in the global climate system. Oxford University Press, Oxford, UK.
Yates, C. J., Norton, D. A., and Hobbs, R. J. 2000. Grazing effects on plant cover, soil and microclimate in fragmented woodlands in south-western Australia: implications for restoration. Austral Ecology, 25: 36–47.
Yuste, J. C., Penuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., Pujol, M. and Sardans, J. 2010. Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Global Change Biology, 17:1475–1486.
Zhang, B., Liang, C., He, H. B. and Zhang, X. D. 2013. Variations in soil microbial communities and residues along an altitude gradient on the northern slope of Changbai Mountain, China. PLoS ONE. 8: e66184.
Zhang, D., Hui, D., Luo, Y. and Zhou, G. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology, 1: 85–93.

Appendices

Appendix A: Agroclimatic Conditions

Table A1. The modal soil type, dominant plant species, mean annual temperature (MAT; °C), mean annual precipitation (MAP; mm), mean elevation (m) and total area (km^2) of nine subregions across southern Alberta.

Subregion	Modal Soil	Dominant Species	MAT (°C)	MAP (mm)	Elevation (m)	Total Area (km^2)
Dry Mixedgrass	Brown Chernozems	*Bouteloua gracilis* *Hesperostipa comata*	4.2	333.3	800	46,937
Mesic Mixedgrass	Dark Brown Chernozems	*Hesperostipa comata* *Pascopyrum smithii* *Pascopyrum dasystachyum*	4.4	394.1	975	20,072
Foothills Fescue	Black Chernozems	*Danthonia parryi* *Poa pratensis* *Festuca campestris*	3.9	469.6	1100	13,623
Foothills Parkland	Black/Dark Gray Chernozems	*Festuca idahoensis* *Festuca campestris* *Hesperostipa comata*	3.0	517.0	1250	3,921
Central Parkland	Black Chernozems	*Festuca hallii* *Populus tremuloides* *Elaeagnus commutata* *Symphoricarpos occidentalis*	2.3	441.2	750	53,706
Northern Fescue	Dark Brown Chernozems	*Bouteloua gracilis* *Pascopyrum dasystachyum* *Koeleria macrantha*	2.7	384.6	800	14,933
Montane	Chernozems Luvisols Brunisols	*Pinus contorta* *Pseudotsuga menziesii*	2.3	588.6	1400	8,768
Subalpine	Brunisols Regosols	*Pinus engelmannii* *Abies lasiocarpa*	-0.1	755.5	2350	25,218
Alpine	Brunisols Regosols	*Phyllodoce empetriformis* *Salix reticulata spp.*	-2.4	989.4	2350	15,084

Appendix B: Natural Subregions of Alberta

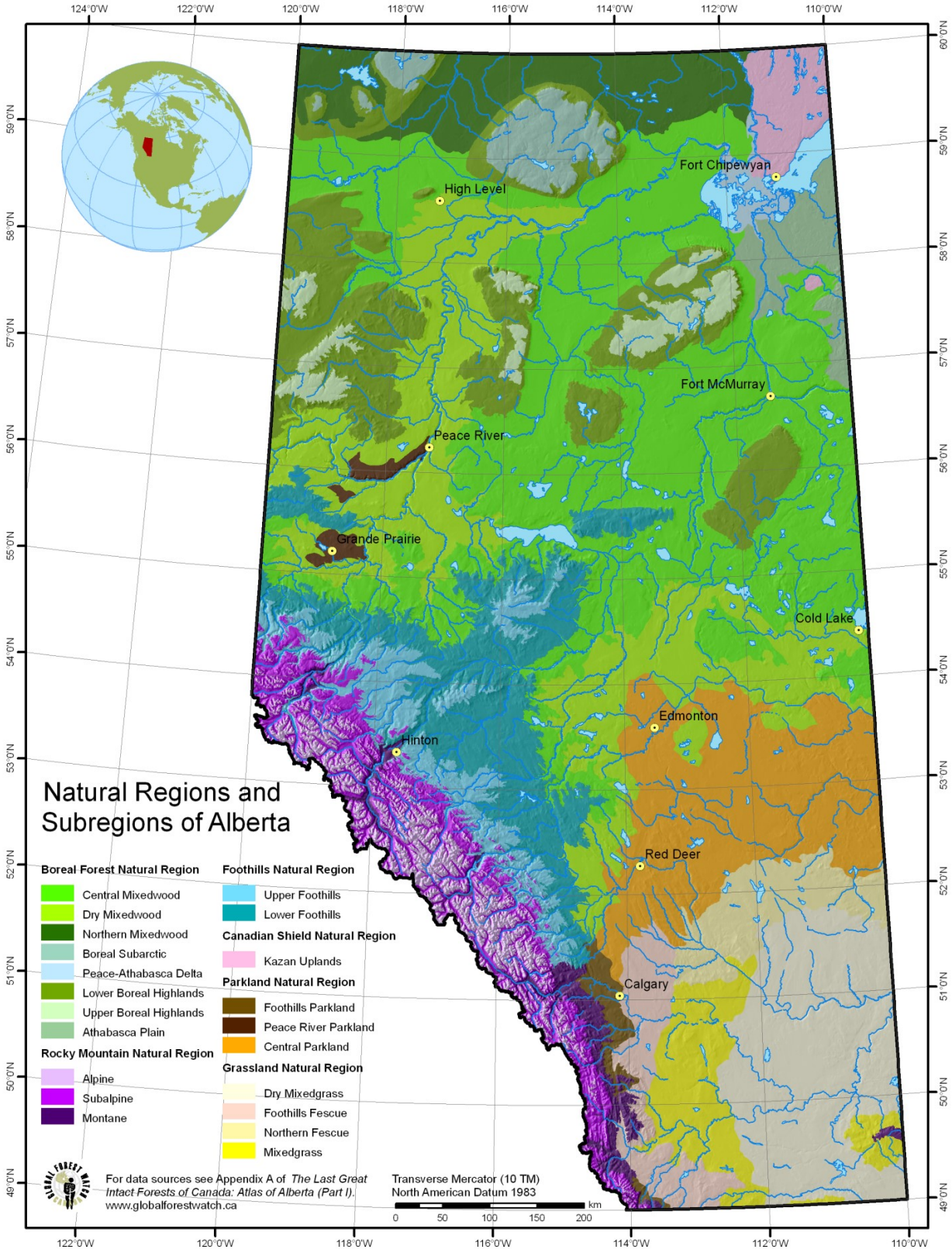

Figure B1. Map of Alberta natural subregions. Map derived from Alberta Biodiversity Monitoring Institute (ABMI).

Appendix C: Study Site Information

Table C1. The coordinates, mean temperature (°C), mean precipitation, soil organic matter (OM; %) and soil pH of each study site. Bruce: BR, Grizzly Bear Creek: GBC, Paradise Valley: PV, Kitscoty: KTS, Kinsella Ranch: KR, Antelope Creek 2: AC2, Antelope Creek 4: AC4, Rainy Hills: RH, Lomond: LO, Mattheis: MA, Castle: CA, Wards: WA, Jim Heath Creek: JHC, Stavely: ST, Waldron Ranch: WR. The mean temperature and mean precipitation were generated from ClimateAB v3.21.

Subregion	Site	Coordinates	Mean Temperature (°C)						Mean Precipitation (mm)						OM	pH
			Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sept-Oct	Nov-Dec	Jan-Feb	Mar-Apr	May-Jun	Jul-Aug	Sept-Oct	Nov-Dec		
Central Parkland	BR	53.25, -112.01	-11.0	-3.1	12.8	16.8	7.5	-1.8	15.8	22.6	57.1	48.3	18.2	19.2	5.05	6.05
	GBC	53.04, -110.17	-11.3	-4.3	13.2	16.4	8.7	-3.2	28.9	17.5	63.0	61.2	23.3	27.5	5.07	5.88
	PV	53.14, -110.69	-11.4	-4.2	13.1	16.3	8.8	-2.3	26.1	16.0	70.0	55.2	19.1	23.5	5.72	5.90
	KTS	53.30, -110.47	-11.1	-3.8	13.3	16.5	8.8	-13.3	25.5	15.5	66.5	55.5	18.5	24.0	4.89	7.40
	KR	53.01, -111.54	-11.5	-3.0	13.5	16.8	9.1	-11.6	23.0	18.0	73.5	57.9	19.2	23.3	5.20	6.06
Dry Mixedgrass Prairie	AC2	50.60, -112.16	-6.2	-0.3	13.2	18.8	10.2	-8.1	15.0	15.5	77.2	34.2	32.1	13.6	3.96	6.50
	AC4	50.58, -112.18	-6.2	-0.3	13.2	18.8	10.2	-8.1	15.0	15.5	77.2	34.2	32.1	13.6	5.98	6.08
	RH	50.48, -111.17	-7.3	-0.4	12.3	19.2	11.6	-8.3	16.2	16.2	72.2	35.3	28.3	12.9	5.31	6.81
	LO	50.51, -112.61	-5.2	1.3	12.9	19.1	12.1	-7.9	15.9	17.5	80.2	36.2	29.1	12.6	5.78	7.18
	MA	50.90, -111.90	-6.3	0.3	11.2	18.9	11.7	-7.8	16.0	18.5	73.2	31.2	34.2	14.1	5.92	6.64
Foothills Fescue	CA	49.42, -114.33	-4.1	-0.1	9.3	16.2	7.9	-4.3	35.1	69.2	106	31.2	57.2	70.1	8.98	6.03
	WA	49.80, -114.12	-4.3	-1.2	10.1	17.3	6.1	-5.0	15.1	40.2	102	35.2	47.2	34.1	8.14	6.60
	JHC	49.82, -113.99	-4.4	-1.0	11.3	16.2	8.3	-5.3	22.1	45.2	112	34.2	42.2	34.6	8.25	6.05
	ST	50.18, -113.90	-4.5	-0.8	8.9	15.7	8.8	-6.3	15.2	35.4	121	47.2	39.2	21.5	8.34	6.21
	WR	49.79, -113.76	-4.1	-0.4	11.2	16.5	8.9	-5.5	16.2	41.2	113	41.5	38.5	29.0	8.15	7.16

Appendix D: Litterbag Deployment and Retrieval Schedule

Table D1. Litterbag deployment plans and total litterbag numbers for each of three subregions. Bruce: BR, Grizzly Bear Creek: GBC, Paradise Valley: PV, Kitscoty: KTS, Kinsella Ranch: KR, Antelope Creek 2: AC2, Antelope Creek 4: AC4, Rainy Hills: RH, Lomond: LO, Mattheis: MA, Castle: CA, Wards: WA, Jim Heath Creek: JHC, Stavely: ST, Waldron Ranch: WR; *Pascopyrum smithii*: PS, *Bouteloua gracilis*: BG, *Festuca campetris*: FC, *Festuca hallii*: FH, *Koeleria macrantha*: KM, *Poa pratensis*: PP, *Hesperostipa comata*: HC, Cellulose: CE, Community mix: CM.

Subregion	Site	Grazing Treatment	Species									Replicates	Collection	Subtotal
			PS	BG	FC	FH	KM	PP	HC	CE	CM			
Central Parkland	BR	Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Non-grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
	GBC	Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Non-grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
	PV	Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Non-grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
	KTS	Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Non-grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
	KR	Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Non-grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
		Continuous Grazing	✓			✓	✓	✓	✓	✓	✓	2	7	98
Dry Mixedgrass Prairie	AC2	Grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Non-grazing	✓	✓			✓		✓	✓	✓	2	7	84
	AC4	Grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Non-grazing	✓	✓			✓		✓	✓	✓	2	7	84
	RH	Grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Non-grazing	✓	✓			✓		✓	✓	✓	2	7	84
	LO	Grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Non-grazing	✓	✓			✓		✓	✓	✓	2	7	84
	MA	Grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Non-grazing	✓	✓			✓		✓	✓	✓	2	7	84
		Continuous Grazing	✓	✓			✓		✓	✓	✓	2	7	84

Foothills Fescue	CA	Grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Non-grazing	✓	✓	✓	✓	✓	✓	2	7	84
	WA	Grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Non-grazing	✓	✓	✓	✓	✓	✓	2	7	84
	JHC	Grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Non-grazing	✓	✓	✓	✓	✓	✓	2	7	84
	ST	Grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Non-grazing	✓	✓	✓	✓	✓	✓	2	7	84
	WR	Grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Non-grazing	✓	✓	✓	✓	✓	✓	2	7	84
		Continuous Grazing	✓	✓	✓	✓	✓	✓	2	7	84
											Total: 2926

Appendix E: Litterbag Processing

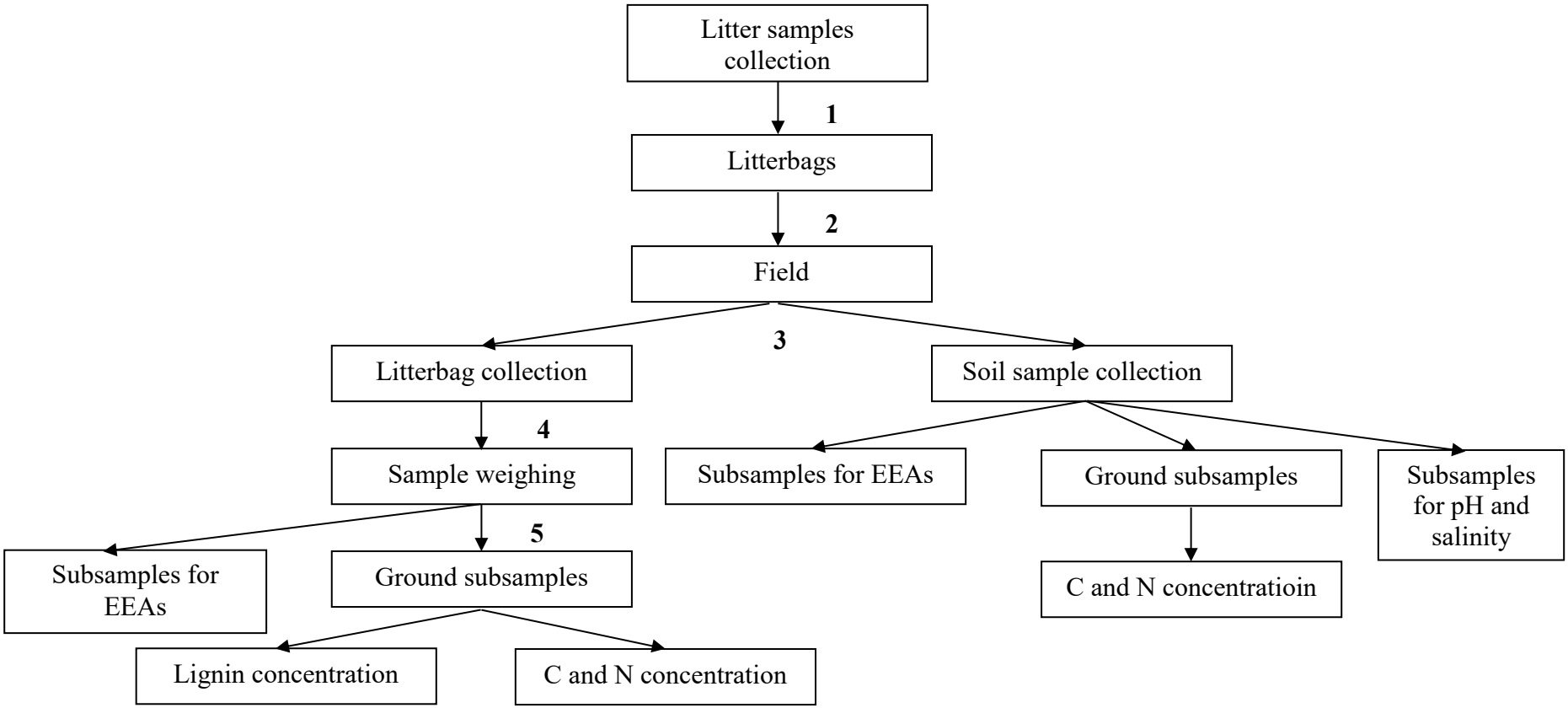

Figure E1. Flow chart depicting detailed litterbag processing and analytical procedures. Numbers indicate the specific processes described below.

1). Litter samples were harvested in the field and air-dried for 72 hours. 2.00 ± 0.02 g of sample was sealed into each bag.
2). Litterbags were transferred to the study sites.
3). Up to 2 litterbags were collected during each collection time (occasionally a bag was missing).
4). Litter samples were removed from each bag and weighed for mass loss.
5). Samples were divided to measure EEAs, and ground for measuring lignin, C and N concentration.

Appendix F: Litter Mass Summary Data

Table F1. Mean (±SE) litter mass remaining at each collection time and associated decay constant k values over the course of the decomposition study. Data were averaged across all sites and agroclimatic zones.

Litter source	Common Name	Ash-free mass remaining (%)						*k* value
		0 month	1 month	3 month	6 month	12 month	18 month	
P. smithii	Western wheatgrass	106.8±1.2	82.0±2.9	65.8±1.7	69.4±3.0	60.7±2.9	42.5±2.3	0.68±0.02
B. gracilis	Blue grama	98.9±2.8	79.2±1.0	57.5±3.0	62.0±2.2	53.4±2.8	25.1±3.0	0.99±0.06
F. campestris	Foothills fescue	101.4±2.5	78.9±2.2	61.0±1.3	61.0±2.9	58.1±2.8	35.1±1.4	0.81±0.03
F. hallii	Plains rough fescue	103.1±3.5	79.5±1.4	61.5±2.8	65.7±1.5	52.6±2.2	30.4±3.7	0.92±0.04
H. comata	Needle-and-thread	100.8±1.6	82.5±2.6	64.2±1.1	68.2±2.6	59.3±2.4	34.0±2.6	0.76±0.02
K. macrantha	June grass	102.4±2.9	80.4±2.1	66.2±2.7	62.3±1.1	54.7±1.4	32.1±2.7	0.85±0.02
P. pratensis	Kentucky bluegrass	105.6±2.5	73.2±3.0	55.1±1.1	58.0±2.5	47.6±2.8	29.3±2.1	1.09±0.05
Cellulose	NA	100.5±1.6	96.4±1.4	97.4±2.4	99.3±1.3	93.1±3.2	92.8±2.9	0.06±0.01
Community mix	NA	121.3±2.1	107.0±1.7	91.1±2.4	85.4±1.7	75.9±2.4	45.8±2.3	0.41±0.02

Appendix G: Mass Loss and *k* Value Summary

Table G1. Mean litter mass loss and *k* value (±SE) for each of cellulose, the community mix, *P. smithii* and *K. macrantha*, in response to grazing treatment and the three subregions.

Treatment Level		*k* value				Final mass remaining (%)			
		Cellulose	Community mix	*P. smithii*[2]	*K. macrantha*[3]	Cellulose	Community mix	*P. smithii*	*K. macrantha*
Grazing	Grazed	0.07±0.02a[1]	0.46±0.04a	0.69±0.02a	0.86±0.05a	90.4±3.6a	35.6±2.5a	37.5±1.9a	26.7±2.3a
	Non-grazed	0.06±0.02a	0.38±0.03a	0.66±0.03a	0.78±0.04a	92.3±1.6a	45.8±2.3b	40.1±2.3a	32.7±2.3b
Subregion	Foothills	0.12±0.03b	0.35±0.05a	0.68±0.05a	0.72±0.06a	82.2±4.8a	42.1±3.6a	39.8±3.4a	33.2±2.6a
	Parkland	0.03±0.01a	0.48±0.03a	0.67±0.03a	0.95±0.03a	95.8±1.5b	39.4±2.4a	35.3±1.8a	27.3±1.8a
	Mixedgrass	0.05±0.01ab	0.43±0.04a	0.66±0.03a	0.80±0.05a	95.1±1.6b	41.8±3.2a	41.8±2.6a	30.3±9.4a

[1] Within a column and treatment, means with different letters differ, $P < 0.05$.
[2, 3] The mean comparison of *P. smithii* and *K. macrantha* was derived from the three-way ANOVA (see Appendix F).

Appendix H: Summary ANOVA Results for the 3-Way Assessment of Litter Mass and *k* Values

Table H1. Three-way ANOVA results showing the effect of litter type (*P. smithii* and *K. macrantha*), grazing, subregion and their interations, on decay constant *k* values and final litter mass. Significant effects are in bold ($P < 0.05$).

Source	*k* value			Final mass remaining		
	F	df	*P*	F	df	*P*
Litter Type (LT)	21.06	1,58	**<0.001**	20.21	1,107	**<0.001**
Grazing (G)	2.78	1,58	0.10	3.03	1,107	0.08
Subregion (Sub)	2.43	2,57	0.13	0.82	2,106	0.465
LT*G	0.45	1,58	0.51	0.82	1,107	0.366
LT*Sub	3.42	2,58	0.06	0.46	2,106	0.63
G*Sub	0.44	2,58	0.65	0.80	2,106	0.45
LT*G*Sub	0.03	2,58	0.97	0.53	2,106	0.59

Appendix I: Summary of Litter Enzyme Activties among 18 Months

Table I1. The mean (±SE) activities of five hydrolytic and two oxidative enzymes of each litter type during 18 months. Phos: acid phosphase; Xylo: β-xylosidase; BG: β-glucosidase; NAG: N-Acetyl-glucosaminidase; Cell: β-Cellobiosidase; POX: Phenol oxidase and Pero: Peroxidase. Note: the unit of hydrolytic enzyme activities is μmol g^{-1} h^{-1}, and the unit of oxidative enzyme activities is nmol g^{-1} h^{-1}.

Species	Enzymes	Time					
		0 month	1 month	3 month	6 month	12 month	18 month
P. smithii	Phos	1.17(0.00)	2.53(0.34)	1.21(0.22)	1.32(0.23)	0.48(0.11)	0.95(0.24)
	Xylo	0.08(0.00)	1.56(0.22)	0.49(0.11)	0.68(0.15)	0.57(0.23)	0.88(0.21)
	BG	1.86(0.00)	13.50(1.81)	5.27(0.91)	6.04(0.81)	2.22(0.45)	6.31(1.05)
	NAG	0.44(0.00)	5.02(0.79)	2.68(0.56)	3.95(0.58)	1.40(0.23)	4.30(0.86)
	Cello	0.24(0.00)	5.41(0.62)	1.36(0.28)	2.56(0.41)	1.31(0.27)	2.45(0.49)
	POX	0(0.00)	0.04(0.02)	0.04(0.01)	0.08(0.03)	0.03(0.01)	0.07(0.02)
	Pero	0(0.00)	0.22(0.08)	0.02(0.01)	0.13(0.06)	0.05(0.02)	0.06(0.02)
B. gracilis	Phos	0.93(0.00)	3.66(0.73)	2.16(0.59)	3.00(0.87)	0.71(0.17)	2.98(0.66)
	Xylo	0.17(0.00)	3.33(0.73)	1.02(0.28)	2.58(0.69)	0.65(0.20)	3.10(0.58)
	BG	1.33(0.00)	18.13(2.75)	7.61(2.16)	14.93(4.46)	2.93(0.87)	18.35(2.65)
	NAG	0.67(0.00)	10.41(1.58)	3.15(0.58)	8.87(2.02)	2.72(0.91)	9.79(1.84)
	Cello	0.47(0.00)	6.21(1.41)	3.09(0.77)	6.86(1.82)	1.80(0.61)	5.78(1.33)
	POX	0.00(0.00)	0.04(0.02)	0.29(0.26)	0.16(0.06)	0.11(0.05)	0.17(0.06)
	Pero	0.00(0.00)	0.03(0.02)	0.03(0.01)	0.09(0.05)	0.16(0.08)	0.15(0.06)
Cellulose	Phos	0.00(0.00)	0.10(0.04)	0.11(0.03)	0.26(0.08)	0.08(0.02)	0.39(0.06)
	Xylo	0.00 (0.00)	0.04(0.02)	0.07(0.01)	0.11(0.03)	0.13(0.05)	0.38(0.09)
	BG	0.00(0.00)	0.31(0.04)	0.61(0.12)	1.30(0.19)	0.77(0.15)	0.26(0.29)
	NAG	0.00(0.00)	0.17(0.05)	0.13(0.03)	0.61(0.15)	0.31(0.093)	0.70(0.09)
	Cello	0.00(0.00)	0.13(0.05)	0.12(0.03)	0.46(0.09)	0.33(0.06)	1.13(0.13)
	POX	0.00(0.00)	0.00(0.00)	0.22(0.15)	0.01(0.01)	0.02(0.01)	0.04(0.01)
	Pero	0.00(0.00)	0.01(0.01)	0.01(0.00)	0.00(0.00)	0.01(0.01)	0.03(0.01)
F. campestris	Phos	0.08(0.00)	2.16(0.31)	1.65(0.55)	2.36(0.56)	0.73(0.22)	2.22(0.52)
	Xylo	0.43(0.00)	1.13(0.37)	0.84(0.17)	0.89(0.30)	1.03(0.64)	1.57(0.34)
	BG	0.02(0.00)	10.05(2.35)	8.83(2.17)	10.22(1.97)	4.05(1.02)	15.93(3.28)

	NAG	0.02(0.00)	6.10(1.61)	4.54(1.17)	7.22(1.93)	2.45(0.79)	6.87(1.83)
	Cello	0.00(0.00)	4.84(1.34)	2.65(0.54)	4.51(0.86)	2.34(0.83)	7.69(1.56)
	POX	0.08(0.00)	0.21(0.09)	0.15(0.05)	0.16(0.06)	0.08(0.04)	0.25(0.1)
	Pero	0.00(0.00)	0.19(0.07)	0.13(0.04)	0.17(0.05)	0.06(0.03)	0.27(0.1)
F. hallii	Phos	0.69(0.00)	1.94(0.32)	1.38(0.21)	4.71(1.08)	0.57(0.25)	3.32(0.84)
	Xylo	0.03(0.00)	1.51(0.47)	1.15(0.43)	1.38(0.43)	0.35(0.16)	3.39(0.62)
	BG	0.82(0.00)	9.00(2.99)	6.85(1.43)	10.15(2.80)	2.31(0.68)	19.14(3.20)
	NAG	0.51(0.00)	3.91(1.31)	4.30(1.01)	7.49(2.08)	1.38(0.63)	7.05(1.50)
	Cello	0.12(0.00)	4.50(1.38)	3.38(0.87)	6.61(1.81)	0.80(0.30)	6.98(1.50)
	POX	0.00(0.00)	0.09(0.03)	0.05(0.02)	0.17(0.05)	0.03(0.02)	0.21(0.11)
	Pero	0.03(0.00)	0.19(0.15)	0.01(0.01)	0.11(0.04)	0.03(0.02)	0.12(0.03)
H. comata	Phos	0.65(0.00)	3.01(0.48)	1.66(0.38)	2.79(0.38)	0.96(0.32)	1.34(0.22)
	Xylo	0.23(0.00)	3.78(0.57)	0.95(0.19)	1.87(0.35)	0.61(0.12)	1.65(0.33)
	BG	2.36(0.00)	16.34(2.33)	7.75(1.79)	9.56(1.48)	3.54(0.61)	11.45(1.72)
	NAG	0.53(0.00)	4.86(0.76)	3.70(0.82)	7.41(1.05)	3.02(0.84)	6.81(1.20)
	Cello	0.46(0.00)	5.80(0.82)	3.46(0.77)	5.19(0.71)	2.27(0.56)	4.78(1.00)
	POX	0.00(0.00)	0.11(0.03)	0.05(0.02)	0.10(0.03)	0.11(0.04)	0.07(0.02)
	Pero	0.42(0.00)	0.11(0.04)	0.05(0.02)	0.11(0.03)	0.12(0.05)	0.06(0.02)
K. macrantha	Phos	0.21(0.00)	2.49(0.21)	1.48(0.19)	2.47(0.41)	0.62(0.10)	2.62(0.31)
	Xylo	0.04(0.00)	2.09(0.33)	1.59(0.30)	1.75(0.31)	0.36(0.07)	2.14(0.33)
	BG	0.76(0.00)	10.42(1.08)	5.56(0.98)	9.29(1.21)	2.63(0.43)	9.04(1.24)
	NAG	0.19(0.00)	4.68(0.59)	3.27(0.48)	6.38(0.81)	1.97(0.46)	5.22(0.82)
	Cello	0.10(0.00)	5.05(0.70)	1.44(0.23)	3.00(0.45)	1.37(0.28)	5.27(0.78)
	POX	0.00(0.00)	0.07(0.02)	0.08(0.02)	0.11(0.03)	0.08(0.02)	0.17(0.03)
	Pero	0.00(0.00)	0.10(0.03)	0.05(0.01)	0.14(0.04)	0.06(0.02)	0.11(0.03)
P. pratensis	Phos	0.93(0.00)	3.32(0.36)	2.83(0.41)	3.28(0.55)	1.01(0.21)	2.76(0.43)
	Xylo	0.01(0.00)	2.17(0.38)	1.74(0.34)	1.30(0.27)	0.71(0.19)	3.01(0.46)
	BG	0.77(0.00)	15.07(2.06)	14.05(2.32)	15.13(2.24)	5.14(0.81)	15.48(1.76)
	NAG	0.31(0.00)	7.67(1.16)	7.04(1.30)	6.01(1.02)	3.52(0.65)	8.67(1.13)
	Cello	0.08(0.00)	7.14(1.02)	3.42(0.52)	6.97(1.29)	2.25(0.55)	6.97(0.87)
	POX	0.00(0.00)	0.04(0.02)	0.06(0.02)	0.04(0.02)	0.09(0.04)	0.29(0.06)

	Pero	0.00(0.00)	0.11(0.06)	0.04(0.01)	0.11(0.03)	0.10(0.03)	0.14(0.04)
Community mix	Phos	1.55(0.18)	3.07(0.35)	1.38(0.20)	2.86(0.36)	1.17(0.32)	2.99(0.45)
	Xylo	0.43(0.05)	1.30(0.23)	1.23(0.27)	1.70(0.27)	0.73(0.17)	2.25(0.37)
	BG	2.89(0.32)	8.87(1.26)	6.43(0.99)	9.78(1.25)	4.83(0.73)	10.11(1.15)
	NAG	1.07(0.13)	4.21(0.72)	3.52(0.78)	4.87(0.73)	2.32(0.46)	6.13(0.95)
	Cello	0.96(0.17)	4.76(0.79)	2.29(0.30)	4.92(0.60)	2.05(0.46)	5.14(0.78)
	POX	0.02(0.01)	0.06(0.02)	0.05(0.02)	0.05(0.02)	0.04(0.01)	0.21(0.04)
	Pero	0.02(0.02)	0.08(0.02)	0.04(0.02)	0.06(0.02)	0.02(0.01)	0.08(0.02)

Appendix J: Supplementary Information for Chaters 2 and 3.

In order to examine the effects of three different grazing systems (i.e., rotational grazing vs. continuous grazing vs. non-grazing) on EEAs and decomposition over a period of 18 months, we also conducted a two-way ANOVA using grazing treatment and subregions as fixed effects. For this analysis, each subregion served as a random factor (i.e. blocks).

In general, our results suggested that no differences were detected among grazing systems on observed litter decay k ($F_{2, 67} = 6.34$, $P = 0.53$; non-grazed = 0.63 ± 0.08; continuous grazed = 0.77 ± 0.11; rotational grazed = 0.71 ± 0.10), nor on the final litter mass remaining ($F_{2, 89} = 4.22$, $P = 0.29$) across subregions.

According to results from ANOVA, the grazing system affected three litter cEEAs, BG, NAG and Cello (Table I1). We found higher higher NAG and Cello activities in non-grazed areas (Table I2). Results also indicated that the activity of BG was followed in such order: $BG_{\text{continuous grazing}}$ (77.0±9.3 μmol g^{-1}) < $BG_{\text{rotational grazing}}$ (105.5±13.5 μmol g^{-1}) < $BG_{\text{non-grazing}}$ (124.7±16.8 μmol g^{-1}).

Table J1. ANOVA results of the effect of grazing system, subregion, sampling time, and their interactions, on five extracellular enzyme activities. Significant effects are bolded ($P < 0.05$).

Source	Phos			Xylo			BG			NAG			Cello		
	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*
							Litter								
Grazing	0.88	2, 67	0.42	1.43	2, 67	0.25	4.20	2, 67	**0.02**	4.31	2, 67	**0.02**	4.89	2, 67	**0.01**
							Soil								
Grazing	1.03	2, 15	0.38	0.07	2, 15	0.93	0.24	2, 15	0.79	0.01	2, 15	0.99	0.07	2, 15	0.93

Table J2. Mean (±SE) cumulative activities of each of five enzymes (μmol g^{-1}) among three contrasting grazing systems.

Source	Grazing system	Phos	Xylo	BG	NAG	Cello
Litter	Continous Grazing	26.9±4.7	16.7±3.5	77.0±9.3a	53.6±8.8a	38.1±6.0a
	Rotational Grazing	24.6±3.5	16.4±2.7	105.5±13.5ab	52.8±7.9a	39.5±5.6a
	Non-grazing	34.2±6.3	24.2±3.5	124.7±16.8b	92.1±14.4b	65.7±8.4b

Appendix K: Summary ANOVA Results for the 3-Way Assessment of Enzyme Activties on Four Common Litter Types

Appendix K1. Results of ANOVAs testing the effect of four common litter types (*K. macrantha*, *P. smithii*, cellulose and community mix), grazing treatments, subregion and their interactions, on the activities of each of five extracellular enzyme. Significant results are in bold ($P < 0.10$).

Source	Phos			Xylo			BG			NAG			Cello		
	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*	F	Df	*P*
Litter type (LT)	6.32	3,238	**<0.001**	4.31	3,238	**0.001**	13.42	3,238	**<0.001**	8.61	3,238	**<0.001**	9.22	3,238	**<0.001**
Grazing (G)	4.18	1,238	**0.04**	6.36	1,238	**0.01**	9.22	1,238	0.69	4.31	1,238	**0.01**	6.42	1,238	**0.071**
Subregion (Sub)	1.33	2,238	0.15	3.51	2,238	**0.046**	5.66	2,238	**0.009**	3.33	2,238	**0.021**	3.83	2,238	**0.031**
LT*G	0.88	3,238	0.44	1.44	3,238	0.28	1.48	3,238	0.15	1.68	3,238	0.29	1.48	3,238	0.17
LT*Sub	0.63	6,238	0.14	0.82	6,238	0.64	1.93	6,238	0.32	1.24	6,238	0.86	0.95	6,238	0.68
G*Sub	1.72	2,238	0.29	2.48	2,238	0.32	3.57	2,238	**0.073**	1.58	2,238	0.42	1.73	2,238	**0.06**
LT*G*Sub	0.78	6,238	0.65	0.97	6,238	0.58	1.60	6,238	0.22	1.43	6,238	0.26	0.58	6,238	0.61