> *"Right here in America is one of the world's most threatened natural systems. The Northern Great Plains is as important as the Amazon or Arctic, and deserves our attention."*
>
> – Martha Kauffman , Managing Director, Northern Great Plains program, WWF

University of Alberta

Plant litter: direct and indirect effects of simulated climate change and clipping on its decomposition, and its effects on plant-plant interactions

by

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To,

My mother, Rebecca, sisters, Mariah and Monicah, and brothers, James and Joshua:

for sticking with me through thick and thin despite being miles away.

Abstract

Terrestrial ecosystems are characterized by an intricate relationship between plants and soils that influence ecosystem and community level processes and properties. At the ecosystem level, plants (producers) provide organic carbon to the decomposer subsystem and obligate root-associated organisms once they die. In turn, the decomposer subsystem breaks down dead plant material and ultimately returns this carbon to the atmosphere. At a lower level of ecological organization, litter can have profound impacts on plant-plant interactions through physical and/or chemical effects. I investigated the role that litter plays at these two levels of ecological interaction.

First, I conducted a short-term factorial decomposition study testing the interactive effects of warming, drought, and land use practice (simulated grazing) on root and shoot litter decomposition. The study was carried out over a 2-year period at three sites across the Canadian Prairie Provinces. I manipulated temperature using open-top chambers (OTCs), reduced precipitation using rain-out shelters, and manually clipped vegetation at varied levels consistent with grazing practices in the region. Additionally, I studied the effect of litter quality on decomposition, whereby litter material pre-exposed to climate manipulations were placed in plots of origin (*in situ*) or in untreated standard plots.

Decomposition varied as function of site and was higher for root than shoot litter. Clipping intensity had no effect on decomposition across all sites. In contrast, drought significantly hampered decomposition whereas warming significantly decreased shoot decomposition but increased root decomposition, although insignificant. Drought generally reduced litter quality consequently retarding decomposition and this effect was further enhanced under drought condition. These findings suggest that in the short-term temperature and precipitation may have direct consequences on carbon storage in these systems.

Second, I studied the interactive effects between litter, root competition and belowground chemical interaction, as well as the effect of root exudates on plant growth, competition and evenness. The nature of belowground interaction switched from negative when aboveground litter was left intact to positive when removed. Root exudates enhanced plant growth, modified competition and enhanced evenness. These results show the existence of other potential non-resource mechanisms that may play a role in the organization of natural plant communities.

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Chapter 1 Introduction

1.1 Litter effects on ecosystem function

Litter decomposition is closely tied to nutrient cycling, accounts for transformation of nearly as much carbon as does photosynthesis, and is therefore a fundamental process of in the functioning of terrestrial ecosystems. Plants influence the functioning of the soil subsystem by providing carbon (C) and nutrients through decomposition. In turn, soil microorganisms that breakdown dead plant materials during this process determine soil fertility and productivity indirectly regulating plant growth and community composition (van der Putten *et al.* 2009). These interactions create a feedback loop between plants and soils (Ehrenfeld, Ravit & Elgersma 2005), implying that litter decomposition is critical in understanding aboveground-belowground linkages (van der Putten *et al.* 2009).

1.1.1 Plant litter decomposition

Decomposition and photosynthesis are two processes that account for a vast majority of biological C processing on earth (McClaugherty & Berg 2008). Photosynthesis and litter decomposition have both been studied extensively. However, decomposition is relatively less understood and more complex compared to photosynthesis as it largely occurs “out of sight.” In spite of this complexity, decomposition is understood to involve physical, biological and chemical transformation of organic matter into its elemental constituents. In broad terms, decomposition is considered a two-phase process, whereby litter is broken down into small pieces by detritivores in the first phase, and chemically transformed into stable forms by bacteria and fungi in the second phase (see

Swift, Heal & Anderson 1979; Cadisch & Giller 1997; McClaugherty & Berg 2008 for details on decomposition). Since both phases operate in concert, decomposition in natural settings can only be ascribed in general terms although consistent patterns can be outlined.

The importance of litter decomposition is reflected in the plethora of studies conducted in different ecosystems since the advent of the litterbag technique by Bocock & Gilbert (1957). Decomposition is closely tied to nutrient cycling, and is essential for regenerating organically bound nutrients important for plant growth (McClaugherty & Berg 2008), thereby maintaining site fertility and productivity (Prescott 2005). Litter decomposition is also an important component of the global C budget (Schimel 1995; Aerts 1997), and is a process by which C fixed during photosynthesis is returned to the atmosphere (Couteaux, Bottner & Berg 1995). Litter and soil organic matter (SOM) decomposition represents one of the major C fluxes from terrestrial ecosystems, estimated at ~ 60 Pg C year $^{-1}$ (Pg= 10^{15} g) (Houghton 2007), which is an order of magnitude greater than fossil fuel C emission (~7 Pg C year^{-1}) (Denman *et al.* 2007). Therefore, understanding litter decomposition processes and factors controlling litter decomposition is important for studying C and nutrient cycling, developing C budgets, and assessing the consequences of global climate change on decomposition.

1.1.2 Factors controlling litter decomposition

Climate, litter quality, and soil organisms are thought to be the main factors that regulate litter decomposition (Swift, Heal & Anderson 1979; Lavelle *et al.* 1993; Cadisch & Giller 1997). It is thought that these controlling factors operate in a hierarchical fashion in the following order: climate > litter quality > soil organisms (Meentemeyer 1984; Lavelle *et al.* 1993; Aerts 2006). Although synergistic effects among these factors may exist, temperature and moisture have predominant and direct effects on decomposition (Lavelle *et al.* 1993; Aerts 2006). In addition, these determinants of litter decomposition operate at different spatial and temporal scales; therefore, their importance in controlling decomposition is contingent upon the biome or environment under consideration (Lavelle *et al.* 1993). For instance, within a climatic region, litter quality, the chemical composition of plant litter, and edaphic properties are believed to be the most important factors regulating decomposition (Heneghan *et al.* 1998; McClaugherty & Berg 1987; Silver & Miya 2001), whereas at larger spatial scales, climate plays a more prominent role (Meentemeyer 1984). Despite this general understanding, litter decomposition results from terrestrial ecosystems including grasslands have been contradictory (e.g. Murphy, Klopatek & Klopatek 1998; Epstein, Burke & Lauenroth 2002). This implies that research on decomposition should continue, taking into consideration the consequences of changes in factors controlling decomposition (e.g. climate change).

1.1.3 Climate change predictions and litter decomposition

The direct linkage between climate and litter decomposition has led to the postulation that climatic change such as warming could accelerate global decomposition rates causing positive feedback of greenhouse gases (GHGs) production to the atmosphere (Kirschbaum 1995; Trumbore, Chadwick & Amundson 1996; Cox *et al.* 2000). There are various drivers of global climate change but warming attracts the most public attention (Woodwell & McKenzie 1995). Warming happens to be the factor that has the potential to greatly affect terrestrial ecosystems because temperature directly affects all ecosystem processes.

Predictions about future climate scenarios are made possible through coupled C cycle-climate simulations that govern our understanding of C cycle feedbacks. Although simulation models differ in their response, they provide valuable information about past, present and future climate scenarios, as well as links to important ecosystem processes (e.g. litter decomposition) (Bonan *et al.* 2013). Global warming resulting from GHGs emissions is expected to increase the mean global temperature by 2-7 °C by the end of 21st century (Allison *et al.* 2009). Precipitation predictions on the other hand are variable and is projected to increase in higher latitudes and decrease in most subtropical regions (IPCC 2007). Therefore, changes in these factors may alter fluxes of C from soil to the atmosphere. Ecologists from around the world have conducted, and are still conducting, climate change experiments in various ecosystems, but general responses of terrestrial ecosystems to factors such as temperature and precipitation changes especially their interactions remain unclear (Wu *et al.* 2011).

1.1.4 Climate change and history of disturbance in the Great Plains

Moisture and temperature strongly influence the structure and function of the Great Plains grasslands, one of the largest prairies in the world. In the Northern and Central Great Plains, temperatures have risen by about 1 °C in the

20th century (Karl *et al*. 1999), and will likely continue to increase, with the largest increases expected in the Western and Northern parts of the Great Plains (Joyce *et al.* 2001). Although precipitation increases are predicted for some parts of the Great Plains, increased evapotranspiration due to a rise in temperatures is projected to lead to soil moisture deficits (Joyce *et al.* 2001).

Grasslands, including the Great Plains, have the potential to impact, and be impacted by global climatic change with implications for both local and global C budgets. Grasslands comprise up to 40% of terrestrial land cover globally (White, Murray & Rohweder 2000), store most of its C belowground, at a much reduced risk for release by fire, and thus are likely to store more soil C than forest ecosystems. However, land use practices (e.g. grazing) have globally degraded rangelands, including grasslands (Lund 2007), thereby accelerating net C losses.

In Canada, grasslands are primarily located in western Canada within the Prairie Provinces, but also some parts of the interior British Columbia. Before the influx of European settlers, the Canadian prairie supported vast herds of grazing animals. The character and distribution of grasslands was influenced by disturbances from fire and grazing by large herbivores, which suppressed woody plants. These natural disturbances historically affected the physical environment and maintained biodiversity on the Great Plains of North America. The disturbances initiated and altered succession in communities by changing composition, structure, and functioning at many ecological scales. Interactions among fire, grazing, and environmental conditions, and their effects on prairies, were historically dynamic throughout the landscape and time. Grazing is a key ecological process in maintaining native prairie health and is equally important as the diversity of living organisms in native prairie (Romo 2007). The diversity of herbivores, including bison, pronghorn, elk, deer, small mammals, and insects, each with different grazing behaviours and impacts were unevenly distributed throughout the landscape and time. This created an ever-changing and variable mosaic of grazed patches throughout the landscape. Grazing by large herbivores may cause changes at various scales ranging from ecosystems to individuals including the physical environment. For instance, herbivores can influence nutrient cycling by consuming plants and depositing the digested plant material in new locations creating heterogeneous environment in the process, reduce plant litter, as well as affect species composition.

Similarly, fire is a natural process, which creates and maintains heterogeneity and biodiversity on the prairie landscapes (Romo 2007). Historic abundance of fire varied among seasons and locations but as a whole the prairies were constantly on fire over a span of several years (Fidler 1991). There we different sources of fire ignition on the prairies; lightning and spontaneous combustion, burning coal beds, intentional and accidental ignitions by Aboriginals (Lewis 1982), and spread from other places. The unevenness in time, frequency, intensity, magnitude and shape of burns created a mosaic that constantly changed through time and throughout the landscape. The effects of burning were further modified by grazing, growing conditions of plants before and after burning, environmental conditions and landscape characteristics and interactions between these elements. However, since the 1900's, the modern society has significantly suppressed fire both in size and frequency with possible negative consequences on vegetation composition, insect populations and soil properties.

Grazing and burning together created and rearranged a mosaic of flora and fauna communities maintaining biodiversity throughout the prairies at different spatial and temporal scales.

1.1.5 Landscape transformation and agriculture after settlement by Europeans in the Canadian prairies

Anthropogenic impacts before European contact existed and were imposed by Aboriginal people; however, the introduction of European farming practices in the early 19th century and natural resource extraction in recent years were and have been ecologically catastrophic (Willms, Adams & McKenzie 2011). Following European contact, livestock and farming were introduced, and grazing by livestock became the primary disturbance factor on the grasslands following fire suppression and bison extirpation. However, in a very short time farming became the predominant primary land-use activity on the prairies (Willms, Adams & McKenzie 2011). As a consequence, most of the grasslands have been fragmented and transformed through cultivation, and those that remain have been altered by grazing, establishment of infrastructure including roads, urbanization, and conduits for natural resource extraction. Land transformation still continues to this day and has led to reduction in size of the once large and intact landscape that was rich in biodiversity.

In western Canada today, the remaining native grassland area is small as annual crops have replaced native grass in all but dry or hilly areas (Bailey, McCartney & Schellenberg 2010). Only about 20% of the fescue prairie and 33% of the mixed prairie grassland remains intact (Federal, Provincial and Territorial Governments of Canada 2010). The grassland biome in Canada covers about 5% (45 million ha) of the total land base, and is where most of the cattle grazing takes place (Horton 1994). Forage resources, the foundation for livestock industry in Canada, is supported by both native rangelands and cultivated crops that take up 36 million ha (36%) of Canada's land base in comparison to 25 million ha for grain and oilseed crops (Horton 1994). This is divided into 72% native range (26 million ha), 11% cultivated pastures (4 million ha) and 17% forage crops (6 million ha) (Horton 1994). The majority of the forage-based livestock industry is situated in western Canada. Of the 26 million ha of Canadian rangeland used for livestock production, 96% is within the four western provinces with 36% in British Columbia, 29% in Alberta, 24% in Saskatchewan and 8% in Manitoba (McCartney & Horton 1997; McCartney 2011). These provinces also have 82% of the nation's cultivated pasture, 64% of the nation's crop area, and 84% of the nation's beef cow herd (McCartney & Horton 1997; McCartney 2011). Agriculture is one of Canada's primary industries and third highest contributor to the gross domestic product after mining and oil (Statistics Canada, 2010).

The forage-based livestock industry makes a significant contribution to the national economy. Canada's beef industry has 4.3 million beef cows and the beef industry accounts for close to 25% of total farm receipts (Statistics Canada, 2010).

The prairie provinces of Alberta, Saskatchewan and Manitoba have 82% of the national beef cow herd, Ontario and Quebec 12%, British Columbia 5% and Atlantic Canada 1% (Statistics Canada 2010; McCartney 2011). Alberta, with its vast rangelands and feed supplies, dominates Canada's beef production (McCartney 2011).The shift to agriculture and other threats such as habitat fragmentation, energy development and climate change have made the northern Great Plains to become one of the most threatened natural systems in the world (WWF 2013).

1.2 Litter effects and plant-plant interactions

At a lower level of ecological organization, litter can have profound impacts on plant species interactions. It is well established that plant litter affects surrounding living organisms through direct and indirect effects on the abiotic environment, both chemically (through mineral nutrient and phytotoxin composition), and physically (by altering micro-environmental conditions) (Facelli & Pickett 1991). The decay of above- and/or belowground plant parts is one of the avenues through which allelochemicals are released into the soil (Patrick & Koch 1958). However, in the field, inhibitory effects of decomposing plant residues may be buffered by soil (Inderjit & Weiner 2001). The accumulation and decomposition litter through various complex mechanisms has long been considered an important factor in structuring plant communities (Grime 1979; Facelli & Pickett 1991; Xiong & Nilsson 1999).

In addition to direct effects, litter can interact with chemicals that living plants release into the environment. Plants release chemicals from roots that make nutrients available (see Metlen, Aschehoug & Callaway 2009 for a review) or in some cases inhibit surrounding neighbours (Lambers, Chapin & Pons 1998), and litter by itself is an important driver of plant growth in grasslands (e.g. Willms, Smoliak & Bailey 1986). Therefore, interactions between litter effects and such resource and non-resource interactions are likely. A central theme in ecology is how plant populations are organized into higher units (Lortie *et al.* 2004), and it has been hypothesized that interactions mediated through chemical compounds are important for this organization (Rabotnov 1982). However, the potential interaction between litter and chemicals released by plants has seldom been included in many discussions about the structure of plant communities.

1.3 Research approach and rationale

I conducted two broad sets of experiments to evaluate the effects of litter on two different levels of ecological processes and function. First, I was interested in litter decomposition and C storage as influenced by simulated land use practice (grazing) and climate change. I used experimental manipulations to test the effects of temperature, precipitation, and clipping on litter mass loss, and C and N dynamics. I conducted this research at three sites in the three prairie provinces of Canada: Alberta, Saskatchewan and Manitoba. Additionally, I conducted a study to test the effect of litter quality on decomposition, whereby I used plant biomass pre-exposed to climatic manipulations as the source of litter material. The aim of this study was to distinguish between litter quality effects and interaction between litter quality and local conditions effects on decomposition.

Besides conservation and biodiversity values, the Canadian prairies are important regions that support beef industry, and thus have direct economic benefits since they are dedicated to native range, cultivated pastures and forage crop production. The study sites were located at the northern edge of the Great Plains, a zone that includes areas with strong changes in climate and associated vegetation (e.g. Vance, Emerson & Habgood 1983). Though there are numerous studies that investigate climate-mediated effects of litter decomposition, there is uncertainty regarding the effects of climate change on soil C pools. In addition, fewer studies include *in situ* root samples (Silver & Miya 2001), and little is known about climate-mediated decomposition at the Northern edge of the Great Plains. My research studies were part of an interdisciplinary design aimed at linking relationships between three main components: plant biomass production, microbial function and process, and soil invertebrate community.

Second, I was interested in the role that aboveground litter plays in regulating belowground species-level interactions. In this regard, I conducted a field experiment at the University of Alberta Research Ranch Station, near Kinsella, where I manipulated litter, competition and belowground organic compounds using activated charcoal. In a greenhouse study, I further evaluated the effects of root leachates on plant growth, competition and evenness. A summary of my research questions is provided below.

1.4 Specific research questions and objectives

Chapter 2

1. Do litter decomposition patterns vary among sites, and between shoot and root samples?

2. How are litter decomposition, and accompanying carbon (C) and nitrogen (N) dynamics influenced by temperature and precipitation (environment effect) at three sites in Canadian Prairie Provinces?

3. Does land use practice (i.e. simulated grazing through clipping) influence litter decomposition, and related C and N dynamics through changes in soil microclimatic conditions?

Chapter 3

1. Does litter quality regulate litter decomposition? *Common site* decomposition – shoot and root litter of different origins i.e. different precipitation and temperature combinations were placed in the same site conditions (in untreated control plots).

2. Do litter quality and local conditions interact to regulate litter decomposition? *In situ* decomposition – shoot and root litter pre-exposed to temperature and precipitation manipulations were placed in the plots of their origin.

Chapter 4

1. Does the presence or absence of shoot litter alter the outcome of root competition?

2. Does the presence or absence of shoot litter alter the outcome of belowground chemical interactions?

Chapter 5

1. Does leachate type alter plant growth, competition, and evenness?

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Chapter 2 Effects of temperature, precipitation regime and clipping on root and shoot decomposition at three sites in the Canadian Prairie Provinces

2.1 Introduction

Litter decomposition is an important process that links above and belowground ecological processes. Carbon (C) fixation through photosynthesis is mainly returned to the atmosphere through litter decomposition (Couteaux, Bottner & Berg 1995), and therefore decomposition is a key component of the global carbon budget (Shaver *et al.* 1992; Couteaux, Bottner & Berg 1995; Aerts 1997; Robinson 2002). Litter decomposition is influenced by many biotic and abiotic factors broadly classified as environmental conditions, chemical and physical composition of litter ('litter quality'), and soil organisms (Tenney & Waksman 1929; Swift, Heal & Anderson 1979; Seastedt 1984; Anderson & Flanagan 1989; Cadish & Giller 1997).These factors act at different temporal and spatial scales, and hence operate in a hierarchical fashion (Lavelle *et al.* 1993; Aerts 2006). Three main levels of litter decomposition control operate in the following order: climate, particularly temperature and precipitation regimes > litter quality > soil organisms (Swift, Heal & Anderson 1979; Meentemeyer 1984; Aerts 2006). It is thought that at larger spatial scales, climate plays a dominant role in controlling litter decomposition (Meentemeyer 1984; Sun *et al.* 2004), whereas, edaphic conditions and litter quality play a more significant role within a climatic region (McClaugherty & Berg 1987; Berg *et al.* 1993; Aerts 1997; Vivanco & Austin 2006). Although litter decomposition has been studied extensively, less is known about predicted climate change on litter decomposition at the northern edge of the Great Plains – an area projected to be strongly impacted in future climate scenarios.

Temperature and precipitation, the two important elements of climate, are key drivers of ecosystem processes that have both direct and indirect effects on litter decomposition (Aerts 1997; Aerts 2006). Over the past century, global mean surface temperature has increased by more than 0.74 °C due to anthropogenic activities (IPCC 2007), and the mean global temperature is expected to increase by 2-7 °C by the end of this century as (Allison *et al.* 2009). Mean precipitation is projected to increase in high latitude and tropical regions, and decrease in most subtropical regions (IPCC 2007). In addition, anthropogenic manipulation of natural systems continues to increase the release of C to the atmosphere further increasing warming (Houghton 2007). Grasslands cover a large extent of the earth's land surface area (40% of the terrestrial land cover, excluding Greenland and Antarctica) (White, Murray & Rohweder 2000), and a significant portion of the global total C stock is stored in grasslands (Scurlock & Hall 1998). Therefore, the predicted global climate change could greatly alter litter decomposition (Chapin *et al.* 2008; Day, Ruhland & Xiong 2008), as well as nutrient cycling in grasslands (e.g. Luo 2007; Parton *et al.* 2007), and this may have a significant impact on global C budget.

Decomposition, like most biological processes, is sensitive to temperature and moisture changes. The Q_{10} coefficient (the factor by which a response increases per 10 °C temperature rise) for biological systems is often assumed to be

~ 2, but higher values have been recorded for temperate and tropical soil decompositions (e.g. Singh & Gupta 1977). Increasing temperature can enhance litter decomposition rates by stimulating microbial activity (Couteaux, Bottner & Berg 1995). However, temperature can also decrease decomposition by inducing drought conditions that reduce the microbial population activity and humification process (De Santo *et al.* 1993; Couteaux, Bottner & Berg 1995). Soil moisture also enhances decomposition, but very wet soils lead to anaerobic conditions that retard metabolic activity of microorganisms involved in decomposition (Couteaux, Bottner & Berg 1995). This has led to the proposition that decomposition rates will only increase when both temperature and moisture increase (Giardina & Ryan 2000; Epstein, Burke & Lauenroth 2002; Aerts 2006; Bontti *et al.* 2009).

Although litter decomposition and the factors that control it have been studied for decades (e.g. Olson 1963; Meentemeyer 1978; Aerts 1997; Vivanco & Austin 2006), surprisingly few include *in situ* root samples from many terrestrial ecosystems (Silver & Miya 2001). Estimating root decay is inherently complicated by problems associated with field sampling of roots e.g. partial decomposition (Scheffer & Aerts 2000). In grasslands, it is critical to include roots in decomposition studies because plants allocate a larger proportion of the total biomass production to belowground organs (Stanton 1988). The magnitude of this sink can be significant in the evaluation of the consequences of climate change on global C budget. In this respect, a factorial experiment was established at three sites in the Canadian prairies to evaluate the effect of temperature and precipitation changes on both root and shoot litter decomposition.

Concurrent with changes in climatic conditions, land use practice is an additional element that greatly impacts the function of terrestrial ecosystems (Vitousek *et al.* 1997). Grasslands in Canada are typically grazed by native and domestic animals causing potential shifts in litter quality and plant species composition (Willms, Smoliak & Dormaar 1985; Milchunas & Lauenroth 1993), as well as alteration of soil microclimatic conditions (Naeth *et al.* 1991). Therefore, grazing can directly alter litter decomposition through changes in soil moisture and soil temperature that occur in short time-scales, or indirectly through litter quality effects that occur at longer time-scales. In this study, grazing was simulated by clipping vegetation annually in mid to late June during peak plant biomass production consistent with local grazing practices. Clipping has been shown to influence microclimatic conditions in grasslands (e.g. Wan, Luo & Wallace 2002; Klein, Harte & Zhao 2005), as well as alter plant species composition and litter quality (Cheng *et al.* 2010), changes that likely affect decomposition dynamics. Studies examining the interactive effects of both grazing or clipping, and environmental condition manipulation, or climate on litter decomposition are rare (Semmartin *et al.* 2004; Cheng *et al.* 2010) and those that include root samples are even rarer (Giese *et al.* 2009). To my knowledge this is the first study to assess the simultaneous impact of changes in environmental conditions (temperature and precipitation), and clipping on shoot and root litter decomposition in a northern temperate grassland ecosystem. The objective of this study was to explore differences in decomposition dynamics in 3 Canadian grassland sites in order to answer the following questions:

Research questions

1. Does litter decomposition patterns vary amongst sites, and between shoot and root samples?

2. How are litter decomposition and the accompanying carbon (C) and nitrogen (N) dynamics influenced by temperature and precipitation (environment effect) at three sites in Canadian Prairie Provinces?

3. Does simulated grazing through clipping influence litter decomposition, and related C and N dynamics through changes in soil microclimatic conditions?

2.2 Materials and Methods

Field sites description

The study was conducted at three sites representative of each of Canada's Prairie Provinces: Alberta (AB), Saskatchewan (SK), and Manitoba (MB) (Figure 2-1). These sites are located on the northern edge of the Great Plains of North America, an area predicted to have reductions in carbon storage in climate change simulations (Burke *et al.* 1991). Specifically, the sites were located in the Aspen Parkland regions of the Canadian Prairie Provinces that extend from southwestern MB, northwestward through SK to central Alberta. The Parkland is a transitional zone between boreal forest to the north and the grasslands to the south (Sims & Risser 2000) an area projected to be impacted in future climate scenarios (Vance 1979). The ecoregion has a transitional grassland ecoclimate defined by short warm summers and long, cold winters with continuous snow cover. The Great Plains were historically grazed by bison that sustained human life before the arrival of European explorers. Most of the area is now cultivated but was originally characterized by oak groves, trembling aspen, mixed tall shrubs, and intermittent fescue grasslands.

All experimental plots were confined in the grassland areas which are more heavily grazed than the Aspen stands (Arthur 1984). The region is underlain by Cretaceous shale and is covered by undulating to kettled, calcareous, glacial till with significant areas of level lacustrine and hummocky to ridged fluvioglacial deposits. The study site in AB (53.016539°N, 111.539898°W) was located at the University of Alberta Ranch Research station in Kinsella, Alberta. The site is a savanna-type habitat containing a mixture of trembling aspen (*Populus tremuloides* Michx.) stands and rough fescue (*Festuca hallii* (Vasey) Piper) prairie on a hummocky moraine landform. Historically, cattle have grazed this area; however, grazing was halted in 2006, prior to the start of the experiment allowing recovery of the plant community.

The SK study site (49.30039°N, 104.633961°W) was located at the GAP Prairie Farm Rehabilitation Administration (PFRA) community pasture which is a Dry Mixed grassland dominated by native perennial grasses and forbs. The Mixed Grass Prairie in SK has five community types comprised of grasses such as needlegrass (*Stipa viridula*, *Stipa richardsonii*, *Stipa curtiseta*), blue grama grass (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) and northern wheat grass (*Agropyron dasystachyum* (Hook.) Scribn.), and less frequent forbs such as pasture sage (*Artemisia frigida* Willd.), and moss phlox (*Phlox hoodii* Richardson) that make up the bulk of forbs. Ungulate herbivores sustained by SK prairies include cattle, elk (*Cervus Canadensis*), deer (*Odocoileus virginianus*) and pronghorn antelope (*Antilocapra Americana*).

The MB study site (MB, 50.781249°N, 100.593395°W) was located at Riding Mountain National Park which is dominated by native perennial grasses, forbs and shrubs. The dominant plant species at the field site are *Poa secunda* J. Presl, *Carex* spp., and *Monarda fistulosa* L.). The park is a crossroad where Fescue Prairies, Aspen woodland, Mixed Boreal and Eastern Hardwood inter-mix. The experimental plots were located within a forest landscape interspersed with grassland. The site has historically been grazed by native ungulates and presently utilized by deer, moose (*Alces alces*), elk and a captive bison herd. See Table 2-1 for additional specific physical characteristics of the study sites.

General Experimental Design

A three-year manipulative study was established in May 2007 to determine the effects of temperature (2 levels), precipitation (3 levels in AB and, 2 levels in SK and MB), and clipping (3 levels) on shoot and root litter decomposition and the related C and N dynamics. Each plot was 2 x 2 m in size, with at least 0.5 m buffer zone separating adjacent plots. The plots were arranged in a randomized block design in AB site to account for topographical gradient and in a completely randomized design in SK and MB because there was no obvious environmental gradient. There were five replicates per treatment combination yielding 90 plots in AB and 60 plots in each of SK and MB.

Warming

High latitude ecosystems are predicted to experience the greatest increase in temperature over the next century (Hengeveld 2000; Houghton *et al.* 2001). In this experiment warming was achieved by the use of passive open-top chambers (OTCs). OTC is a valuable tool for temperature manipulation in high altitude ecosystems while minimizing confounding ecological effects e.g. overheating (Marion *et al.* 1997). There are different types of OTCs each with its advantages and disadvantages (see Marion *et al.* 1997 for an overview). In this study a cone-shaped OTC 40 cm high and, 2 m outer and 1.5 m inner diameters was used. The OTCs were made of fibreglass (Sunlite-HP Components Corporation/Kalwall Corporation, Manchester, NH, USA) with the sides inclined at a 60° angle with respect to the ground surface (Figure 2-2; Plate 2-1). The fibreglass allows transmission of visible, but not infra-red light wavelength, increasing the temperature inside the chambers by around 2 – 4 °C above ambient (Marion *et al.* 1997). This range is consistent with the Canadian Centre for Climate Modelling and Analysis (CCCma) Coupled Global Climate Model (CGCM1) future projections which predicts mean annual temperature increase from 2 to 4.5 °C for the Prairies by 2040 – 69.

Precipitation

Although realistic, precipitation predictions under climate change are more difficult to evaluate than temperature effects and there is considerable variability from region to region (Hengeveld 2000). Precipitation is predicted to increase at higher latitudes (IPCC 2007), and the CGCM1 model predicts a precipitation increase over most of Canada over the next century. However, the forecasted warmer temperatures will lead to net soil moisture deficits in the summer, and more frequent extreme events, including severe droughts particularly in regions such as Canada's southern prairies (Hengeveld 2000).

In this study, plots were individually modified to receive ambient, reduced or added (only in AB) growing season precipitation using a modified design of Zhou *et al.* (2006). Precipitation manipulation was achieved by fitting transparent plastic (Dura-Film Super 4^{TM} 6-mil polyethylene film; AT Plastics, Edmonton, AB, Canada) on wooden rain-out structures (Figure 2-2). For the reduced precipitation treatment, small slits were made on the plastic that intercepted approximately 60% of rainfall. For the ambient precipitation treatment, larger slits were made that allowed complete rainfall penetration. At the AB site, water intercepted from reduced precipitation plots was collected by gravity feeding rainfall into reservoir tanks which was subsequently reapplied to the 'added precipitation' treatment. Water addition was done within 24 hours after any rainfall event. Ambient and added precipitation treatments intercepted the same amount of rainfall but the latter treatment received the additional water collected from the 'reduced precipitation' treatment. All plots had similar shelters built around them to control for potential confounding effects of these structures on air temperature and shading. This approach only alters rainfall amount but not the frequency and it depends on the actual rainfall event.

Due to logistical constraints, the MB and SK sites did not have the 'added precipitation' treatment and installation of rain-out shelters was delayed until June in the AB site in first year. In subsequent years, rain-out shelter plastic tops, OTCs and data loggers were installed in May and removed in September of each year which corresponded with the growing season at all sites.

Clipping

The presence of OTCs precluded grazing of the experimental plots by ungulates. Instead, to simulate grazing, vegetation was manually clipped within the plots using a lawn mower and clippers at the AB and SK sites, and a string trimmer at the MB site at the peak of growing season (mid to late June) which is similar to what is done by local producers. The vegetation was either unclipped, or clipped to a stubble height of approximately 7.5 ('low intensity") or 2.5 cm ("high intensity") which coincides with the conservative and excessive use of native rangelands. Clipping with a lawn mower or string trimmer was applied throughout the area under the rain-out shelter, except over a 50 cm by 50 cm permanent sampling subplot (Figure A-1), which was hand clipped to estimate vegetative biomass removal.

Sampling

Each plot had three random areas allocated for destructive sampling that included soil cores for chemical analysis and aboveground biomass harvest (Appendix A, Figure A-1). Two soil cores 5 cm in diameter were taken at two depths (0-5 cm and 5-15 cm) at each site each year when the plots were clipped. Soil samples were air-dried, ground with a mortar and pestle and passed through a 250 µm sieve before C and N determination. Total C and N concentration (%) was determined by dry combustion using a CE440 Elemental Analyzer (Exeter

Analytical Inc., North Chelmsford, MA, USA) at the University of Alberta Biogeochemical Analytical Service laboratory. At peak biomass in late July each year, shoot biomass was clipped to ground level from a 10 cm by 100 cm quadrat to quantify annual aboveground production. Accumulated litter from previous years was separated from standing live shoot biomass, and live shoot biomass was later sorted to graminoid, forbs and shrubs. The samples were then dried and weighed (65 °C, 72h). Root biomass was obtained from two root cores, 5 cm diameter and 20 cm deep, taken adjacent to the shoot biomass harvest strip. Root cores were washed over a 2 mm sieve, dried (65 °C, 72h) and weighed. This harvest biomass from within the plots was used as litter material to study the effect of litter quality and local site conditions on litter decomposition (Chapter 3).

Plant available nitrogen was determined using PRS^{tm} (Plant Root Simulator) probes (Western Ag Innovations, Saskatoon, SK) that were deployed in the plots between late June and early September. Four sets of PRS probes per plot, consisting of both a cation (NH_4^+) and anion (NO_3^-) probe, were evenly positioned around and within 10 cm of the permanent sampling subplot (Appendix A, Figure A-1). Probes were pooled prior to elution and colourimetrical analysis was performed by Western Ag Innovations, Saskatoon, SK. Total inorganic N was the sum of NH_4^+ and NO_3^- (mg total nitrogen/10 cm^3 ion-exchange membrane surface area/time of burial).

Litter collection, preparation, litterbag incubation & processing

Shoot and root litter decomposition rates were determined using the litterbag method (Bocock & Gilbert 1957; Shanks & Olson 1961). Litter material used was harvested from live plants around the general area of the experimental plots in each respective site, pooled, oven-dried (65 °C, 72h), thoroughly mixed and redistributed among the plots. Using mixed species samples is more realistic, and some studies have even shown that in this case decomposition rate is faster than single species (e.g. Liu *et al.* 2010). Furthermore, decomposition of single species litter alone is not sufficient for understanding decomposition in real ecosystems. Secondly, because of the small plot size, a single species experiment would have increased the unintended plot disturbance.

Approximately 3 g of plant material was placed in each litterbag (13 cm x10 cm, 1 mm^2 pore size) made of charcoal fibreglass screen (Phifer Inc., USA). This opening was small enough to prevent major litter loss but large enough to allow access by most fungi, bacteria and dominant soil invertebrates in these systems (e.g. Clapperton, Kanashiro & Behan-Pelletier 2002). Soil organisms, the drivers of litter decomposition and nutrient release patterns, were not directly studied in this experiment. Root litterbags were buried at 15 cm depth to mimic the natural environment of root decomposition and because most of root biomass in the Canadian prairies is within 10-20 cm of soil surface (e.g. Steinaker & Wilson 2005; Coupe, Stacey & Cahill 2009). Shoot litter bags were incubated on the soil surface and held in place by steel anchor pins. Litterbags were deployed in September 2007, with replicate bags retrieved after 6, 12, and 24 months. One litter bag from each treatment combination was retrieved each time. After retrieval any extraneous debris was gently removed and residual litter oven-dried (65 °C, 72h) immediately after returning from the field. The remaining litter was weighed and percent remaining mass was determined as:

$$\frac{\text{Remaining mass}}{\text{Initial mass}} \times 100$$

Each sample was then individually reweighed, ground to <1mm using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and soil contamination was corrected by loss-on-ignition to determine percentage ash-free dry mass (% AFDM) following Blair (1988). For soil, samples were ground with a mortar and pestle then passed through a sieve (120 µm) to ensure homogeneity. Both soil and remaining litter samples were individually analyzed for total %C and N by dry combustion using a CE440 Elemental Analyzer (Exeter Analytical Inc., North Chelmsford, MA, USA) at the University of Alberta Biogeochemical Analytical Service Laboratory. The final remaining litter mass %C and N content were expressed as a percentage of initial %C and N content. Initial %C and N content was determined from the pooled litter material for both shoot and root litter (n=5, for each), and were thus common to all treatments at each site. Nutrient concentration in the residual litter is also affected by accumulated soil in litterbags and was therefore corrected for soil contamination as recommended by Blair (1988).

Environmental measures

Environmental variables were measured to confirm the effect of the treatments imposed. Air temperature, soil temperature and soil moisture were measured with two replications for each treatment combination at each site between May and September in 2008 and 2009. Air temperature was measured 7.5 cm aboveground near the plot centre at 30-min intervals using Onset HOBO Pendant Temperature data loggers (Onset Computer Corporation, Bourne, MA, USA). Soil temperature and soil moisture were measured at 30-min intervals using Decagon ECH2O EC-TM probes buried at 0-5 cm depth. Additionally, ambient precipitation was recorded using two Davis Rain Collector II buckets (Davis Instruments, Hayward, CA, USA).

Statistical analysis

The response variables were shoot and root litter % remaining dry mass and the related C: N ratio. Shoot litter % remaining mass satisfied parametric statistics assumptions without transformations. However, root litter % remaining mass for all sites was log-transformed for normality of residuals before analysis. To assess the effects of treatments, data were analyzed using repeated measures ANOVA with precipitation, temperature, clipping and sites as fixed factors and the three collection times were used as replicates. Despite the sites being located in the Canadian grasslands they slightly differed in their physiognomy especially the Manitoba site which was located within a forest, hence site is treated as a fixed factorto allow comparison of sites. A separate analysis for AB site was conducted for % remaining mass to assess the effect of added precipitation using the linear mixed model procedure. The model for this supplemental analysis included site, precipitation, temperature, and clipping as fixed factors and block as a random factor because AB site was blocked to account for a slight slope gradient. Since the construction of the rain-out shelters was delayed in the first year (2007), only data from12 and 24 months of litterbags incubation were considered for this analysis. Data were analyzed with PASW STATISTICS 18 for Windows (*Release Version 18.0.,* Chicago: SPSS, Inc.). Decomposition rate was estimated as annual decomposition constant *k* using the negative exponential decay function (Olson 1963),

$$X_t/X_0= e^{-kt}$$

where X_0 is the initial litter mass, X_t is the amount of mass remaining at time *t*, which is the time in years. *K*-values for decomposition after 6 and 12 months were adjusted to 1 year for comparability.

To evaluate efficacy of the treatments in altering microclimatic conditions, 2008 and 2009 average daily soil moisture, soil temperature and air temperature were analyzed using a general linear model. Environmental data were averaged for the whole day from mid-May to early September. This data met the assumptions for parametric statistics and the model included site, precipitation, temperature and clipping as fixed factors.

2.3 Results

Treatment efficacy

Approximately 29% (low intensity) and 54% (high intensity) of shoot biomass was removed per plot in AB; 34% and 63% in SK; and 48% and 69% in MB (White 2013). Environmental conditions within experimental plots were influenced by the imposed treatments. OTCs increased average ambient air temperature by between 0.5 – 1.2 °C in 2008 and by between 0.3 – 1.6 °C in 2009 among sites, but rain-out shelters significantly increased average air temperature only in 2008 (Table 2-2).

Clipping significantly influenced average soil temperature in both years but significant effects of OTCs on average soil temperature were only found in 2008 (Table 2-3). Warming increased average soil temperature by between 0.1 and 0.8 °C among sites in 2008, and high intensity clipping increased soil temperature by between 0.7 – 1.2 °C among sites in 2008 and by between 0.8 – 1.2 °C in 2009. Low intensity clipping increased average soil temperature by between 0.5 – 1 °C in 2008 and by 0.3 – 0.8 °C in 2009. Rain-out shelters significantly increased average soil temperature in 2009 (but not in 2008) by between 0.3 – 0.6 °C. Rain-out shelters significantly reduced soil moisture in both years (Table 2-4). Percent volumetric water content (%VWC) was between 4 – 13.3% and 5 – 8.2% higher in ambient than reduced precipitation plots among sites in 2008 and 2009, respectively. Clipping levels had varied effects on soil moisture, whereby it increased, decreased or had no effect on average daily volumetric water content.

Site differences and differences between shoot and root decomposition

The sites differed significantly in decomposition of shoot and root litter (Tables 2-6 and 2-7). After two years of *in situ* decomposition, the average ash-free percent litter mass remaining ranged between 52 and 59% and corresponding k-values between 0.13 and 0.17 for shoot litter. Shoot litter decomposition followed an exponential decay pattern and was greatest in the Alberta site initially (after half a year of deployment), but was greatest in the Manitoba site 1 and 2 years after deployment. Root decomposition followed a similar pattern. Percent remaining litter mass varied between 38% and 48% and corresponding k-values between 0.19 and 0.25 after two years of incubation (Table 2-5). Within sites, mass loss rates for root litter were consistently greater than for shoot litter except at the Manitoba site after 0.5 and 1 years of incubation. This result suggests that root litter mass loss may have greater implication on C and N flux compared to shoot litter.

Effects of warming and precipitation on litter decomposition

Precipitation significantly influenced both shoot and root litter decomposition, but temperature only significantly affected shoot litter decomposition (Tables 2-6 and 2-7). Warming decreased shoot litter mass loss across all sites (Figure 2-3 and Figure 2-5). Although insignificant, warming increased root litter mass loss for Alberta and Manitoba sites (Figure 2-5). This difference between shoot and root litter in response to temperature suggests that root samples should be considered in decomposition studies, especially in grassland ecosystems.

When only temperature was considered, the collective % average remaining mass recorded after two years of incubation was 49% and 61% for shoot litter, and 43% and 39% for root litter under control and warming conditions, respectively (Table 2-8). This corresponded to k-values of 0.17 and 0.12 for shoot litter, and 0.21 and 0.25 for root litter under control and warming conditions, respectively.

At all sites, drought decreased mass loss for both shoot and root litter (Figures 2-3a and 2-4a). When considering only the Alberta site, significant differences in shoot and root litter mass losses were also found (Table 2-9). At this site, added precipitation further increased litter decomposition (Figure 2-6). Percent remaining dry mass for added precipitation treatment was consistently lower for both shoot and root litter mass after 1 and 2 years of incubation (Table 2-10). This finding further suggests that moisture enhances litter decomposition in these grassland systems.

There was no significant interaction between moisture and temperature effects on either shoot or root litter decomposition (Table 2-6). However, there was a significant interaction between site and precipitation treatments on shoot litter mass loss suggesting that differences in local precipitation regimes are likely to control mass loss.

Effect of clipping on litter decomposition

Clipping had pronounced effects on aboveground plant biomass production, and temperature and volumetric water content (%VWC) changes attributed to clipping were comparable to the effect of OTCs and rain-out shelters.

However, these changes as a result of clipping did not significantly affect shoot or root litter decomposition after 2 years of incubation (Table 2-6, Figures 2-3 and 2-4). This finding suggests that the level of clipping imposed did not alter the manipulated soil microclimatic conditions enough to cause dramatic change in litter decomposition.

Effect of precipitation, temperature and clipping on carbon and nitrogen dynamics

The pooled shoot litter initial C: N ratio was 31.5, 44.2 and 44.6, whereas pooled root litter C: N was 32.3, 32.3 and 26.9 for the AB, SK and MB sites, respectively (Table 2-11). The sites and the two litter types differed in their N immobilization and mineralization patterns over the 2 years of decomposition. Site, precipitation and temperature significantly affected aboveground N immobilization and mineralization during the 2 years of decomposition as indicated by %C: N ratio remaining, whereas belowground N immobilization and mineralization was significantly affected only by site (Table 2-12, Figure 2-7). At the AB site, shoot litter N was initially mineralized (after 6 months) and then immobilized, whereas at SK and MB sites the trend was in the opposite direction. However, shoot litter generally immobilized N after 2 years of decomposition at all sites. MB root litter released about 55% of their initial N concentrations over a period of 6 months but N was generally immobilized or maintained among sites.

Relationship between environmental intermediary variables

No relationship was found between % remaining dry mass and total available nitrogen (Appendix A; Figures A-2 and A-3), as well as with other environmental variables.

2.4 Discussion

In this study, litter decomposition patterns significantly varied among sites. For example, after 2 years of incubation, root and shoot percent remaining mass was lowest and highest, respectively at MB site. These results could be attributed to differences in site characteristics such as edaphic conditions or litter quality i.e. chemical composition (Sanchez 2001; Vivanco & Austin 2006), and/or indirect effects of regional climate on species composition of plants that have different litter quality (Aerts 2006). Possible explanations for low root decomposition at the MB study site is that the experimental plots were located within a forest and aspen stand landscape dominated by shrubs where soils are expected to be wetter and colder than in open grassland.

Decomposition differed between shoots and roots with the latter having lower mass loss after the second year of decay. Literature shows that there is no consensus on the differences between shoot and root litter decomposition, both in forest and grassland ecosystems. In some studies, roots decomposed more slowly than shoots (Bloomfield, Vogt & Vogt 1993; Lehmann, Schroth & Zech 1995; Biondini, Patton & Nyren 1998; Vivanco & Austin 2006), while in others roots decomposed more rapidly (Seastedt, Parton & Ojima 1992; Hobbie 1996; Ostertag & Hobbie 1999; Moretto, Distel & Didoné 2001), or no difference between shoot and root decomposition was found (Giese *et al.* 2009). Litter quality (Bloomfield, Vogt & Vogt 1993; Vivanco & Austin 2006; Fujii & Takeda 2010), and location of decomposition of materials (soil surface vs. soil fabric) (Gholz *et al.* 2000; Cusack *et al.* 2009; Fujii & Takeda 2010) have been ascribed as the reasons why roots and shoots differ in mass loss and nutrient dynamics. Higher lignin content or high C: N ratios of roots than have also been reported in studies that found slower root decay rates (Bloomfield, Vogt & Vogt 1993; Silver & Miya 2001). The results of the present study do not support that hypothesis although lignin content was not measured. Despite having 3–4 times higher lignin content than roots, faster decomposition has been reported for roots in a semi-arid grassland ecosystem (Giese *et al.* 2009). This suggests that in grassland systems differences in these dynamics are more likely due to climatic variables than chemical differences.

In this study, experimental warming significantly decreased shoot litter decomposition. There was a trend of increased root decomposition with increased temperature in two of the sites (AB and MB) although this effect was not significant. The abundance of prostigmata mites, some of the dominant invertebrates in Albertan grasslands (Clapperton, Kanashiro & Behan-Pelletier 2002) increased with warming in the AB site (Newton 2013). This response of soil organisms that may play a role in decomposition may explain the increased root decomposition with warming. Although climate warming may accelerate global scale decomposition rates (Kirschbaum 1995; Trumbore, Chadwick & Amundson 1996), at a local scales increased temperature can also intensify soil moisture deficits and hinder decomposition rates (Shaw & Harte 2001; Gavazov 2010). Litter decomposition in grassland ecosystems have shown variable results whereby it was affected by litter quality but not climate (Hamadi *et al.* 2000), influenced by precipitation and not temperature (Epstein, Burke & Lauenroth 2002), determined by precipitation or nutrient availability (Liu *et al.* 2006), correlated with precipitation or interaction between climate variables (Bontti *et al.* 2009; Murphy, Klopatek & Klopatek 1998), or not correlated with mean annual precipitation (Semmartin *et al.* 2004). Some of these inconsistencies could be due to indirect climate effects (e.g. Yahdjian, Sala & Austin 2006), interaction between climate and litter quality (e.g. Murphy *et al.* 2002) or lack of separation between shoot and root decomposition. The findings of this study suggest that exposure to different environment (soil surface vs. mineral soil) can determine the outcome of climatic factors on litter decomposition.

Temperature increase seems to increase litter decomposition in high-latitude and high-altitude ecosystems (Hobbie 1996; Shaw & Harte 2001), where decomposition is temperature limited (Hobbie, Nadelhoffer & Högberg 2002; Robinson 2002). In this study, a 0.5 – 1.3 °C increase in daily average air temperature among the sites led to a 2 – 9 % decrease in volumetric water content at the AB and SK sites in 2008. The potential stimulatory effects of warming on litter decomposition could have been offset by moisture deficits within the plots resulting in lower shoot decay rates (Lauenroth *et al.* 2004; Cheng *et al.* 2010; Butenschoen, Scheu & Eisenhauer 2011). Unlike root tissues, which were buried within the soil, shoot litter was placed on the soil surface where it is less buffered from direct effects of climate. Average daily soil warming by about 1 °C, the most induced by OTCs in this experiment, might not have been sufficient enough to stimulate root litter decomposition, or may have been counterbalanced by cooling effect by increased soil moisture. Temperature is likely not a limiting factor for decomposition in temperate grassland ecosystems.

Grasslands are comparatively water-limited ecosystems (Vivanco & Austin 2006; Bontti *et al.* 2009), implying that litter decomposition will likely be increased by precipitation (soil moisture) than by slight temperature increase. In this study, drought retarded both shoot and root litter mass loss. Additionally, the sites differed in shoot litter decomposition patterns as a result of precipitation and this can be attributed to the variability in mean annual precipitation (MAP) among sites as well as the potential inherent sites differences in litter quality. In US Great Plains grasslands, Bontti *et al.*, (2009) found that MAP and other indices that included moisture or precipitation better explained root decay rates. In the AB site, where there was an added precipitation treatment, percent remaining dry mass under drought conditions were consistently higher than under the added precipitation treatment after both 12 and 24 months of incubation (Figure 2-6). Other studies that manipulated rainfall with rainout shelters reported similar findings, where litter decomposition was lower under drought conditions (Yahdjian, Sala & Austin 2006; Saura-Mas *et al.* 2012). In others that did not manipulate climate variables, mean annual precipitation (MAP) explained more variability in shoot (Epstein, Burke & Lauenroth 2002) and root (Bontti *et al.* 2009) decomposition dynamics in the US Great Plains, and root decomposition in

Inner Mongolia (Giese et al. 2009). Interception in of precipitation in this study significantly reduced soil volumetric water content by between 4 – 13% in 2008 and 5 – 8% in 2009 among the sites. These drought conditions limit metabolic activity and soil microbial activity (Griffin 1981; De Santo *et al.* 1993), and increase unfavourable soil and soil surface conditions potentially hindering decomposer community and the humification process (Couteaux, Bottner & Berg 1995). A study conducted within the framework of this experiment showed that drought generally the decreased abundance and biomass of most soil microarthropods (Newton 2013). These soil fauna likely contribute to litter breakdown in these grassland systems that are devoid of larger soil fauna such as earthworms.

The opposite is true for precipitation effects since moisture beyond an optimum point can lead to anaerobic conditions that may suppress decomposition (Couteaux, Bottner & Berg 1995). This idea has consequently led to the hypothesis that synergies between temperature increase and high moisture content may increase decomposition rates (Giardina & Ryan 2000; Shaw & Harte 2001; Epstein, Burke & Lauenroth 2002; Aerts 2006; Bontti *et al.* 2009; Butenschoen, Scheu & Eisenhauer 2011). However, the findings of this study do not support the hypothesis that higher decomposition rates occur only under increased temperature where soil moisture is not limiting (e.g. Bontti *et al.* 2009). In this study, higher moisture by itself led to increased mass loss suggesting that precipitation may influence the fate of C more than temperature shifts in these grassland systems. This is especially so because grasslands allocate more biomass belowground than aboveground (Sims & Singh 1978; Stanton 1988; Milchunas & Lauenroth 2001; Steinaker & Wilson 2005). In this study, soil moisture availability affected root litter decomposition to a greater extent compared to shoot litter. Although decomposition in grasslands is considered slow (Gill & Burke 2002), the implication of higher belowground decomposition on the C budget could be significant. Precipitation is the only manipulated factor that significantly affected root litter decomposition in this study. Therefore, it can be speculated that in the short term, precipitation increases as a result of climate change may lead to decreased C storage in these water-limited grassland ecosystems.

Despite having pronounced effects on soil moisture and temperature, clipping did not influence shoot or root litter decomposition. Although there was a trend of increased root decomposition at the MB site under ambient precipitation and clipping treatments, this pattern was not consistent across sites. This finding is similar to Giese *et al*. (2009),who found no grazing effects on shoot and root litter decomposition in semi-arid grasslands of Inner Mongolia. Conversely, Cheng *et al*. (2010) found that clipping increased decomposition rates in US Great Plains. In the present study, it is unclear why clipping did not affect decomposition despite the magnitude of its effect on soil moisture and temperature being comparable to the effects of OTCs and rain-out shelters. Nonetheless, this suggests that in the short-term current clipping in the Northern edge of the Great Plains may not affect C and N dynamics through alteration of soil microclimatic conditions.

Many decomposition studies have found that the relative concentration of nutrients in decomposing litter generally increases over time (e.g. Allison & Vitousek 2004), although the final phase of litter decomposition culminates with nutrient release that parallels mass loss (Prescott *et al.* 1993). This is in agreement with the findings of this study for shoot litter; N concentration of shoot litter generally increased after two years of decomposition. This increase has been attributed to translocation of nutrients from soil substrate to decomposing litter by soil microflora (Gallardo & Merino 1992; Aubert *et al.* 2010) and this may serve an important role of maintaining nutrients in a system (Ostertag & Hobbie 1999). Higher initial shoot litter N concentration at AB site than at the SK and MB sites, as expected, corresponded with much more N mineralization initially, and a similar pattern was found for the MB roots that had a higher initial N concentration. It is well established that low initial litter N concentration (poor litter quality) usually leads to net N immobilization (Aerts & deCaluwe 1997; Aerts, van Logtestijn & Karlsson 2006). Temperature and precipitation significantly influenced shoot litter N immobilization and mineralization. These dynamics can be attributed to microflora activity, specifically fungi, which enhance nutrient uptake from surrounding soil substrate (Berg & Laskowski 2005; Aubert *et al.* 2010). Fungi are known to be positively influenced by soil moisture (Ormeno *et al.* 2006), and in some cases have been shown to be resistant to seasonal moisture and temperature variations (Yuste *et al.* 2011). Although increased mass loss was observed in this study, evidence of immobilization and the trend toward maintenance of N levels for root litter suggests that long-term studies are necessary to provide meaningful information about decomposition and nutrient release dynamics. A lot less is known about litter decomposition at the latter stages when reliable insights into nutrient cycling and carbon storage become more apparent (Prescott 2005).

To my knowledge, this is the first study to simultaneously test the effects of temperature; precipitation and clipping (simulated grazing) on both shoot and root litter. Simulated grazing did not influence shoot or root litter decomposition suggesting that in the short-term land-use practice in Canadian prairies may not directly regulate C and N cycling through alteration of microclimatic conditions. However, climate variables differentially controlled litter decomposition; drought retarded both shoot and root litter decomposition while temperature retarded shoot but generally stimulated root litter decomposition. Furthermore, root litter decomposed more compared to shoot litter potentially reducing C storage. The knowledge on root decomposition patterns and accompanying nutrient release is limited compared to shoot decomposition (Silver & Miya 2001), and the results of this study highlight the need to consider root decomposition dynamics in order to reliably predict the consequences of climate change on C storage in grasslands. In grasslands, belowground biomass production outweighs aboveground biomass production by far (Milchunas & Lauenroth 2001), and root decomposition dynamics may play a more prominent role in the regulation of C and nutrients turnover, and soil organic matter (SOM) formation in these systems. Whether Canadian grasslands will be a C source or sink will largely depend on the balance between above- and belowground decomposition, as well as the differential effect of predicted climate change factors on litter decomposition in these contrasting environments at least in the short-term.

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Table 2-1. Characteristics of the three study sites

Attribute	Alberta	Saskatchewan	Manitoba
Latitude	53.016539°N	49.30039°N	50.781249°N
Longitude	111.539898°W	104.633961°W	100.593395°W
MAP (mm)	431.3	386.3	529.6
MAT (^{0}C)	2.8	3.6	1.4
2007, 2008, 2009 growing season precipitation (mm)	241.8, 204.2, 168.9	273.2, 348.4, 241.2	369.1, 405.1, 277.3
2007, 2008, 2009 growing season average temperature (^{0}C)	13.7, 13.5, 13.8	16.5, 15.1, 15.2	14.7, 13.8, 14.2
Long-term growing season precipitation	314.7	262.4	349.3
Long-term growing season daily average temperature (^{0}C)	14	15.2	14.4
Natural sub-region	Aspen Parkland	Mixed grassland	Aspen Parkland
Soils	Chernozemic Orthic black	Chernozemic Orthic dark brown	Chernozemic Orthic dark grey
Total soil C and N content			
%N 0-5 cm	0.90	0.59	1.51
%N 5-15 cm	0.44	0.33	1.05
%C 0-5 cm	9.64	6.52	16.42
%C 5-15 cm	4.97	3.58	10.72

The climate information is from the nearest climate station to the study sites i.e. Viking for AB, Ceylon for long-term data and Weyburn for short-term data for SK, and Gilbert Plains for MB (Environment Canada, 2013). MAP (mean annual precipitation), MAT (mean annual temperature), long-term growing season precipitation and average temperature values presented here are based on at least 15 years of data between 1971 – 2000. Soil classification follows Soil Classification Working Group (1998). Total %C and N content was determined using CE440 Elemental Analyzer (Exeter Analytical Inc., North Chelmsford, MA, USA).

Table 2-2. Results of general linear model for the effects of site (AB, SK, MB), precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on 2008 and 2009 air temperature. Subscripts under F ratio indicate the numerator and denominator degrees of freedom, respectively.

	(a) 2008		(b) 2009	
Effect	$\mathbf{F}_{\mathrm{df}}$	***P***	$\mathbf{F}_{\mathrm{df}}$	***P***
Site	$114.752_{2,31356}$	**<0.001**	$4.663_{2,1980}$	**0.010**
Temperature	$69.543_{1,31356}$	**<0.001**	$5.696_{1,1980}$	**0.017**
Clipping	$0.954_{2,31356}$	0.385	$0.179_{2,1980}$	0.836
Precipitation	$28.946_{1,31356}$	**<0.001**	$1.670_{1,1980}$	0.196
Site x Temperature	$8.803_{2,31356}$	**<0.001**	$1.448_{2,1980}$	0.235
Site x Clipping	$0.248_{4,31356}$	0.911	$0.114_{4,1980}$	0.978
Site x Precipitation	$2.532_{2,31356}$	0.080	$0.316_{2,1980}$	0.729
Temperature x Clipping	$3.095_{2,31356}$	**0.045**	$0.631_{2,1980}$	0.532
Temperature x Precipitation	$0.023_{1,31356}$	0.879	$0.026_{1,1980}$	0.872
Clipping x Precipitation	$2.148_{2,31356}$	0.117	$0.115_{2,1980}$	0.891
Site x Temperature x Clipping	$0.996_{4,31356}$	0.408	$0.136_{4,1980}$	0.969
Site x Temperature x Precipitation	$0.865_{2,31356}$	0.421	$0.025_{2,1980}$	0.975
Site x Clipping x Precipitation	$0.379_{4,31356}$	0.824	$0.053_{4,1980}$	0.995
Temperature x Clipping x Precipitation	$0.651_{2,31356}$	0.521	$0.056_{2,1980}$	0.945
Site x Temperature x Clipping x Precipitation	$0.527_{4,31356}$	0.716	$0.911_{4,1980}$	0.985

Table 2-3. Results of general linear model for the effects of site (AB, SK, MB), precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on 2008 and 2009 soil temperature. Subscripts under F ratio indicate the numerator and denominator degrees of freedom, respectively.

	(a) 2008		(b) 2009	
Effect	**F**$_{df}$	***P***	**F**$_{df}$	***P***
Site	$137.567_{2,1844}$	**<0.001**	$144.373_{2,1928}$	<**0.001**
Temperature	$3.699_{1,1844}$	**0.055**	$1.964_{1,1928}$	0.162
Clipping	$10.115_{2,1844}$	**<0.001**	$11.199_{2,1928}$	<**0.001**
Precipitation	$1.720_{1,1844}$	0.190	$8.721_{1,1928}$	**0.003**
Site x Temperature	$1.193_{2,1844}$	0.304	$3.697_{2,1928}$	**0.025**
Site x Clipping	$0.301_{4,1844}$	0.877	$0.424_{4,1928}$	0.792
Site x Precipitation	$0.229_{2,1844}$	0.795	$0.366_{2,1928}$	0.694
Temperature x Clipping	$0.639_{2,1844}$	0.528	$2.851_{2,1928}$	**0.058**
Temperature x Precipitation	$0.002_{1,1844}$	0.963	$0.789_{1,1928}$	0.374
Clipping x Precipitation	$0.901_{2,1844}$	0.406	$0.288_{2,1928}$	0.750
Site x Temperature x Clipping	$1.984_{4,1844}$	0.094	$0.892_{4,1928}$	0.468
Site x Temperature x Precipitation	$0.305_{2,1844}$	0.737	$0.353_{2,1928}$	0.703
Site x Clipping x Precipitation	$1.626_{4,1844}$	0.165	$2.525_{4,1928}$	**0.039**
Temperature x Clipping x Precipitation	$4.014_{2,1844}$	**0.018**	$2.289_{2,1928}$	0.102
Site x Temperature x Clipping x Precipitation	$3.267_{4,1844}$	**0.011**	$1.258_{4,1928}$	0.285

Table 2-4. Results of general linear model for the effects of site (AB, SK, MB), precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on 2008 and 2009 soil moisture. Subscripts under F ratio indicate the numerator and denominator degrees of freedom, respectively.

	(a) 2008		(b) 2009	
Effect	**F_{df}**	***P***	**F_{df}**	***P***
Site	$254.945_{2,1844}$	**<0.001**	$133.355_{2,1928}$	<**0.001**
Temperature	$65.049_{1,1844}$	**<0.001**	$15.621_{1,1928}$	<**0.001**
Clipping	$46.879_{2,1844}$	**<0.001**	$39.174_{2,1928}$	<**0.001**
Precipitation	$433.158_{1,1844}$	**<0.001**	$273.705_{1,1928}$	<**0.001**
Site x Temperature	$84.761_{2,1844}$	**<0.001**	$62.007_{2,1928}$	<**0.001**
Site x Clipping	$28.601_{4,1844}$	**<0.001**	$17.276_{4,1928}$	<**0.001**
Site x Precipitation	$63.913_{2,1844}$	**<0.001**	$5.646_{2,1928}$	**0.004**
Temperature x Clipping	$10.522_{2,1844}$	**<0.001**	$25.151_{2,1928}$	<**0.001**
Temperature x Precipitation	$127.412_{1,1844}$	**<0.001**	$22.544_{1,1928}$	<**0.001**
Clipping x Precipitation	$8.235_{2,1844}$	**<0.001**	$11.651_{2,1928}$	<**0.001**
Site x Temperature x Clipping	$37.500_{4,1844}$	**<0.001**	$8.518_{4,1928}$	<**0.001**
Site x Temperature x Precipitation	$2.009_{2,1844}$	0.123	$14.321_{2,1928}$	<**0.001**
Site x Clipping x Precipitation	$27.336_{4,1844}$	**<0.001**	$15.664_{4,1928}$	<**0.001**
Temperature x Clipping x Precipitation	$14.817_{2,1844}$	**<0.001**	$20.917_{2,1928}$	<**0.001**
Site x Temperature x Clipping x Precipitation	$15.755_{4,1844}$	**<0.001**	$22.844_{4,1928}$	<**0.001**

Table 2-5. Shoot and root litter decomposition in the AB, SK and MB sites. Data reported as % remaining dry mass; k-values calculated as $X_t/X_0 = e^{-kt}$ (Olson 1963); where X_0 is the initial litter mass, X_t is the mass remaining at time *t*, and *t* is the time in years.. K-values for harvest of litterbags after 0.5 and 2 years of incubation were adjusted to 1 year for comparability between collection times. Values represent means ± 1SE; n=3).

	Shoot litter					
Site	Remaining dry mass (%)			k-values		
	0.5yrs	1yr	2yrs	0.5yrs	1yr	2yrs
Alberta	77.5(±1.22)	71.7(±1.41)	58.6(± 1.21)	1.02(±0.06)	0.33(± 0.02)	0.13(± 0.02)
Saskatchewan	78.3(±1.37)	71.7(±1.57)	55.0(±1.35)	0.99(±0.07)	0.33(±0.02)	0.15(±0.01)
Manitoba	78.8(±1.34)	63.7(±1.54)	51.7(±1.32)	0.97(±0.06)	0.47(± 0.02)	0.17(± 0.02)
		Root litter				
Alberta	57.1(±2.43)	51.1(±1.84)	38.3(±1.59)	2.34(±0.16)	0.69(±0.03)	0.25(±0.01)
Saskatchewan	66.6(±2.50)	51.2(±1.90)	36.7(±1.64)	1.79(±0.16)	0.70(±0.03)	0.26(±0.01)
Manitoba	83.1(±2.38)	67.1(±1.81)	48.0(±1.56)	0.78(±0.16)	0.40(±0.03)	0.19(±0.01)

Table 2-6. Results of repeated measures ANOVA testing the effects of site (AB, SK, MB), precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on shoot (a) and root (b) litter decomposition at the three collection times. Subscripts under F ratio indicate the numerator and denominator degrees of freedom, respectively.

	(a) Shoot litter		(b) Root litter	
Effect	**F_{df}**	***P***	**F_{df}**	***P***
Site	$6.855_{2,57}$	**0.002**	$43.024_{2,69}$	<**0.001**
Temperature	$42.542_{1,57}$	**<0.001**	$1.088_{1,69}$	0.301
Clipping	$0.064_{2,57}$	0.938	$0.616_{2,69}$	0.543
Precipitation	$37.694_{1,57}$	**<0.001**	$12.169_{1,69}$	**0.001**
Site x Temperature	$1.318_{2,57}$	0.276	$2.081_{2,69}$	0.133
Site x Clipping	$1.197_{4,57}$	0.322	$0.615_{4,69}$	0.653
Site x Precipitation	$5.007_{2,57}$	**0.010**	$0.425_{2,69}$	0.656
Temperature x Clipping	$2.002_{2,57}$	0.144	$0.628_{2,69}$	0.537
Temperature x Precipitation	$0.181_{1,57}$	0.672	$0.316_{1,69}$	0.576
Clipping x Precipitation	$1.823_{2,57}$	0.171	$2.005_{2,69}$	0.142
Site x Temperature x Clipping	$2.088_{4,57}$	0.094	$1.133_{4,69}$	0.348
Site x Temperature x Precipitation	$0.318_{2,57}$	0.729	$1.402_{2,69}$	0.253
Site x Clipping x Precipitation	$0.120_{4,57}$	0.975	$2.057_{4,69}$	0.096
Temperature x Clipping x Precipitation	$1.033_{2,57}$	0.362	$2.867_{2,69}$	0.064
Site x Temperature x Clipping x Precipitation	$1.426_{4,57}$	0.237	$0.201_{4,69}$	0.937

Table 2-7. Results of repeated measures ANOVA testing the effects of site location (AB, SK, MB) and temperature (control; warming) on decomposition of shoot (a) and root litter (b) at the three collection times.

	(a) Shoot litter		(b) Root litter	
Effect	**F_{df}**	***P***	**F_{df}**	***P***
Site	$3.897_{2,87}$	**0.024**	$44.674_{2,99}$	**<0.001**
Temperature	$30.683_{1,87}$	**<0.001**	$1.319_{1,99}$	0.254
Site x Temperature	$1.071_{2,87}$	0.347	$1.161_{2,99}$	0.317

Table 2-8. Shoot and root litter decomposition in AB, SK and MB sites as influenced by temperature. Data reported as % remaining dry mass; k-values calculated as $X_t/X_0 = e^{-kt}$ (Olson 1963); where X_0 is the initial litter mass, X_t is the mass remaining at time *t*, and *t* is the time in years. K-values for harvest of litterbags after 0.5 and 2 years of incubation are adjusted to 1 year for comparability between collection times. Values represent means ± 1SE; n=3).

Level	Remaining dry mass (%)			k-values		
	0.5yrs	1yr	2yrs	0.5yrs	1yr	2yrs
Shoot litter						
Control	76.6(±1.11)	66.1(±1.27)	49.4(±1.09)	1.08(±0.05)	0.42(±0.01)	0.17(±0.01)
Warming	79.8(±1.04)	72.0(±1.19)	60.8(±1.02)	0.91(±0.05)	0.34(±0.01)	0.12(±0.01)
Root litter						
Control	70.6(±1.98)	54.9(±1.50)	43.4(±1.30)	1.53(±0.13)	0.64(±0.02)	0.21(±0.01)
Warming	67.3(±2.00)	58.0(±1.51)	38.6(±1.31)	1.74(±0.13)	0.56(±0.02)	0.25(±0.01)

Table 2-9. Results of repeated measures ANOVA testing the effects of precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) shoot (a) and root litter (b) decomposition for Alberta after 12 and 24 months. Subscripts under F ratio indicate the numerator and denominator degrees of freedom.

	(a) Shoot litter		(b) Root litter	
Effect	**F_{df}**	***P***	**F_{df}**	***P***
Temperature	$3.260_{1,33}$	0.080	$8.355_{1,33}$	**0.007**
Clipping	$1.582_{2,33}$	0.221	$1.186_{2,33}$	0.318
Precipitation	$13.014_{2,33}$	<**0.001**	$9.217_{2,33}$	**0.001**
Temperature x Clipping	$1.260_{2,33}$	0.297	$1.251_{2,33}$	0.300
Temperature x Precipitation	$0.634_{2,33}$	0.537	$2.113_{2,33}$	0.137
Clipping x Precipitation	$0.611_{4,33}$	0.658	$1.219_{4,33}$	0.322
Temperature x Clipping x Precipitation	$0.397_{4,33}$	0.810	$0.651_{4,33}$	0.630

Table 2-10. Shoot and root litter decomposition in Alberta as influenced by precipitation and temperature. K-values (X_t/X_0= e-kt (Olson 1963); where X_0 is the initial litter mass, X_t is the mass remaining at time *t*, and *t* is the time in years. K-values for harvest of litterbags after 0.5 and 2 years of incubation are adjusted to 1 year for comparability between collection times. Values represent means ± 1SE; n=3).

	Shoot				Root			
Level	Remaining dry mass (%)		k-values		Remaining dry mass (%)		k-values	
	1yr	2yrs	1yr	2yrs	1yr	2yrs	1yr	2yrs
Drought	74.8(±2.68)	62.5(±2.16)	0.29(±0.04)	0.11(±0.01)	53.8(±2.71)	41.3(±2.41)	0.64(±0.05)	0.22(±0.02)
Ambient	68.6(±2.68)	54.7(±2.16)	0.38(±0.04)	0.15(±0.01)	48.4(±2.82)	35.2(±2.51)	0.74(±0.06)	0.27(±0.02)
Added	62.9(±2.68)	46.3(±2.16)	0.49(±0.04)	0.20(±0.01)	44.9(±3.13)	28.9(±2.79)	0.83(±0.06)	0.33(±0.02)

Table 2-11. Percent of initial total N and C for pooled shoot and root litter from each site (n=5).

Site	Shoot litter		Root litter	
	N %	C %	N %	C %
Alberta	1.41	44.45	1.25	40.35
Saskatchewan	0.99	43.75	1.16	37.42
Manitoba	0.96	42.83	1.45	39.06

Table 2-12. Results of repeated measures ANOVA testing the effects of site (AB, SK, MB), precipitation (reduced; ambient), temperature (control; warming), and clipping (no clipping; low intensity; high intensity) on C:N ratio of the decomposed shoot (a) and root litter (b) in the three collection times. Subscripts under F ratio indicate the numerator and denominator degrees of freedom.

	(a) Shoot litter		(b) Root litter	
Effect	**F_{df}**	***P***	**F_{df}**	***P***
Site	$38.920_{2,56}$	<**0.001**	$187.761_{2,66}$	**<0.001**
Temperature	$4.771_{1,56}$	**0.033**	$1.195_{1,66}$	0.278
Clipping	$1.662_{2,56}$	0.199	$0.614_{2,66}$	0.544
Precipitation	$5.874_{1,56}$	**0.019**	$0.491_{1,66}$	0.486
Site x Temperature	$0.189_{2,56}$	0.828	$0.527_{2,66}$	0.593
Site x Clipping	$0.501_{4,56}$	0.735	$0.846_{4,66}$	0.501
Site x Precipitation	$0.900_{2,56}$	0.412	$0.422_{2,66}$	0.657
Temperature x Clipping	$0.695_{2,56}$	0.503	$2.373_{2,66}$	0.101
Temperature x Precipitation	$0.295_{1,56}$	0.589	$1.661_{1,66}$	0.202
Clipping x Precipitation	$0.455_{2,56}$	0.637	$0.272_{2,66}$	0.762
Site x Temperature x Clipping	$1.020_{4, 56}$	0.405	$0.277_{4,66}$	0.892
Site x Temperature x Precipitation	$0.120_{2,56}$	0.887	$0.010_{2,66}$	0.990
Site x Clipping x Precipitation	$0.446_{4,56}$	0.775	$0.366_{4,66}$	0.832
Temperature x Clipping x Precipitation	$0.044_{2,56}$	0.957	$0.952_{2,66}$	0.391
Site x Temperature x Clipping x Precipitation	$0.224_{4,56}$	0.924	$1.777_{4,66}$	0.144

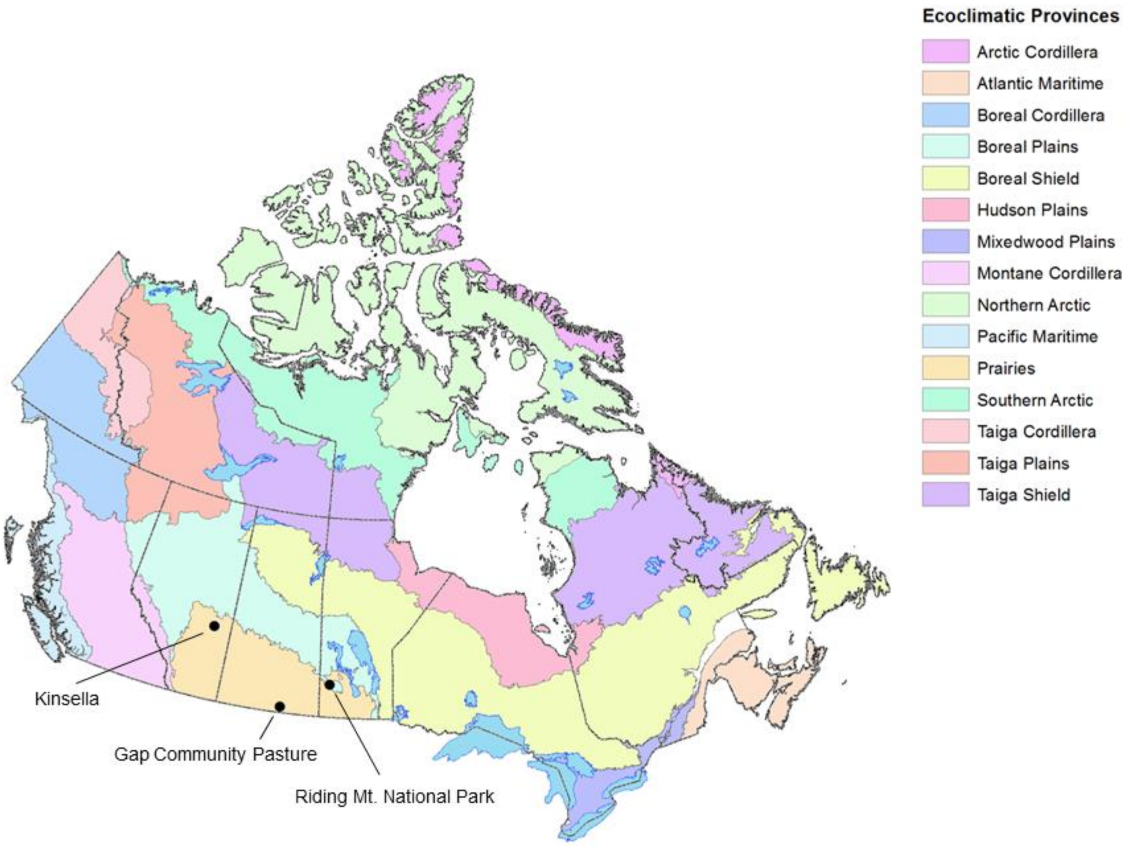

Figure 2-1. Locations of the sites and the distribution of ecoclimatic provinces of Canada.

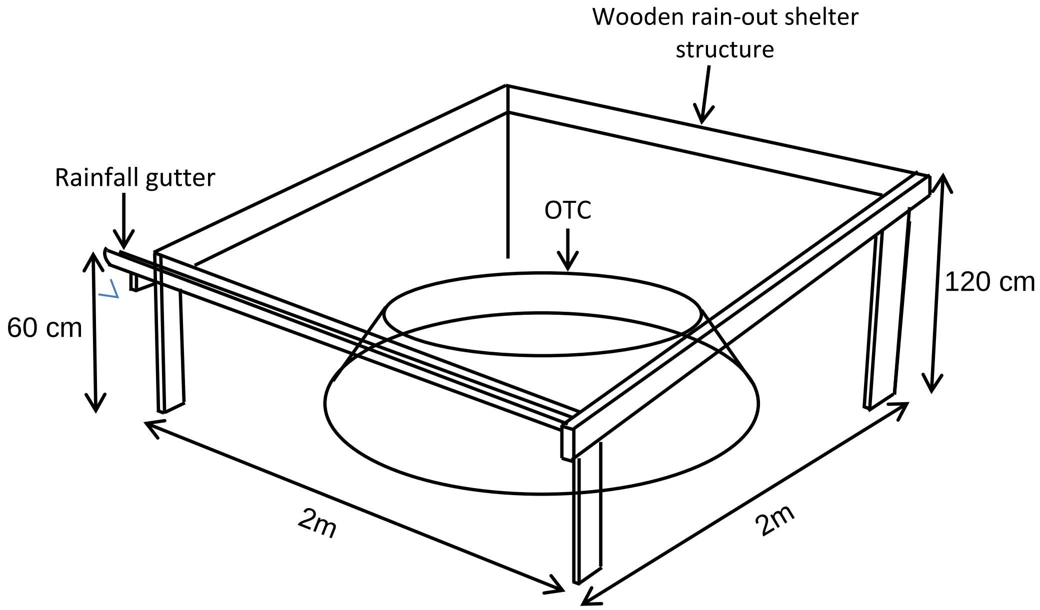

Figure 2-2. Structural design of the experimental plots showing placement of the OTC under the rain-out shelter. The rainfall gutter (in reduced precipitation plots) directed water into reservoir tanks that was subsequently reapplied to water addition plots within 24 hours after each rainfall event.

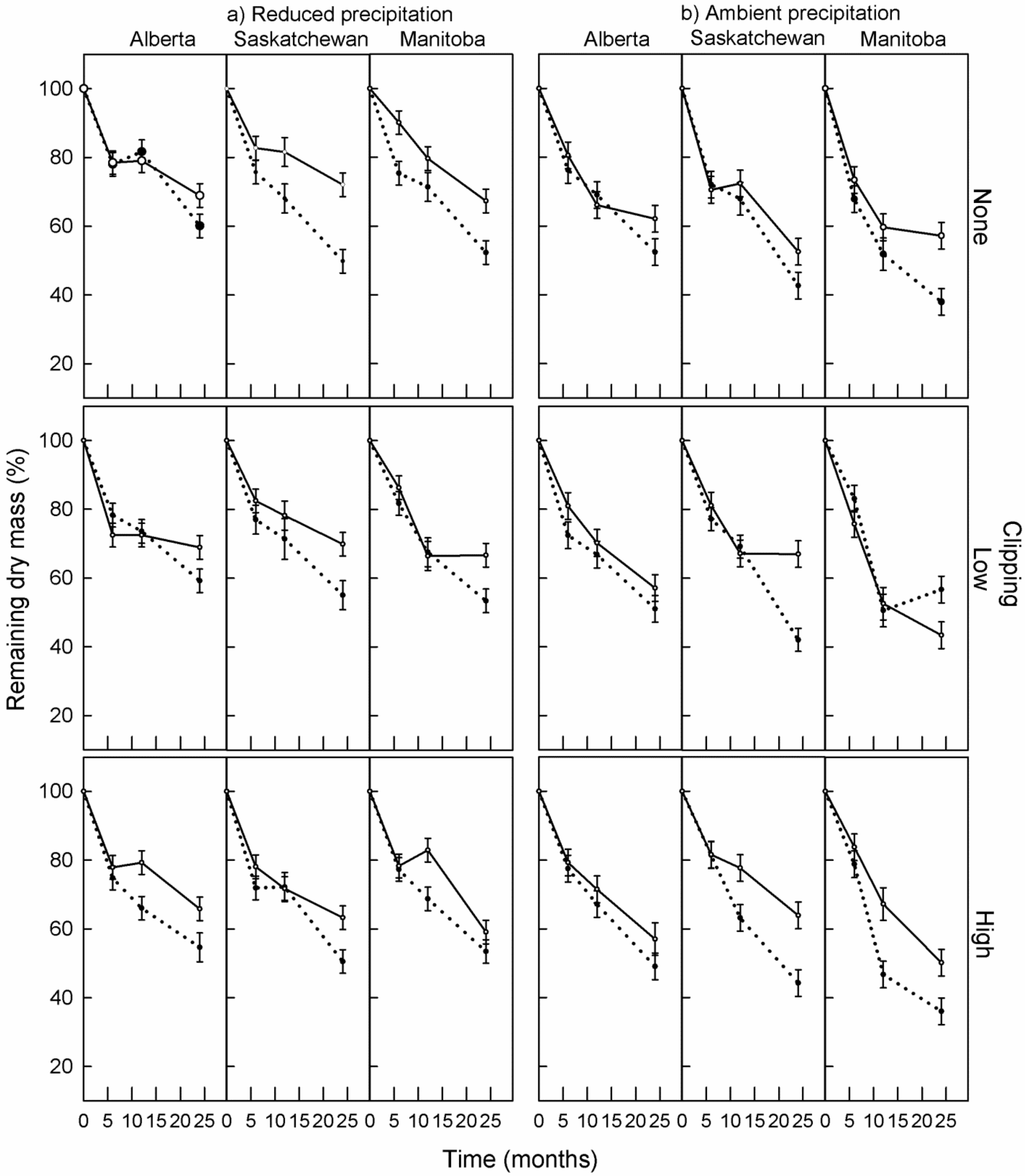

Figure 2-3. Effects of precipitation (reduced, a; ambient, b), warming (control, dashed lines; warming, solid lines), and clipping (no clipping, "None"; low intensity, "Low"; high intensity, "High") on shoot litter decomposition (% mean remaining mass ± 1 SE; n=3) for the AB, SK and MB sites at the three collection times.

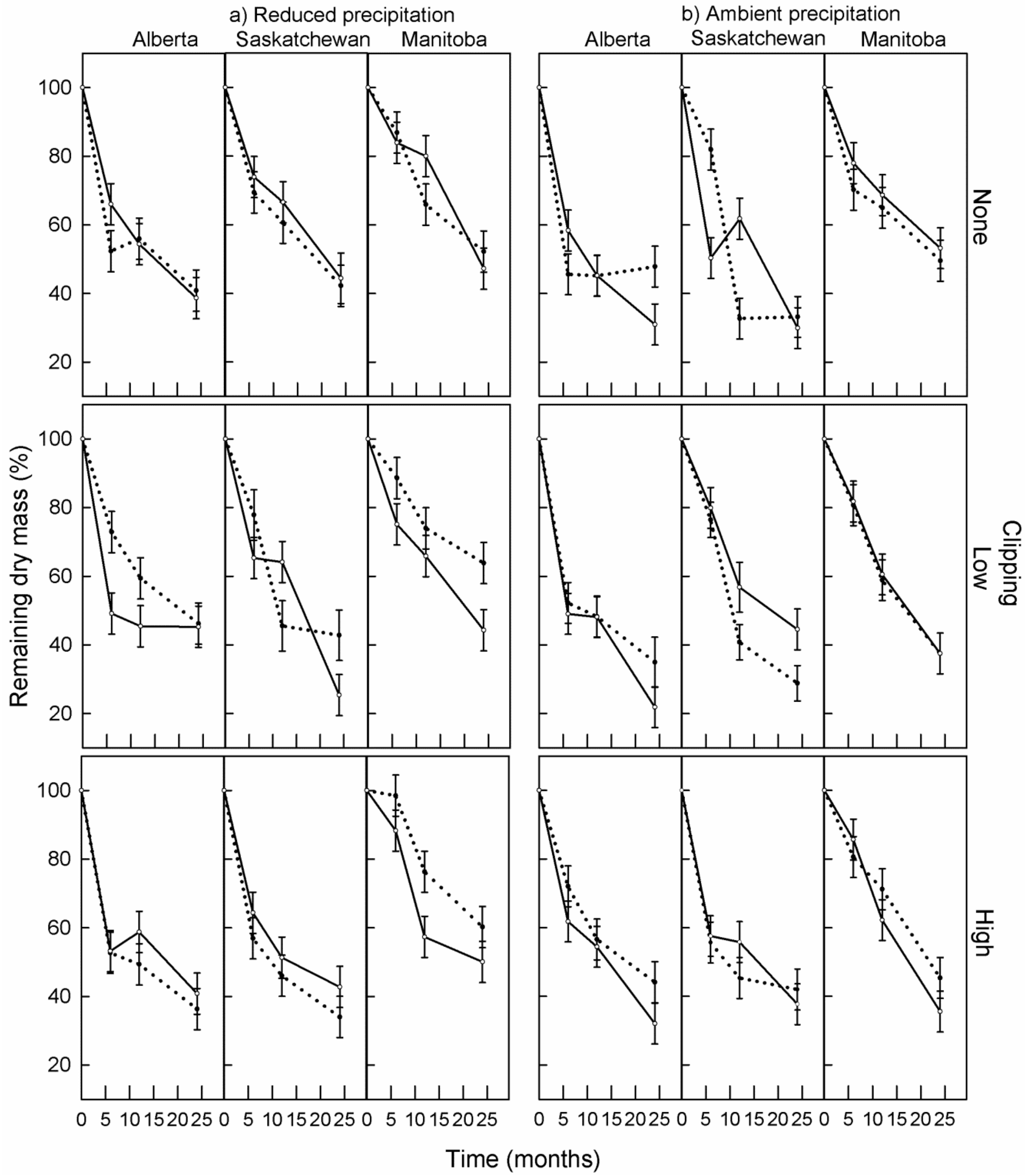

Figure 2-4. Effects of precipitation (reduced, a; ambient, b), warming (control, dashed lines; warming, solid lines), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on root litter decomposition (% mean remaining mass ± 1 SE; n=3) for the AB, SK and MB sites at the three collection times.

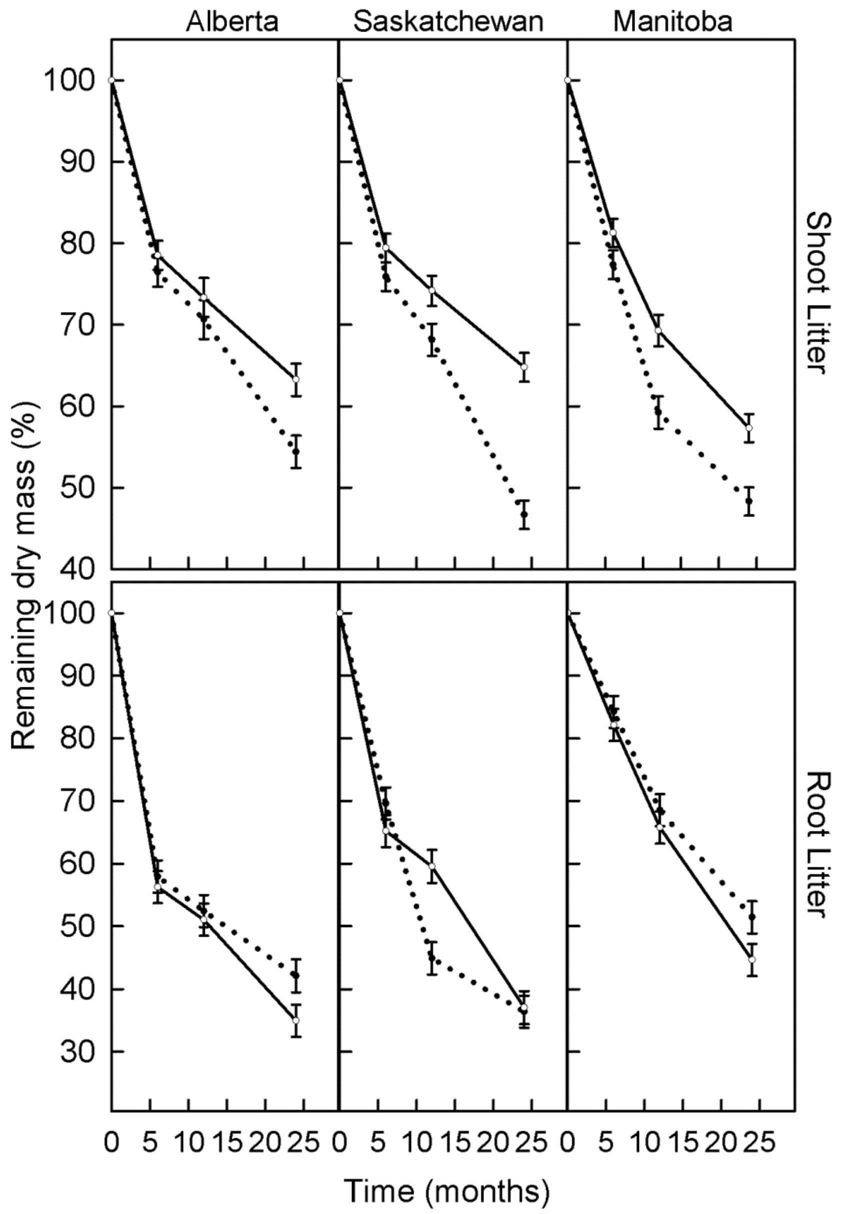

Figure 2-5. Effect of warming (control, dashed lines; warming, solid lines) on shoot and root litter decomposition (% mean remaining mass ± 1 SE; n=3) for the AB, SK and MB sites.

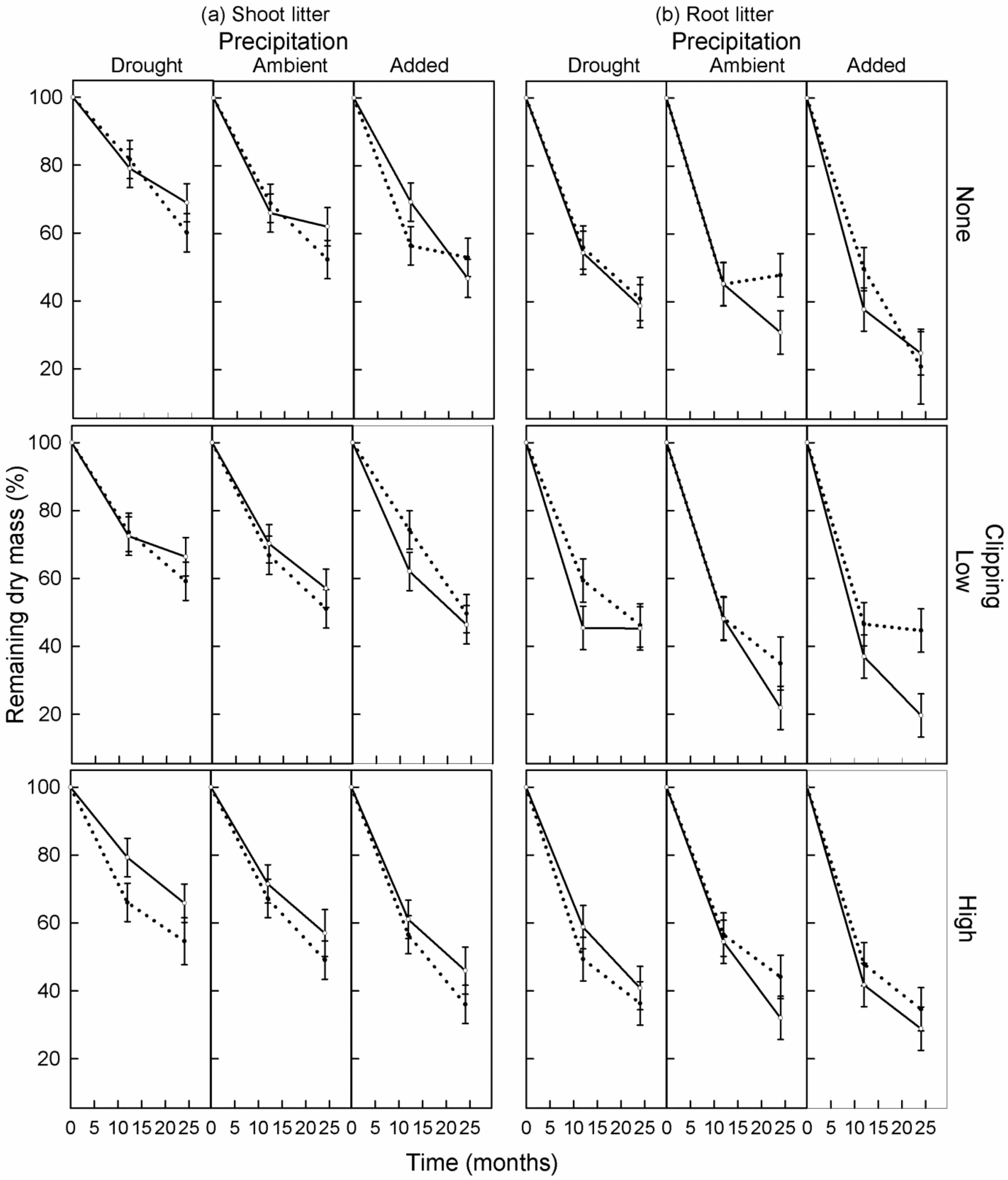

Figure 2-6. Effects of precipitation (reduced, ambient, added), warming (control, dashed lines; warming, solid lines), and clipping (no clipping, “None”; low intensity, “Low”; high intensity, “High”) on shoot & root litter decomposition (% mean remaining mass ± 1 SE; n=3) for the AB site at two collection times.

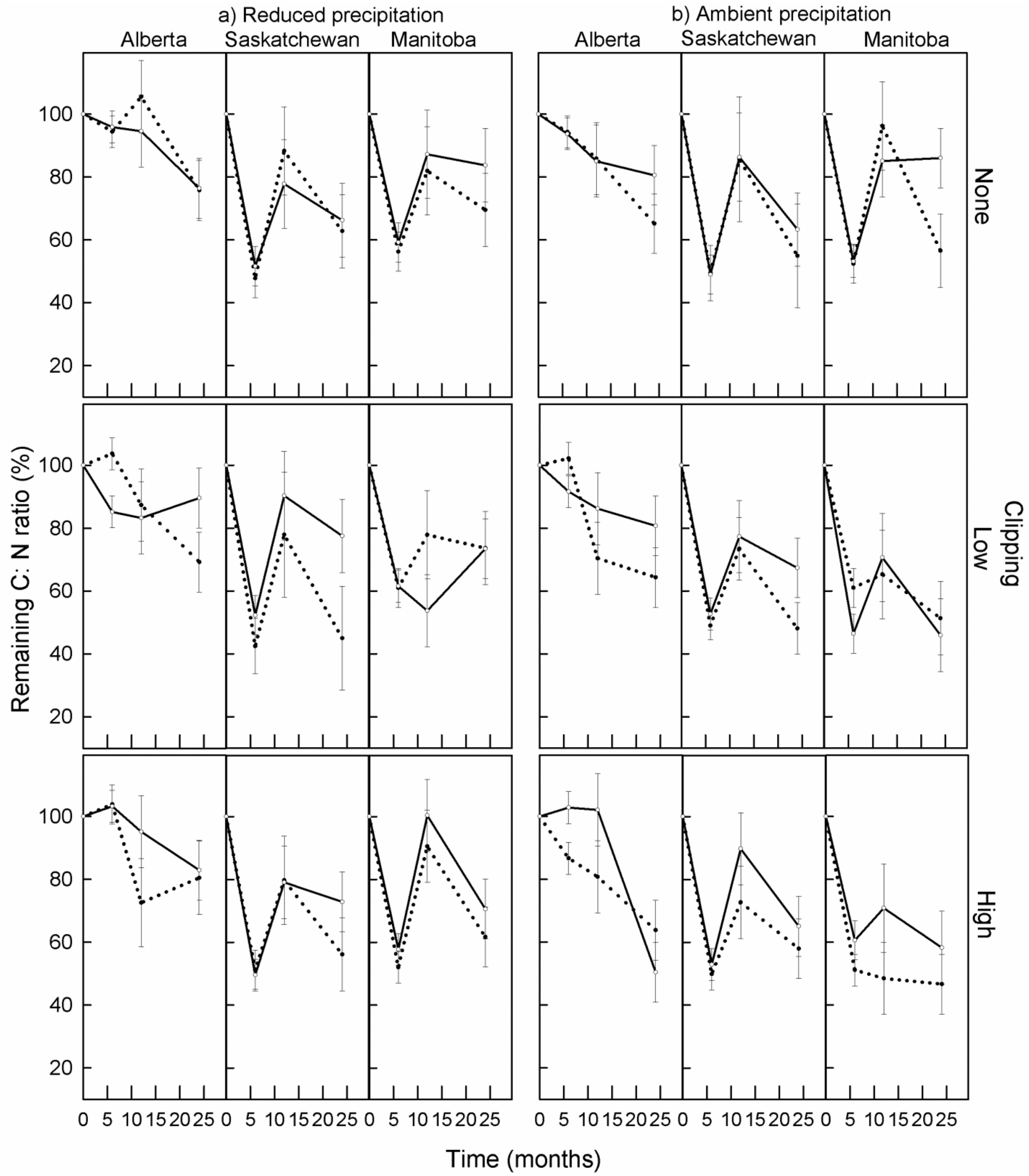

Figure 2-7. Effects of precipitation (reduced, a; ambient, b), warming (control, dashed lines; warming, solid lines), and clipping (no clipping, "None"; low intensity, "Low"; high intensity, "High") on shoot litter % remaining C: N ratio (means± 1 SE; n=3) for the AB, SK and MB sites at the three collection times.

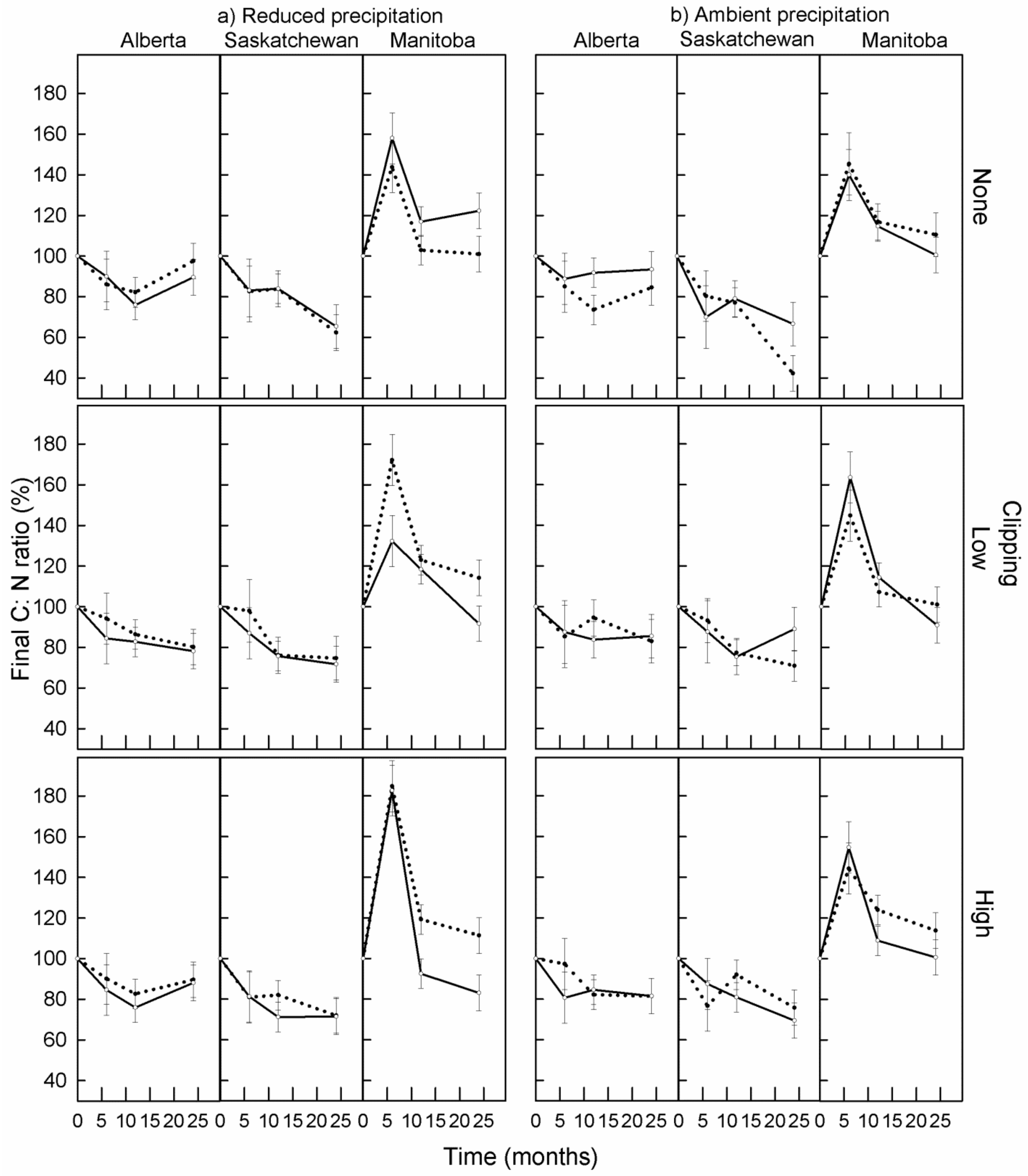

Figure 2-8. Effects of precipitation (reduced, a; ambient, b), warming (control, dashed lines; warming, solid lines), and clipping (no clipping, "None"; low intensity, "Low"; high intensity, "High") on root litter % remaining C: N ratio (means± 1 SE; n=3) for the AB, SK and MB sites at the three collection times.

Plate 2-1. Picture showing experimental plots with rain-out shelters. The one directly above has slits to allow rainfall penetration and has an OTC underneath.

Chapter 3 Litter decomposition as affected by litter quality and local conditions in a fescue grassland ecosystem

3.1. Introduction

Litter decomposition, an important process and direct source of CO2 process to the atmosphere (Couteaux, Bottner & Berg 1995; Aerts 1997), is known to be controlled by both biotic factors (e.g. litter quality and soil biota) and abiotic factors (e.g. climate and edaphic properties) (Swift, Heal & Anderson 1979). It is commonly assumed that the drivers of decomposition in decreasing order of importance are climate (mainly temperature and precipitation), litter quality (e.g. carbon: nitrogen (C: N) ratio, lignin content, chemical recalcitrance, physical protection) and decomposer communities (e.g. fungi, bacteria and other soil organisms) (Meentemeyer 1984; Lavelle *et al.* 1993; Aerts 2006). Accordingly, there is a strong influence of climate and litter quality on litter decomposition (Meentemeyer 1978; Melillo, Aber & Muratore 1982) which has led to concerns that climate change could alter decomposition rates through changes in soil temperature and moisture (Aerts 2006). For instance, it has been stipulated that climate change (e.g. global warming) could accelerate decomposition rates (Kirschbaum 1995) due to sensitivity of biological processes to temperature increase (Aerts 2006) and ultimately having feedbacks to climate (Trumbore, Chadwick & Amundson 1996; Cao & Woodward 1998). However, in grassland ecosystems, litter decomposition studies have shown variable results (e.g. Hamadi *et al.* 2000; Epstein, Burke & Lauenroth 2002; Yahdjian, Sala & Austin 2006; Bontti *et al.* 2009; Cheng *et al.* 2010). Some of these contradictions may be due to indirect effects of climate or precipitation (Aerts 2006; Yahdjian, Sala & Austin 2006), interaction between environment and litter quality (Aerts 1997; Austin & Vitousek 2000), or indirect litter quality changes through shifts of plant species composition (Shaw & Harte 2001; Li *et al.* 2011).

Litter quality by itself, particularly initial N concentrations (e.g. Meentemeyer 1978; Moore *et al.* 1999) has been proposed to be strong driver of decomposition rates and nutrient release (Swift, Heal & Anderson 1979; Couteaux, Bottner & Berg 1995), especially in systems where there is no apparent linkage of litter decomposition to climate variables. At local scales or within a climatic region, litter quality and site edaphic conditions are the primary controllers of decomposition (McClaugherty & Berg 1987; Aerts 1997; Silver & Miya 2001; Vivanco & Austin 2006; Parton *et al.* 2007). However, contradictory results of the effects of litter quality on decomposition have also been reported in grassland systems (e.g. Moretto, Distel & Didoné 2001; Semmartin *et al.* 2004; Liu *et al.* 2006; Giese *et al.* 2009). Litter quality indices that are related to decomposition in temperate ecosystems, include lignin: nitrogen ratios (Melillo, Aber & Muratore 1982) and C: N ratios (Berg, Wessen & Ekbohm 1982; Taylor, Parkinson & Parsons 1989). In general, higher quality litter (e.g. lower C: N ratio) decomposes faster promoting nutrient release (Adams & Attiwill 1982; Kochy & Wilson 1997; Aerts, van Logtestijn & Karlsson 2006) while that of lower quality decomposes slower inducing nutrient immobilization (Aber & Melillo 1991).

In temperate grasslands, litter quality varies as a consequence of two major forces: mean annual precipitation and grazing (Semmartin *et al.* 2004). Grazing is known to alter plant species composition (Milchunas & Lauenroth 1993), which inherently leads to differences in litter quality. Similarly, climatic variables exhibit comparable effects. Litter quality is inversely related to precipitation (e.g. Murphy *et al.* 2002; Bontti *et al.* 2009), whereas warming decreases plant litter quality (An *et al.* 2005; Cheng *et al.* 2010), as well as causes shifts in plant species composition (Chapin *et al.* 1995; Hobbie 1996; Shaw & Harte 2001; Luo *et al.* 2009). In a study conducted within this same experiment, found that warming and drought generally decreased herbage quality (White 2013). Additionally, warming and precipitation generally decreased and stimulated species richness, respectively. This suggests that future climatic changes may not only have direct effects on how elements cycle in grasslands (Chapter 2), but may also do so through indirect effect of species composition affecting litter quality. These shifts likely affect decomposition dynamics by changing the diversity, quality and quantity of plant litter entering the microbial decomposer system.

Despite these potential effects, studies of the interactive effects of litter quality and environmental conditions or climate are limited, and many predictions of global change focus only on the direct effects of abiotic factors (Melillo *et al.* 1993). Furthermore, little is known about how multiple climatic factors affect litter quality and the consequence of these effects on decomposition (Walter *et al.* 2013). The knowledge about how such changes in litter quality may interact with the local environmental conditions remains scarce.

In this study, I first examined how both shoot and root biomass that had simultaneously been pre-exposed to temperature and precipitation manipulations decomposed in an untreated control plot (*common site* decomposition experiment). Secondly, in an *in situ* decomposition experiment, I assessed the effects of precipitation and temperature on shoot and root biomass that was pre-exposed to these climatic factors. This approach aimed at disentangling litter quality effects and the interaction between litter quality and local conditions on decomposition and this may improve the assessment and prediction of climate change consequences on nutrient cycling.

Specific research questions

1. Does litter quality regulate litter decomposition? *Common site* decomposition – shoot and root litter of different origins i.e. different precipitation and temperature combinations were placed in the same site conditions (in untreated control plots).

2. Do litter quality and local conditions interact to regulate litter decomposition? *In situ* decomposition – shoot and root litter pre-exposed to temperature and precipitation manipulations were placed in the plots of their origin.

3.2 Materials and methods

Study site

The research was conducted over one growing season (May to October 2009) in a native grassland at the University of Alberta Research Ranch near Kinsella, Alberta, Canada (53.016539°N, 111.539898°W) located 150 km southeast of Edmonton, Alberta, within the Aspen Parkland ecoregion (Strong 1992). The landscape is characterized by undulating topography known as “knob and kettle” terrain interspersed with intermittent wetlands. The study site is savannah-type habitat, dominated by rough fescue (*Festuca hallii* [Vasey] Piper) interspersed with trembling aspen (*Populus tremuloides* Michx.) groves (Sims & Risser 2000b). Other dominant plant species include: *Hesperostipa curtiseta* A.S. Hitchc. Barkworth, *Artemisia frigida* Willd., *Commandra umbellata* L., and *Aster falcatus* Lindl. The majority of soils at the site are classified as thin Orthic Black Chernozems, well drained over a glacial till (Howitt 1988; Soil Classification Working Group 1998). The area experiences continental climate, with long-term (1971 – 2000) average annual temperature of 2.8 °C and precipitation of 431.3 mm, and long-term average growing season (May – September) temperature and precipitation of 14 °C mm and314.7 (Environment Canada, unpublished data). In 2009, the average temperature and precipitation during the growing season recorded from the nearby weather station (Viking, Alberta) was 13.8 °C and 168.9, respectively. The study site was in a 20-ha area with no history of cultivation, however, historically the site has been grazed by cattle but grazing was halted prior to the start of the experiment.

Experimental design

In May 2007, a three year manipulative experiment was established to determine the effects of temperature (warming and ambient control), precipitation (reduced, ambient, and added precipitation), and clipping (none, low and high intensity) on a suite of response variables including plant biomass responses. To account for topographic variability, 2 x 2 m plots with at least 0.5 m buffer zone were arranged in a randomized complete block design with five replicate blocks. Each block consisted of 18 treatment combination randomly assigned to plots.

However, this study was conducted in 2009, the third year of the climate change and clipping manipulative experiment and only at the AB site. Additionally, clipping treatment was not considered. Each block (n=5) for this litter decomposition experiment thus consisted of six treatment combinations (temperature =warming, ambient; and precipitation = ambient, reduced, added) randomly assigned within the blocks.

Treatments

Passive warming was achieved using open-top chambers (OTCs, Sunlite-HP Components Corporation/Kalwall Corporation, Manchester, NH, USA) that were installed directly above experimental plots. OTCs have widely been used and are a low-cost standard method of increasing air temperature in the field (Marion *et al.* 1997; Aronson & McNulty 2009). Previous studies have reported an average daily temperature increase of 1-2 °C (Marion *et al.* 1997; Klein, Harte & Zhao 2005). The OTCs used in this study were cone shaped with 2 m in diameter at the bottom and 1.6 m diameter at the top, and 40 cm high with the sides positioned at 60° angles with respect to the ground (Marion *et al.* 1997).

Plots received ambient, reduced (approximately 60% less) and added (approximately 60% more) precipitation. Rain-out shelters were used to exclude natural precipitation from the plots. They were a modification of a design by Zhou *et al.* (2006), made of wooden frames, 60 cm and 120 cm high at the back and front, respectively. Plastic tops (Dura-Film Super 4^{TM} 6-mil polyethylene film; AT Plastics, Edmonton, AB, Canada), 2.5 m x 2.5 m in size were installed directly over all plots. Reduced precipitation treatment had slits that allowed approximately 40% of ambient rainfall through and intercepted rainfall was collected by gravity, channeled through a rainfall gutter and collected in reservoir tanks. To control for any unintended effects of the rain-out structures on micro-environmental conditions, the shams shelters were also installed over ambient and added precipitation plots. Ambient and added precipitation plots had larger slits to allow complete rainfall to pass through. The water collected from reduced precipitation treatment was redistributed by hand to added precipitation treatment within 24 hours of a rainfall event. This approach increases the amount of precipitation without altering the timing or frequency of precipitation and depends on the actual rainfall event. OTCs and rain-out shelter tops were installed in May and removed in mid-October each year.

The presence of OTCs was an obstacle to impose grazing by ungulate herbivores; instead grazing was simulated by clipping the vegetation. Plots were either not clipped, or clipped in mid-summer to a stubble height of 7 cm ("low intensity") or 3 cm ("high intensity") aboveground, corresponding to 39 and 56% of total annual standing biomass in 2008, respectively (White 2013). Clipping was achieved using a lawn mower over the entire area under the rain-out shelters, except over a central 50 cm x 50 cm permanent sampling plot, which was clipped by hand to minimize disturbance. Additionally, root biomass was harvested by taking two soil cores, 5 cm diameter and 20 cm deep, washed over a 2mm sieve, dried (65 °C, 72h), and weighed. This above- and belowground biomass exposed to climate manipulations in 2008 (harvest details are provided in Chapter 2) was used as the source material for litter to study litter quality and local conditions effects on decomposition.

Litter source, incubation in common site and in situ decomposition experiments, collection and processing

Clipping, as a mimic of grazing by ungulates, has been shown to cause shifts in plant species composition (White 2013) or improves forage quality (Klein, Harte & Zhao 2007; Cheng *et al.* 2010; Walter *et al.* 2012; White 2013), which may in turn, indirectly or directly alter litter decomposition. However, in this study, biomass from low and high intensity clipping treatments was not considered, first, to reduce the complexity of the study, and second, to minimize plot disturbance of already intensely sampled plots.

Shoot and root biomass from temperature and precipitation treatment combinations were homogenized by thoroughly mixing the samples and then divided equally for *common site* and *in situ* decomposition experiments to separate the effects of litter quality from interaction effects of litter quality and local site conditions (see below).

In the first experiment (*common site decomposition experiment*), shoot and root biomass material (considered litter from here henceforth) previously exposed to climate manipulations were placed in untreated control plots to assess litter quality effects. In the second experiment (*in situ decomposition experiment),* litter pre-exposed to temperature and precipitation manipulations was incubated in the plots from which they were collected. The effect of species-specific initial litter quality on decomposition has been studied before (e.g. Moore *et al.* 1999); however, here I used a different approach. Litter material used in this study was a mixed species sample from a simulated climate change experiment, and therefore litter samples of specific treatment combinations were the same.

Three grams of shoot biomass and 0.3g of root biomass were filled in fibre glass screen bags (13 cmx 10 cm) with a mesh size of 1 mm. Negligible amounts of litter fell out of the bags during filling and transportation to the field. This pore size allows fungi, bacteria, most soil organisms to colonize the detritus (Chapin, Matson & Mooney 2002). The litter bags were deployed on May 25th 2009 and retrieved on October 6th 2009. Shoot litterbags were placed on the soil surface and held in place by steel anchor pins, whereas root litterbags were buried at 15 cm depth, the main rooting zone in this grassland ecosystem (Coupe, Stacey & Cahill 2009), to mimic the natural conditions for root decomposition.

After retrieval, the litterbags were gently scraped to remove extraneous debris, opened and the remaining contents were oven-dried (65 °C, 72h), and then weighed. Ash-free dry weight was determined to correct for soil contamination following recommendations by Blair (1988). The mass remaining of each litterbag was expressed on the basis of initial dry mass. Subsamples of initial litter material and final litter material was milled to less-than-1mm size using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and sent to University of Alberta Biological Sciences Biogeochemical Analytical Service Laboratory to determine nutrient concentrations. Litter C and N concentration was determined by dry combustion using a CE440 Elemental Analyzer (Exeter Analytical Inc., North Chelmsford, MA, USA).

Generally, C: N ratio is positively correlated with lignin: N, a common index of litter quality (Silver & Miya 2001), and it is a better predictor of decomposition for litter substrates with low lignin content (Taylor, Parkinson & Parsons 1989), such as grassland plant litter material (Lambers, Chapin & Pons 2008). Additionally, in the early stages of decomposition, commensurate with this short-term study, N content controls decomposition. In the later stages of decomposition other compounds such as lignin play a more prominent role (Fog 1988; Liu *et al.* 2007). Furthermore, initial litter C: N ratios in this study were less than 75 – 100, above which other indices are preferred (Heal, Anderson & Swift 1997). Final remaining litter was also corrected for soil contamination following Blair (1988).

Statistical analysis

Plant response variables included remaining shoot and root biomass, and final C: N ratio expressed as % of initial (Appendix B). Prior to analyses, the assumptions of ANOVA, normality and equality of variances, were tested and only final C: N ratio for root litter was log transformed to meet these assumptions. To test the effect of litter quality and site conditions on mass loss and C and N dynamics, analyses of variance (ANOVA) were separately performed for *common site* and *in situ* experiments using the mixed model procedure in IBM SPSS Statistics for Windows (*version 19.0.,* Armonk, NY: IBM Corp.). The models included temperature and precipitation as fixed factors, and block as a random factor.

3.3. Results

Common site decomposition

The effect of precipitation on shoot litter mass loss was highly significant (Table 3-2). Overall, % remaining mass under drought, ambient and the added precipitations regimes were 68, 61 and 59%, respectively. In addition, final C: N ratio, an index of decomposability, was significantly higher under drought conditions (Appendix B, Table B-1and Figure B-1). These findings suggest that quality of litter from the previous year herbage harvest was influenced by pre-exposure to precipitation and temperature manipulations, and these changes regulate the following year shoot litter decomposition. The results further suggest that poor litter quality under drought conditions result in slower shoot litter decomposition.

On average, root litter previously exposed to high moisture treatments resulted in lower root decomposition in comparison to those pre-exposed to drought treatment (Figure 3-2). Percent remaining root litter mass was 18 and 4% lower than ambient and added precipitation treatments, respectively, in comparison with reduced precipitation. This result suggests that higher precipitation in the previous year reduced root litter quality that resulted in lower decomposition. Litter pre-exposed to increased precipitation treatment generally had the lowest initial C: N ratio (Table 3-1).

In situ decomposition

Drought significantly reduced shoot litter decomposition after 4 months of incubation (Table 3-2). Generally, added precipitation increased mass loss by 55%, suggesting that increased moisture hastens decomposition for shoot litter of higher quality. Warming significantly interacted with drought to further reduce mass loss for shoot litter. Under reduced precipitation, % remaining mass was 8% greater under warmed conditions compared to control (Figure 3-1). These findings suggest that drought compounds the effect of poor litter quality, further resulting in slower decomposition. On the other hand, increase moisture and warming increased mass loss.

Litter quality and environmental conditions (temperature and precipitation) altered root decomposition. Warming generally increased root decomposition by 10%, implying that root litter quality alteration as a result of pre-exposure to warming, and present soil warming increase root litter decomposition. Added precipitation generally increased root litter mass loss (Figure 3-2), and consistent with this result, C: N ratio decreased significantly under added precipitation treatment (Appendix B, Figure B-2 and Table B-1). Surprisingly, root litter mass loss under ambient precipitation condition was lower compared to drought condition.

3.4 Discussion

Pre-exposure to precipitation manipulation influenced shoot and root litter decomposition in a 4 months study. As expected, shoot biomass material from drought plots were of poor quality and decomposed significantly less than those from ambient and added precipitation treatments. This finding can be explained by higher C: N ratio which leads to slower decomposition (e.g. An *et al.* 2005) or alteration of species composition (Wan *et al.* 2005; Vivanco & Austin 2006; Luo *et al.* 2009), causing changes in decomposition patterns. These effects can be attributed to phenotypic changes of plant species in the community as well as changes in plant species composition since mixed species litter was used in this study. In a study conducted with the framework of this experiment, increased precipitation stimulated herbage quality as well as species richness (White 2013), in spite of the belief that such changes take place over long time scales (Aerts 2006; Walter *et al.* 2013). Similarly, other studies have reported shifts in community composition (e.g. Yang *et al.* 2011; Hoeppner & Dukes 2012), suggesting that change of future precipitation regime may have indirect effects on litter decomposition and consequently on C and N cycling. Contrary to findings of this study, other researchers found that litter quality decreased with increasing precipitation in grasslands in North America (Murphy *et al.* 2002; Bontti *et al.* 2009) and this was attributed to the ability of plants to utilize nitrogen more efficiently under this condition (Murphy *et al.* 2002).

In the *common site* experiment, root litter decomposition patterns differed from those of shoot litter. Root biomass material exposed to the ambient precipitation treatments in the previous years had higher remaining dry mass compared to root biomass exposed to drought conditions. This indicates decreased decomposability of root biomass under these conditions, and this is supported by higher initial C: N ratio for root biomass pre-exposed to ambient and added precipitation treatments than to drought conditions (Table 3-1). Decreased root litter quality as a consequence of increased precipitation might indirectly decrease decomposition, a trend that might possibly increase C storage under future climatic scenarios.

Warming can negatively alter chemical composition of litter (An *et al.* 2005; Bontti *et al.* 2009; Cheng *et al.* 2010) or can cause shifts in composition of plant functional types that inherently have different litter quality (Aerts 2006; Klein, Harte & Zhao 2007; Luo *et al.* 2009). These alterations as a result of warming can have indirect impacts on litter decomposition (e.g. Hobbie 1996). A study conducted in the same experiment, warming by itself did not alter quality (crude protein) of grasses and forbs, the two functional groups that comprise the bulk of plant biomass in this system but interacted with drought to reduce forage quality, a measure derived from plant nitrogen (White 2013). Additionally, warming decreased species richness (White 2013); nevertheless, these changes did not translate into decreased shoot decomposition. Although there was a trend of decreased N concentrations with warming (Table 3-1), these results suggest that this changes might not have been enough to cause significant alteration in litter decomposition.

There was evidence of interaction between *in situ* conditions and litter quality changes as a result of pre-exposure to environmental manipulations. Herbage quality decreased with drought (White 2013), and the *in situ* experiment shows that those effects resulted in slower decomposition suggesting that drought has compounding effects on decomposition. On the other hand, added precipitation increased mass loss, and this result was not surprising since litter from the added precipitation treatment generally had the highest initial N concentration (Table 3-1). Similar to this finding, Liu *et al*. (2006) found that decomposition of higher quality litter was controlled by soil moisture regimes than nutrient availability which was a determinant of low litter quality decomposition.

In this study, warming significantly interacted with drought to further reduce *in situ* shoot litter decomposition, implying non-additive effects of these factors in relation to decomposition. Drought conditions reduce microbial activity involved in the decomposition process (Borken *et al.* 2006), and this stress is compounded by increased temperature which can promote soil drying (e.g. Shaw & Harte 2001; Gavazov 2010). These conditions further retarded the decomposition of poor quality litter (i.e. litter of higher C: N ratio). These findings show an interaction between litter quality and climate manipulation (Couteaux, Bottner & Berg 1995; Aerts 1997; Murphy *et al.* 2002), and support the notion that the effect of soil microclimate on litter decomposition depends on litter quality and the severity of climate (Fierer *et al.* 2005). Shoot litter decomposition was also enhanced by interaction between warming and increased moisture mediated through (higher) litter quality in the *in situ* decomposition experiment.

Root biomass in i*n situ* decomposition experiment responded differently to climate manipulations. Warming generally decreased litter quality, but accelerated root decomposition. This finding is not mirrored in C: N ratio that occurred in common site decomposition experiment, suggesting that local environmental condition (warming in this case) overrides the effect litter quality. Similar patterns were found with respect to the added precipitation treatment, whereby root litter decomposition increased with increased soil moisture, despite the initial N concentration being generally low in the added precipitation treatment. Consistent with this finding, root litter biomass incubated in the added precipitation treatment had the highest final N concentration, suggesting immobilization of N from soil. In contrast, litter pre-exposed to ambient precipitation had the lowest initial C: N ratio (high litter quality), but had the highest final C: N ratio suggesting N mineralization. This finding was consistent with findings from other studies that reported that poor quality litter usually immobilizes N from soil into decomposing detritus (Aerts & deCaluwe 1997; Cusack *et al.* 2009; Aubert *et al.* 2010), enhancing decomposition in the process.

The findings of this study demonstrate that predicting the implications of climate change on litter decomposition is complicated by indirect effects that these changes may have on the litter decomposition process. Warming can directly enhance litter decomposition (Kirschbaum 1995), but this effect could also decrease soil moisture consequently hindering decomposition rates (Shaw & Harte

2001; Bontti *et al.* 2009; Gavazov 2010). Warming may also indirectly alter decomposition through changes in litter quality as a result of phenotypic and species composition changes (Aerts 2006). In this experiment, clipping increased herbage quality but decreased species richness. Thus, these opposing effects suggest that litter quality should be taken into consideration when assessing the effect of climate change on litter decomposition and feedbacks to climate. Furthermore, balance between direct and indirect effects on litter decomposition may be crucial in determining whether grasslands in the short-term will be C sinks or sources. There is a need for more studies to ascertain the consequence and extent of these opposing effects

Drought reduced aboveground litter decomposition through synergistic effects, potentially increasing C storage of soil carbon. In grassland systems, decomposition of belowground material is of particular importance because most of the phytomass is belowground (Milchunas & Lauenroth 1992; Coupe, Stacey & Cahill 2009).

Root litter in this study generally decomposed more slowly than shoot litter, a trend attributed to the poor quality of root litter (Bloomfield, Vogt & Vogt 1993; Vivanco & Austin 2006; Fujii & Takeda 2010), although contradictory results have been reported, with root decomposition being faster (e.g. Moretto, Distel & Didone´ 2001) or slower (Biondini, Patton & Nyren 1998) than shoot litter decomposition. The contrasting responses of shoot and root litter pre-exposed to climatic manipulations could be important in understanding the consequences of climate change more so because research is biased towards aboveground decomposition in terrestrial ecosystems (Silver & Miya 2001) and many studies concentrate on direct effects (e.g. Melillo *et al.*, 1993). In conclusion, the short-term findings of this study show that indirect effects should be taken into account when assessing the implications of climate change on litter decomposition.

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Table 3-1. Initial shoot and root litter C: N ratio from different treatment combinations. Litter material was obtained from 2008 harvest.[1]

Material ratio	Treatment combination	C: N
Shoot	Ambient temperature, reduced precipitation	18.42
	Ambient temperature, ambient precipitation	17.73
	Ambient temperature, added precipitation	18.51
	Warming, reduced precipitation	25.41
	Warming, ambient precipitation	22.51
	Warming, added precipitation	16.96
Root	Ambient temperature, reduced precipitation	28.99
	Ambient temperature, ambient precipitation	33.89
	Ambient temperature, added precipitation	36.81
	Warming, reduced precipitation	39.06
	Warming, ambient precipitation	27.29
	Warming, added precipitation	36.54

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[1] Harvest details are provided in Chapter 2

Table 3-2. Mixed model ANOVA results for the effects of temperature and precipitation on *common site* (litter quality) and *in situ* (litter quality and local conditions) shoot and root litter decomposition.

		(a) Shoot litter		(b) Root litter	
Decomposition	Effect	**F_{df}**	***P***	**F_{df}**	***P***
Common site					
	Temperature	$1.449_{1,21}$	0.242	$2.559_{1,21}$	0.125
	Precipitation	$8.126_{2,21}$	**0.002**	$9.553_{2,21}$	**0.001**
	Temperature x Precipitation	$1.391_{2,21}$	0.271	$1.890_{2,21}$	0.176
In situ					
	Temperature	$0.293_{1,22}$	0.593	$4.056_{1,21}$	**0.058**
	Precipitation	$94.308_{2,22}$	**<0.001**	$6.346_{2,21}$	**0.008**
	Temperature x Precipitation	$4.200_{2,22}$	**0.029**	$0.496_{2,21}$	0.617

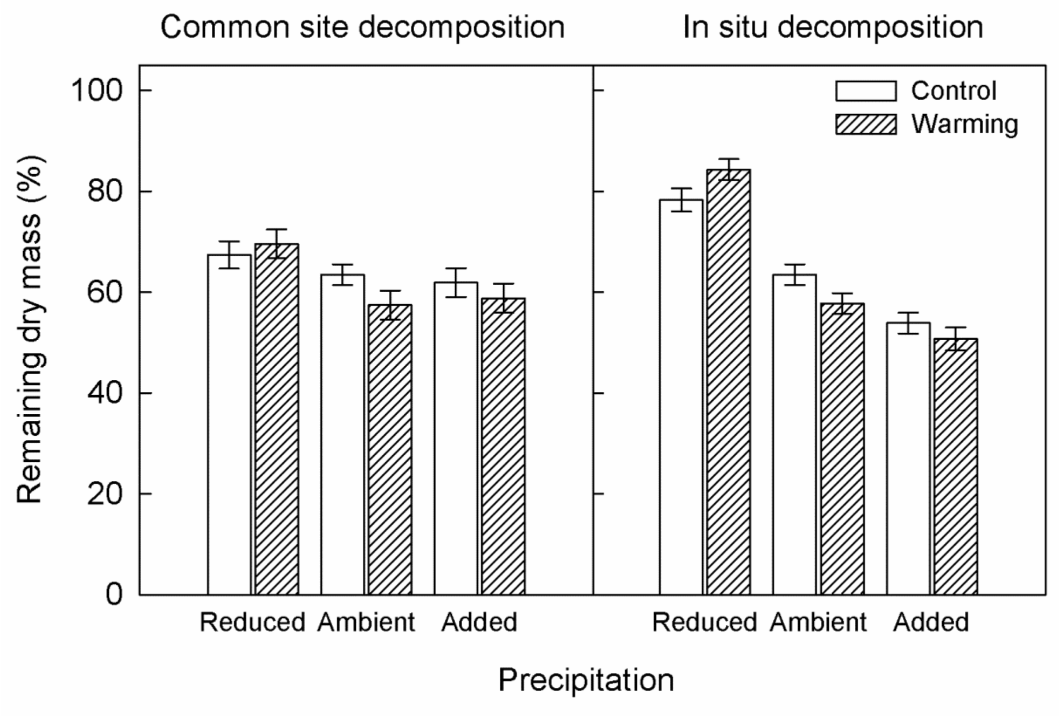

Figure 3-1. Effects of precipitation and temperature variability on mass loss after 4 months of incubation in the field of, 1) shoot litter material of different origins placed in untreated control plots (*Common site decomposition*), and 2) shoot litter materials placed in the same plots from which they were collected. The bars represent means ± 1 SE; n=5.

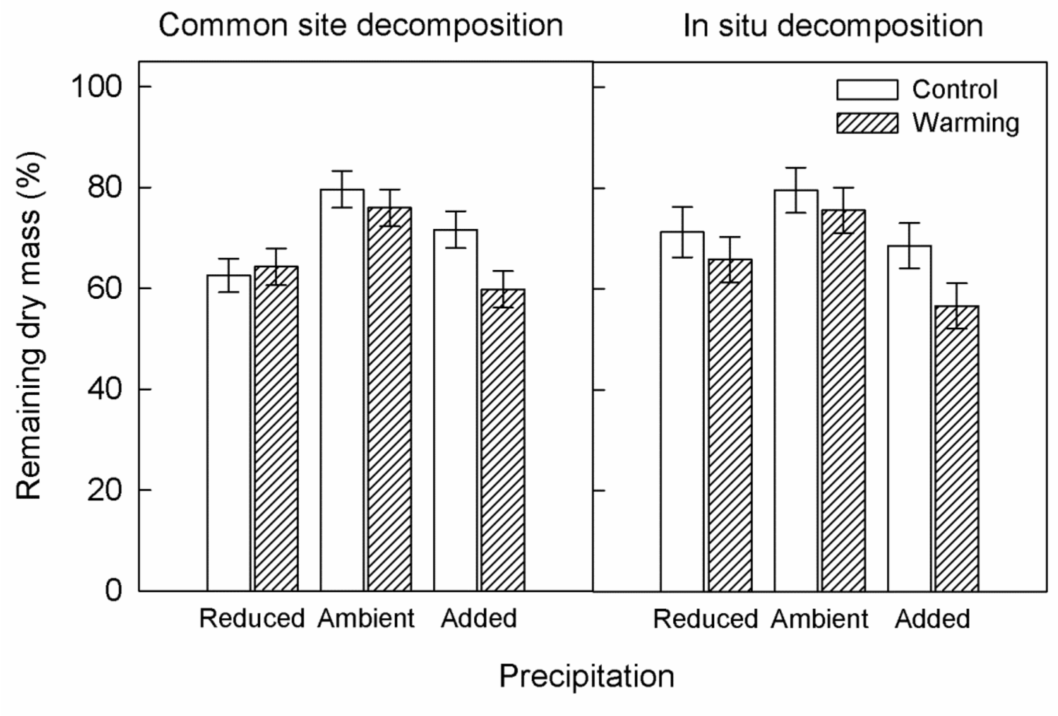

Figure 3-2. Effects of precipitation and temperature variability on mass loss after 4 months of incubation in the field for, 1) root litter material of different origins placed in untreated control plots (*Common site decomposition*), and 2) root litter materials placed in the same plots from which they were collected. The bars represent means ± 1 SE; n=5.

Chapter 4 Effect of aboveground litter on belowground plant interactions in a native rough fescue grassland[2]

4.1 Introduction

Interactions among plants can be positive or negative, each generated by a number of ecological mechanisms. For example, interference, the negative effect of one plant on the growth of another can be due to both competition and allelopathy (Harper 1961). As a result, plant removal experiments often attribute the negative effects surrounding neighbours impose on target plants to competition for limiting resources (Wilson & Tilman 1991; Cahill 1999) but ignore other factors for example root exudates. Although separating these two forms of interference is critical to understand their ecological role, they do not occur in isolation and it is nearly impossible to separate them in nature (Harper 1977; but see Weidenhamer, Hartnett & Romeo 1989; Nilsson 1994). On the contrary, it may be more useful to estimate the effect of both types of interference in plant interaction studies (Hoffman *et al.* 1996).

In addition to living plants, other factors such as plant litter are known to affect plant–plant interactions. Plant litter can directly impact the performance of plants by physically altering micro-environmental conditions (Facelli & Pickett 1991; Foster & Gross 1997), indirectly by releasing nutrients and phytotoxic substances that affect the chemical environment (Putnam & DeFrank 1983; Facelli & Pickett 1991) and influence the plant community structure (Facelli &

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Pickett 1991). Chemical interference can occur through various avenues: litter decomposition (Patrick & Koch 1958; Guenzi, McCalla & Norstadt 1967), leaching after rainwash or fog drip (Moral & Muller 1969) volatilization (Weaver & Klarich 1977) or exudation from living plant roots (Overland 1966). Evidence on the negative effects of litter leachates on germination and plant growth is extensive (Patrick & Koch 1958; Rice 1979; de Jong & Klinkhamer 1985; Zackrisson & Nilsson 1992; Bonanomi *et al.* 2006). Although, the allelopathic effects of litter are well known, to our knowledge no study has looked at the potential effects of shoot litter physically, allelopathically or both changing belowground plant–plant interactions. Chemical interference by living plants by itself may have been given more attention because it has mostly been alluded to as a mechanism by which some plants, especially exotic species, establish monocultures and reduce the diversity of local plant communities (Callaway & Aschehoug 2000).

However, overlooked in chemical ecology is the notion that chemically mediated plant interactions can be beneficial among native plants. For instance it has recently been shown that some root exudates facilitate nutrient acquisition in plants (Uren 2007), and, at least in one scenario, the chemical compound was also phytotoxic to neighbours (Tharayil *et al.* 2009). Other context-dependent interactions in plants occur, for example conditionality for competition (Connell 1983) and allelopathy (Pollock *et al.* 2009), whereby these interactions varied depending on environmental conditions. However, whether belowground chemical interactions among plants can switch from facilitative to inhibitory depending on the presence or absence of shoot litter and its potential allelopathic effects are unknown. In different studies, litter has been shown to have both, positive (e.g. Willms, Smoliak & Bailey 1986), and negative (e.g. Goldberg & Werner 1983) effects on plant growth depending on the productivity of the site while in others non-resource effects of litter e.g. phytotoxicity of decomposing residue has been reported (e.g. Putnam & DeFrank 1983). The goal of this study was to address two specific questions:

1. Does the presence or absence of shoot litter alter the outcome of root competition?

2. Does the presence or absence of shoot litter alter the outcome of belowground chemical interactions?

To accomplish this goal, I separately transplanted seedlings of naturally co-occurring target plant species into experimental plots in which I manipulated litter (intact or removed), root competition (with or without), and soil chemical environment using activated charcoal (plus or minus).

4.2 Materials and Methods

Study site and target species

The experiment was conducted in a 50-ha field at the University of Alberta Research Ranch near Kinsella, Alberta, Canada (53.016539°N, 111.539898°W), located in the Aspen Parkland Ecoregion (Sims & Risser 2000). The study site is a savanna-type habitat containing a mixture of trembling aspen

(*Populus tremuloides* Michx.) stands and rough fescue (*Festuca hallii* [Vasey] Piper) prairie. Historically cattle have grazed this area; however, grazing was halted four years prior to the start of the experiment allowing recovery of the plant community. The soils are classified as thin Orthic Black Chernozerms with thin organic-matter-enriched topsoil horizons and moderately drained, over glacial till (Howitt 1988; Soil Classification Working Group 1998).

Four native target species were selected based on their historic presence at this field site and/or because they have been reported to contain chemicals that inhibit growth of other plants (e.g. Bokhari 1978; Lipinska & Wanda 2005)). Two perennial forbs (*Artemisia frigida* Willd., *Solidago missouriensis* Nutt.) and two perennial grasses (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths, *Poa pratensis* L.) were chosen because they exhibit large differences in their competitive abilities (Cahill *et al.* 2008).

Seeds of *A. frigida*, *S. missouriensis* and *B. gracilis* were purchased from a local native seed supplier (Bedrock seeds, Edmonton, Canada), while seeds of *P. pratensis* were purchased from Apache Seeds Ltd. (Edmonton, Canada). Seeds of *A. frigida* and *S. missouriensis* were wet and cold stratified at 4 °C for 3 weeks, and sown into seedling trays filled with sterilized seedling starter (Sunshine Professional Peat Light Growing Mix, Sun Gro Horticulture Canada Ltd.) on 5 and 6 April 2009. All seedlings were grown in the greenhouse for approximately 4 weeks and then hardened to withstand field conditions by placing them outside for 2 weeks before transplanting them into the field. Similarly sized seedlings of each species were selected and transplanted (one seedling per plot) into experimental plots between 26 and 28 May 2009.

Experimental design

I established 120 1 m × 1 m plots in a 24 m × 20 m area on a dry south-facing slope at the field site. A 1 m bufferzone separated plots in each direction. Plots (30 per species) were then assigned to two litter treatments in stripes (Figure 4-1). The two litter treatments were applied in May, 2009, whereby litter was either left intact or removed from plots. Litter removal was done by hand using a garden rake, taking care to minimize plot disturbance. Plots were further divided into contiguous four 25 cm × 25 cm subplots, with each subplot randomly assigned to one of the four root competition × activated carbon treatment combinations (see below: *'Root competition and chemical interaction treatments'*). I used this split-plot design because litter removal can alter local micro-environmental conditions (Facelli & Pickett 1991), and a litter removed subplot adjacent to a litter intact subplot may not maintain the integrity of the treatments.

Plots were initially watered by hand at a rate of 7.5 L per plot every other day to assist with seedling establishment. After 1.5 weeks, the amount was reduced to 3.75 L per plot every other day. Supplemental watering ended on June 9, with the exception of two watering events in late June (3.75 L per plot) due to an extended drought. In total, each plot received 26% more water than the mean natural rainfall (190 mm) recorded for the months of June, July and August at the nearest weather station, Viking, Alberta (53.0926° N, 111.7793° W) during the same period (Environment Canada National Climate Archive; http://www.climate.weatheroffice.gc.ca).

Root competition and chemical interaction treatments

Root exclusion tubes (PVC tubes 10 cm diameter × 15 cm deep) were used to manipulate root interactions between target plants and their neighbours (e.g. Wilson 1993). Eliminating root competition using root exclusion tubes, however, does not allow full separation of the effects of chemical interference and competition. The tubes were pressed into the soil until the upper surface was flush with the soil surface (in and outside the tube), and then the soil was excavated and pulverized inside the tubes. To control for this disturbance I did this procedure in all four subplots but left the PVC tube in place for the no-root competition treatment and removed the PVC tube in the root competition treatment. Although shoot competition intensity is low compared to root competition at this field site (Lamb, Shore & Cahill 2007), I wanted to ensure that plant interactions were confined belowground. I eliminated plant neighbour shading by installing plastic deer netting (2 cm × 2 cm mesh size; 1 mm thick), to tie back neighbour shoots in all treatments. A 50 cm × 50 cm netting was centred over the plot, its centre fastened to the soil surface using steel pins, and the outer four corners anchored approximately 10 cm above the soil surface. The centre of the netting was cut (6 cm × 6 cm) to allow transplants to grow uninhibited. The netting was installed between 24 and 25June 2009. Neighbouring plants beneath the netting were pulled toward the netting edges and the integrity of this shade elimination was maintained by periodically pulling the growing neighbours beneath the netting and away from the target plants.

To alter plant chemical interactions, I used finely ground activated carbon (Reef Carbon, Kent Marine, Franklin, WI, USA). Care was taken to select a brand of carbon that did not leach phosphates. In the plus carbon treatment, I thoroughly mixed carbon at a rate of 20 mL carbon/L of soil (Callaway & Aschehoug 2000) with an excavated volume of soil (diameter:10 cm, depth:15 cm) which was equal to the volume of the root exclusion tubes (see above). Activated carbon (AC) has a high affinity for organic compounds and can reduce the negative effect of suspected allelopathic chemicals (e.g. Mahall & Callaway 1991a; Nilsson 1994). While side effects of activated carbon are a concern (e.g. Lau *et al.* 2008), activated carbon is still one of the only methods available to potentially manipulate allelopathic chemicals in the field.

Target plant harvest

All target plants were harvested in the last week of August 2009, after 13 weeks of growth. At harvest, only *Bouteloua* plants had started to flower in significant numbers, but this variable was not considered in the analyses. Shoots were clipped at the soil surface, dried at 65 °C for 72 h, and weighed. Roots of plants in minus PVC treatment were extracted but were highly intermeshed with those of neighbouring plants. Therefore, root data was not analyzed because it was not feasible to accurately assign roots to individuals (Cahill 2002).

Data analysis

Each species was analyzed separately to reduce the complexity of the statistical models. I analyzed the effects of activated carbon, root competition and litter on shoot biomass for each species using a linear mixed model procedure in R version 2.12.0 (R Development Core Team 2010) using nlme library package (Pinheiro, Bates, DebRoy, Sarkar, & the R Development Core Team 2010).

Shoot mass data for all species was tested for normality and log transformed (x + 1) prior to analysis whenever necessary. Each model included activated carbon (±), root competition (without/with), litter (intact/removed) and their interactions as fixed effects. Plots (nested in litter treatment) were included as a random variable. A significant root competition × litter interaction effect would indicate that litter influences the outcome of belowground competition. A significant carbon × litter interaction effect would also indicate that litter influences belowground plant–plant chemical interactions.

4.3 Results

Generally, *A. frigida*, *S. missouriensis* and *B. gracilis* plus activated carbon plants (+AC) had similar final shoot biomass as minus activated carbon plants (−AC), without root competition and when litter was either left intact or removed (Figure 4-2E–G). For *P. pratensis* however, −AC plants were 25% larger than +AC plants without root competition, and when shoot litter was present (Figure 4-2H). In contrast, +AC plants were 29% larger than −AC plants for *P. pratensis* in the absence of both shoot litter and neighbour roots (Figure 4-2H). However, these findings were non-significant (Table 4-1). These findings together with the similarity of final shoot biomass for +AC and −AC plants biomass in the no-competition treatments for the other three species is a strong indication, but not proof, that the fertilizing side effects of AC were limited. Root competition significantly reduced shoot biomass by 15–75% for *A. frigida*, *S. missouriensis* and *B. gracilis* focal plants (Figure 4-2A – C), and by 31–42% for *P. pratensis*, although this was not significant (Table 4-1 and Figure 4-2D). These results are a strong indication for intense root competition in this system. On average, litter removal reduced the biomass of *A. frigida*, *S. missouriensis*, *B. gracilis* and *P. pratensis* plants by 14%, 6%, 6% and 9%, respectively. The effects of neighbour removal, litter and AC were intertwined in two-way interactions described below.

Effect of litter on root competition and belowground chemical interaction

Shoot litter altered root competition and belowground chemical interaction only in *A. frigida* as indicated by a significant litter by root competition interaction, and litter by carbon interaction (Table 4-1). Average shoot biomass for *A. frigida* increased by 21% with root competition, and when litter was left intact and AC added to the soil (Figure 4-2A). This result suggests an allelopathic effect on *A. frigida* target plants from surrounding neighbours. In contrast, when litter was removed and with root competition, AC suppressed shoot biomass production by 18% (Figure 4-2A). This suggests that root chemicals potentially adsorbed by AC reduce the strength of competition, implying a facilitative effect when litter is removed. *B. gracilis* showed a similar trend when litter was removed. Adding AC decreased shoot biomass by 36% in the presence of root competition, although this was not significant (Table 4-1 and Figure 4-2C). *S. missouriensis* and *P. pratensis* showed no significant litter interactions.

Competitive effect increased from 25% when litter was intact to 42% when it was removed for *A. frigida* which suggests that litter ameliorates stress on target plants.

4.4 Discussion

Shoot litter significantly influenced the net outcome of belowground plant interactions only for *A. frigida*. With root competition, belowground interactions switched from negative (allelopathic) when litter was present (+AC plants' shoot mass was larger than −AC plants') to positive (facilitative) when litter was removed (+AC plants' shoot mass was smaller than −AC plants', Figure 4-2A). Similarly, addition of AC to the soil with root competition suppressed *B. gracilis* shoot mass production when shoot litter was removed. However, this result was non-significant. These results demonstrate context-dependency of belowground plant interactions. Although, the role that litter plays in interaction among plants has been investigated before (Ladd & Facelli 2008), to my knowledge this is the first time that an attempt has been made to link litter effects to both belowground chemical interaction and resource competition.

Interactions in plant communities may assume a positive or a negative nature depending on a number of factors including habitat type and the species involved. Negative effects of one plant on another can occur through competition or chemical interference (Harper 1961; Fuerst & Putnam 1983) . However, this tends to look at net effects, which really are combinations of potentially opposing effects. This study showed variation in belowground interaction and that conditional interference is contingent on the presence of aboveground litter, although this only occurred in a quarter of the species studied. In nutrient limited systems such as the University of Alberta Research Ranch near Kinsella (Lamb, Shore & Cahill 2007), reduced performance in the presence of AC could imply sequestration of root exudates involved in nutrient acquisition (Tharayil *et al.* 2009). Increased performance by −AC plants suggests that resource competition is mitigated by root exudates when microclimatic conditions possibly become stressful after litter removal. Positive interactions among plants under stressful conditions has been well documented (Bertness & Callaway 1994; Callaway 1995), and it is understood to generally increase with abiotic stress (Callaway *et al.* 2002). Litter in this system is likely to alter microclimatic conditions e.g. soil moisture but have little direct shading effect on surrounding plants because more than 80% of the biomass in this site is belowground (Lamb, Shore & Cahill 2007).

Although, not the focus of the present study, I found evidence for strong root competition in this system, an attribute that has been shown before (e.g. Lamb, Shore & Cahill 2007). The increased performance of plants in +AC, with root competition and intact litter treatments (Figure 4-2A) imply an allelopathic effect from litter, neighbours or both. However, this response occurs in one out of the four species tested, this could be a phenomenon that is not widespread across species.

Litter can mediate positive plant interactions by mitigating stressful environmental conditions especially in unreproductive environments (Willms, Smoliak & Bailey 1986). I found that, at least for one species (*A. frigida*), removing litter significantly increased competitive effect. Additionally, root exudates are known to mediate positive interactions in the rhizosphere through beneficial symbiotic associations with microbes such as mycorrhizae (Badri & Vivanco 2009) or the chelation of limiting minerals (see Metlen, Aschehoug & Callaway 2009 for reviews). Tharayil et *al.* (2009) found a conditional effect of phytotoxin 8-hydroxyquinoline (8HQ) from *Centaurea diffusa*; whereby its toxicity reduced when it complexed with metals. They concluded that 8HQ could primarily be an agent for resource foraging. Similarly, I show conditionality for chemical effects in *A. frigida*; the net outcome of this interaction switches from inhibitory to facilitative depending on the absence or presence of shoot litter. This implies that other factors (e.g. environmental condition) that influence the net outcome of plant interactions should be taken into consideration (Inderjit, Weston & Duke 2005). Embracing the possibility of the existence of a stimulatory-inhibitory continuum ('allelopathy phenotype') – analogous to the mutualism-parasitism continuum in arbuscular mycorrhizal fungi (Johnson, Graham & Smith 1997) – may improve our understanding of the role played by the myriad of chemical compounds that plants exude. To my knowledge this is the first time that context-dependency of chemical effects (mediated through shoot litter) has been demonstrated in plants. However, since the context-dependency of chemical effects is exhibited only in one out of four species in this study, I underscore that this is a proof of concept and not the ‘rule’. Additionally, the absolute magnitude of the results suggests that allelopathy and litter play only minor roles in this system.

To date, allelopathy research has mainly focused on negative effects of chemical compounds while tending to ignore the positive effects of plant-produced chemicals (Romeo & Weidenhamer 1998). It is known that root exudates (which include secondary metabolites such as allelochemicals) mediate both positive and negative interactions in the rhizosphere (Badri & Vivanco 2009) and are involved in vital soil processes (Zhang, Romheld & Marschner 1991). However, it has not been explicitly established whether the production of secondary metabolites could be a mechanism by which plant species facilitate each other (Metlen, Aschehoug & Callaway 2009). This observation implies that a narrow focus on negative effects may not capture the potential role that chemical compounds may play in mediating the outcome of plant–plant interactions. I believe new terminology that defines these positive ecological interactions may be necessary. Here I coin the term ‘*allelarexis*’ for this potential ecological interaction, based on the Greek roots *allelo* meaning “one another” or “reciprocal” and *arexis* meaning “help”, “aid” (Brown 1956).” Since this study used plant species that naturally coexist, I postulate that naturally coexisting plants species may have evolved mechanisms to tolerate and facilitate each other through chemicals exuded from roots. Recently, the role that positive interactions play in natural plant communities has reignited research interest (see Brooker *et al.* 2008). However, the focus is still on the traditional amelioration of stressful physical stress while ignoring the potential effects of chemical compounds. Incorporating chemical effects may shed light on an important potential mechanism that might further improve our understanding of coexistence among plants. In conclusion, the results of this study suggest a potential mechanism of positive interaction among plants that has not been given much attention in the discussions on plant facilitation. I have shown that shoot litter can significantly alter the net outcome of plant–plant interactions from inhibitory to facilitative although the size effect of this interaction is smaller compared to root competition. A similar trend was shown in *B. gracilis* though non-significant. Similar to ‘mycorrhizal phenotype’ in AMF-plant association I speculate the existence of ‘allelopathy phenotype’. This is determined by a number of factors such as plant species identity and environmental factors including litter. I have argued that this context-dependency of chemical effects, if common in nature may change how I view plant interactions and species coexistence. Finally, I suggest that chemical effects be considered in discussions about facilitation in plants.

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Table 4-1. Results of linear mixed model testing the effects of activated carbon (AC), root competition (RC) and litter (L) on target plants' shoot biomass. Degrees of freedom are numerator degrees of freedom followed by denominator degrees of freedom.

	A. frigida		*S. missouriensis*		*B. gracilis*		*P. pratensis*	
Effect	**F_{df}**	***P***	**F_{df}**	***P***	**F_{df}**	***P***	**F_{df}**	***P***
AC	$3.3184_{1, 81}$	0.0722	$2.3522_{1, 77}$	0.1292	$0.0043_{1, 74}$	0.9477	$0.2030_{1, 74}$	0.6536
RC	$3.9241_{1, 81}$	**0.0510**	$52.8566_{1, 77}$	**<0.0001**	$56.9831_{1, 74}$	**<0.0001**	$1.8836_{1, 74}$	0.1741
L	$14.0769_{1, 28}$	**0.0008**	$1.4335_{1, 28}$	0.2412	$0.1124_{1, 28}$	0.7399	$0.0000_{1, 28}$	0.9973
AC x RC	$2.3769_{1, 81}$	0.1270	$1.9151_{1, 77}$	0.1704	$0.0982_{1, 74}$	0.7549	$2.1290_{1, 74}$	0.1487
AC x L	$5.5195_{1, 81}$	**0.0212**	$1.2272_{1, 77}$	0.2714	$1.3858_{1, 74}$	0.2429	$0.0463_{1, 74}$	0.8302
RC x L	$6.7788_{1, 81}$	**0.0110**	$0.1719_{1, 77}$	0.6796	$1.8287_{1, 74}$	0.1804	$0.5603_{1, 74}$	0.4565
AC x RC x L	$3.2523_{1, 81}$	0.0750	$0.2679_{1, 77}$	0.6062	$1.1036_{1, 74}$	0.2969	$2.4537_{1, 74}$	0.1215

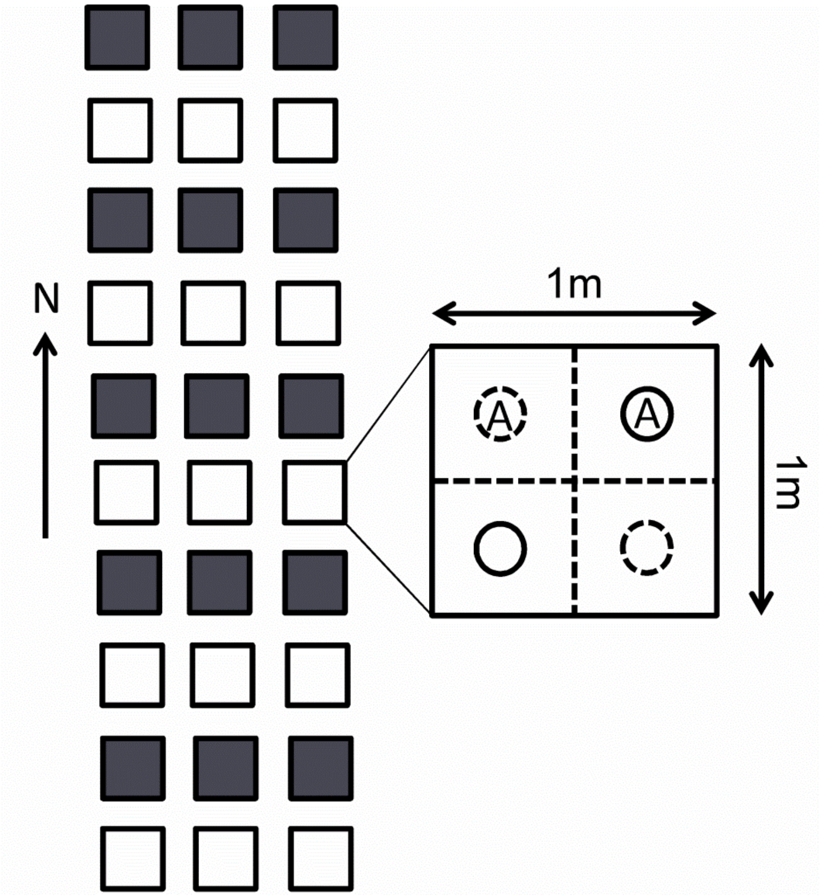

Figure 4-1. Experimental layout for each species separately. Plots were alternately assigned to two litter treatments defined by intact litter (dark squares) and removed litter (white squares). The plots were further divided into four contiguous 25 x 25 cm subplots (indicated by dashed lines) that were each randomly assigned one of the four root competition (dashed circles) x activated charcoal (AC) treatment combinations. Root exclusion tubes (PVC tubes diameter: 10 cm, depth: 15 cm) were used to manipulate root interactions between target plants and their neighbours.

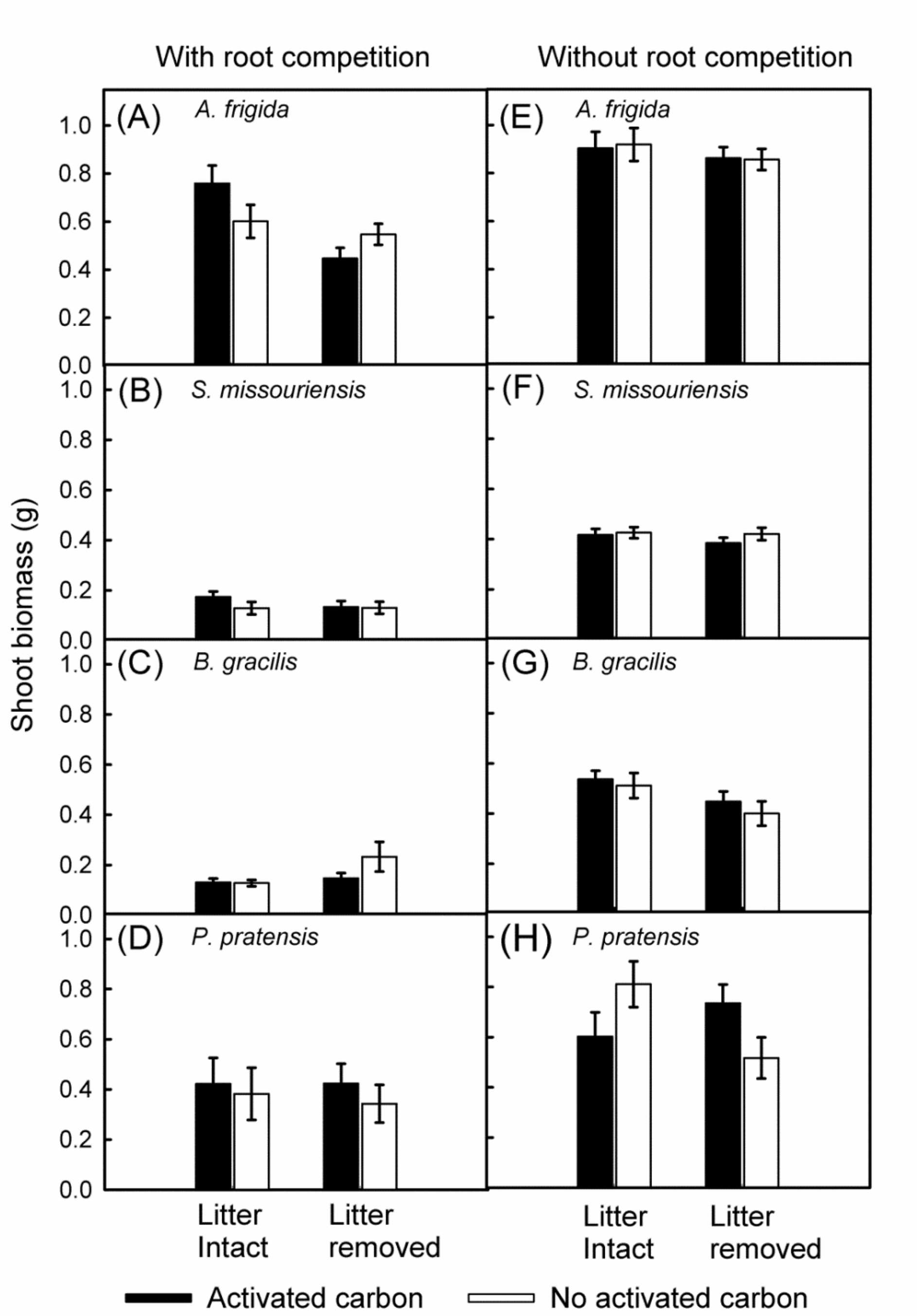

Figure 4-2. Shoot biomass (means ±SE) of *A. frigida, S. missouriensis*, *B. gracilis*, and *P. pratensis* when litter was left intact or removed, grown in soil amended with (black bars) or without (open bars) activated carbon, and with or without root competition , panels A – D and E – H, respectively.

Chapter 5 Does leachate type alter plant growth, competition and evenness?

5.1 Introduction

Roots aid plants in performing basic functions such as anchorage, water and nutrient absorption; in addition, they are also sites of complex processes such as production and release of growth regulating chemical compounds (Bertin, Yang & Weston 2003). The arrays of chemical compounds that roots exude are products of primary metabolism and/or are produced in response to both biotic and abiotic stresses. They can therefore influence plant growth directly, as well as indirectly, through interaction with surrounding organisms. The diversity of these exudates is remarkable (Bais *et al.* 2001) but they can be classified either as *excretions*, compounds without known function or byproducts of primary metabolism, or *secretions*, compounds with some known function (Bertin, Yang & Weston 2003) or organic compounds indirectly involved in primary metabolic processes (Metlen, Aschehoug & Callaway 2009). Therefore, excretions are deemed to facilitate internal primary metabolic processes, whereas secretions facilitate external processes (Uren 1988).

These root exudates, a mixture of both classes, can have positive, negative or neutral effects on rhizospheric interactions, contingent upon environmental conditions affecting the involved organisms. Exudates directly influence growth and development of surrounding organisms (Bertin, Yang & Weston 2003), enable plants to adapt to changing environmental conditions (Metlen, Aschehoug & Callaway 2009), and may also influence the growth of the same plants synthesizing them, e.g. through facilitation of nutrient acquisition (Zhang, Romheld & Marschner 1991). However, to date, belowground plant chemical ecology research has mainly focused on elucidating negative interactions e.g. the novel weapon hypothesis (Callaway & Ridenour 2004), while ignoring positive effects, likely because the former has been suggested as a mechanism that leads to a decrease in plant biodiversity (Foy & Inderjit 2001; Hierro & Callaway 2003). By far most reports on belowground positive interactions among plants have been resource-based (e.g. Callaway 1995), and if they are non-resource based, elegant examples have mostly been from aboveground interactions, such as the release of volatile compounds during herbivory as a warning signal to other plants (Baldwin & Schultz 1983; Dicke & Baldwin 2010).

Secretions that negatively affect the growth or occurrence of other plants have been termed ‘allelochemicals’ (Whittaker & Feeny 1971). However, the prolific diversity of secretions as well as other attributes such as indirect allelopathy, species-specificity of the compounds, contingency of allelopathy on soil biotic and other abiotic factors (see Inderjit & Weiner 2001), and potential dual activity of secretions (Inderjit & Duke 2003) have made it difficult to come to a consensus about which compounds are allelopathic. Furthermore, skepticism still remains whether allelopathy occurs in nature (Harper 1977; Vaughan 1991; Watkinson 1998) because field studies on this subject are limited. In an attempt to address this problem, Blum *et al.* (1993) classified organic and inorganic components as promoters, inhibitors or neutral substances, and showed that some compounds may not be classified as allelopathic but may modify the effect of allelochemicals. Berenbaum (1995) suggested that focus should be on the role that chemicals play rather than their identity or biosynthetic origin. These discussions indicate that the focus for non-resource interactions among plants should be on the effect of root exudate mixtures rather than single (known) allelopathic compound(s) and/or their direct effects, particularly in natural systems.

The function of all constituents of root exudates is not yet known because of the technical difficulties of studying plant roots which is further complicated by the degradation and/or transformation of released compounds (Inderjit & Weiner 2001). Despite these difficulties, response of plants biochemically to biotic and abiotic factors in very specific ways has been well documented. For instance, De-la-Pena *et al*. (2008) clearly showed that the composition of exudates changes in response to a particular microbial neighbor, and exudation of proteins by different microbes was triggered by a specific plant neighbour. Similarly, Kong *et al.* (2004) showed that two compounds in an allelopathic rice accession were particularly induced by the presence of another plant (*Echinochloa crus-galli).* From these examples, it can be inferred that if plants biochemically respond to particular neighbours by changing their secretion profile, it is possible that secretion profile will differ in response to environmental stress, nutrient limitation or when plants are grown alone.

The aim of this study was to test whether leachate type alters plant growth, competition, and evenness. To achieve this, I watered target plants with leachates from donor plants or control donor pots (pots containing only soil) (Figure 5-1). I speculated that ‘plant-cultured’ leachates would have effects on target plants that are not resource driven; this is because I expected a change in root exudation profile induced by donor plants. Further, to evaluate the nature of this change, I manipulated the effect of leachates using activated charcoal (AC), which is a strong adsorber of organic compounds (Cheremisinoff & Morresi 1978) and has widely been used as a tool to investigate root-mediated chemical interaction in plants (e.g. Mahall & Callaway 1991). It is expected that if leachates from donors are inhibitory, growth of recipient plants would be suppressed in minus AC treatment but if they are stimulatory growth of target plants in minus AC treatment would be enhanced. These effects could consequently affect plant competition and evenness.

5.2 Materials and methods

Experimental design

The experiment was conducted in a greenhouse at the University of Alberta, Biological Sciences Biotron facility, with a 16:8 light: dark cycle and 25 ˚C ambient temperature throughout the experiment. Soil used in all pots in this experiment was low nutrient 3:1 sand: commercial top soil mixture. DONOR plants and DONOR soil were contained in 6 inch standard pots; whereas RECIPIENT plants were grown in 4 inch standard pots (see 'Donor plant growth and treatments', and 'Recipient plant species' below). Seeds of all species were obtained from a local native seed distributor (Bedrock Seed Bank, Edmonton, Alberta, Canada) who propagates seed from local populations. Plant species used as source of leachates (hereafter DONOR plants) were: *Festuca saximontana* Rydb., *Artemisia frigida* Willd., *Aster laevis* L., *Oenothera biennis* L., and *Carex praticola* Rydb. These species were chosen based on differences in growth form, taxonomic distinctness, seed availability, and because they naturally occur in Alberta grasslands, and some species - *Artemisia frigida* Willd., and *Aster laevis*

L.- naturally co-occur in a local native rough fescue grassland (Kinsella, Alberta, Canada) where factors that structure the plant community are known. All these species are morphologically different thus were expected to compete differently (Cahill *et al.* 2008). Additionally, the difference in the quality and quantity of root exudation is also expected to be greater since the species are distantly related (Fletcher & Hegde 1995). In natural systems, plants rarely occur in monocultures, therefore including plant species from different taxa in this experiment was thought to trigger a root secretion profile similar to what is expected in nature.

Donor plants growth and treatments

Five to 10 seeds per DONOR species were equidistantly sown (5cm apart) in a concentric circle on the soil surface. After germination the plants were thinned to one similar sized plant of each species per pot resulting in five plants (species) in total per pot. Seeds of forbs (*A. laevis*, *A. frigida*, and *O. biennis*) were wet and cold stratified at 4°C for 3 weeks before sowing. For control, similar sized pots were used but they only contained soil. In half of the pots with only soil, finely ground activated carbon (Reef Carbon, Kent Marine, Franklin, WI, USA) was thoroughly mixed with DONOR soil in the plus carbon treatment at a rate of 20 mL carbon/ L of soil (Callaway & Aschehoug 2000). Activated carbon (AC) is a strong adsorber of organic compounds, such as root exudates, and is expected to have minimal effects on inorganic compounds such soil nutrients (Cheremisinoff & Morresi 1978), but see Lau *et al*. (2008). Thoroughly mixing activated charcoal in DONOR pots created an environment in which the effects of root exudates and consequently leachates were reduced. DONOR pots were fitted on wooden racks, whereby approximately 10 cm of the pots protruded above the surface of the rack (Figure 4- 2) and were watered daily throughout the experiment with an automatic drip system. Additionally, DONOR pots were alternately fertilized weekly with half strength Hoagland solution to ensure optimal growth, whereas RECIPIENT plants were not. This low fertilization ensured sufficient nutrients for plant growth for the duration of the experiment, while reducing potential unintended effects of high fertilization. Plastic funnels were attached at the bottom of DONOR pots that directed leachates dripping from them into reservoir pots below them (Figure 4-2). The collected leachates were then used to water RECIPIENT plants.

Recipient plant species

A. frigida and *A. laevis*, both common in the local grassland, and *F. saximontana* which is similar to *Festuca hallii* (Vasey) Piper, the dominant grass species in the local grassland, were chosen as the three phytometer species (hereafter RECIPIENT(s) or RECIPIENT plants) to assess the effect of leachates on plant performance. These species were chosen as phytometers because of their different growth form thus they were expected to have different competitive responses (Cahill *et al.* 2008). In addition, response to chemical effects is species-specific (e.g. Gomez-Aparicio & Canham 2008) and plants respond differently to root exudates depending on whether they are their own, from siblings or strangers (Biedrzycki *et al.* 2010). Therefore, using multiple species from different taxa may make the results more generalizable or may be extended to similar plant taxa in the local system.

RECIPIENT plants were either grown alone or in competition. In no competition pots, 5-10 seeds of *A. frigida, A. laevis*, and *F. saximontana* were separately sown in the centre of a pot and thinned to one plant per pot after germination. In competition treatments, 5-10 seeds of each of the 3 species were sown equidistantly from each other (6.5 cm apart) in the same pot. After germination, the plants were also thinned to one similarly sized plant of each species per pot resulting in 3 plant species in total per pot.

DONORs and RECIPIENTs were arranged in a randomized block design in nine blocks. Each block consisted of 4 DONOR leachate (plants, soil) by activated charcoal (plus, minus) treatment combinations that were imposed on RECIPIENT plants of each species grown with or without competition. This design eliminated confounding effects of DONOR plants sharing the same physical space with the RECIPIENT(s) which is a concern in pot experiments (see Semchenko, Hutchings & John 2007).

Harvest

Plant harvest was initiated 162 days after sowing when plants started senescing. Blocks were harvested sequentially between 22 and 29 January 2009. Shoots were clipped at the soil surface, dried (65 ºC, 72h) and weighed. Roots were washed over a 1-mm sieve, dried (65 ºC, 72h) and weighed. Roots that could not be immediately processed were frozen (including soil) until they could be extracted. These roots were then thawed before washing, dried, and weighed. It is possible to extract and assign roots to individual plants in pot experiments (e.g. Wang *et al.* 2010). However, in this experiment roots for RECIPIENT plants in competition pots were too highly intermeshed to accurately be assigned to individual plant species, and were consequently not analyzed.

Plant community structure can be characterized by its species diversity, which is defined by species richness and evenness and it can be altered by dominant plant species via competition for limited resources and/or space (e.g. Whittaker 1965; Souza, Weltzin & Sanders 2011). In this experiment, effect of leachates on community structure was measured as evenness (of shoot biomass) since all pots in competition treatments had the same number of species. Evenness was calculated as Simpson's Diversity (1/D) divided by the number of species (Smith & Wilson 1996).

Effects of activated carbon on soil nitrogen and phosphorus

The use of activated carbon (AC) as a technique to neutralize chemical exudates has been around for a long time (Schreiner & Reed 1907). AC has been a successful tool for allelopathy studies because of its large surface area and strong adsorpstive capacity to bind organic compounds (Cheremisinoff & Morresi 1978). It has minor impacts on nutrient dynamics allowing for the separation of resource completion from chemical interference (Inderjit & Callaway 2003). However, recently a number of studies have reported undesired potential confounding effects AC, including fertilization effects (Lau *et al.* 2008; Weisshuhn & Prati 2009) and disruption of plant symbioses (Wurst & van Beersum 2009). The effects of AC seem to be species-specific and depend on the type of AC used (Lau *et al.* 2008). The effect of the AC used in the experiment, Reef Carbon from Kent Marine, Franklin, WI, USA, was tested on concentration of total N and P. Since Hoagland solution was used to water DONOR plants and the resulting leachates used to water RECIPIENT plants, a comparison of total N and P content in leachates from different soil treatment and a control was performed. The treatments were: Hoagland solution (Control), Soil + Hoagland solution, Soil + Hoagland + AC solution, and Soil + Deionized water. For treatments containing soil, pots were set up following the procedure for DONOR pots the only difference is that there were no plants. There were 5 replicates per treatment.

Each pot was initially watered to saturation with 400 ml of either deionized water or Hoagland solution. The following day, an additional 200 ml was added. One week later the samples were flushed with 200 ml deionized water and the resulting leachates were filtered and collected. This a modified method described in Lau *et al.*, (2008). Total N (N µg/L) based on addition of NO_3^- + NO_2^- and NH_4^+, and total P were determined at the University of Alberta Biogeochemical Analytical Laboratory. The results of these analyses can be found in Appendix C.

Statistical analysis

Data for each species was analyzed separately to reduce the complexity of the statistical models using linear mixed model procedure in IBM SPSS Statistics for Windows, Version 20.0. (Released 2011, IBM Corp. Armonk, NY). The model for shoot biomass included donor leachates, activated carbon and competition as main effects, and block as a random factor. Root biomass, total biomass and evenness models only included donor leachates and activated carbon as main effects, and block as a random factor. Root biomass for *A. frigida* and *F. saximontana*, and total biomass data for *A. frigida* were log transformed (x+1) for normality prior to analysis.

5.3 Results

Effect of leachates on plant growth

Plants watered with soil leachates were generally larger than those watered with plant leachates. However, the effect of donor leachates was only significant for *A. frigida* shoot and total biomass produced (Table 5-1). Plants watered with potting soil leachates were 29% and 27% larger than those watered with plant leachates for shoot (in no competition treatment) and total biomass, respectively (Figures 5-3 & 5-5). This result suggests nutrient effects from DONOR soil pots or exudates from DONORs suppress plant growth.

In 2 out of 3 species tested (i.e. for *A. frigida* and *A. leavis*), when RECIPIENTs were watered with plus AC soil leachates, they were larger than those watered with minus AC soil leachates by between 19 – 28% for shoot, root and total biomass. This finding suggests a fertilization effect of AC; however, the effect of AC was insignificant. We would also expect a similar pattern when RECIPIENTs were watered with plant leachates which we do not see (Figures 5-3, 5-4 & 5-5). In no competition treatment, RECIPIENTs watered with plus AC DONOR plant leachates had similar or smaller final biomass than RECIPIENTs watered with minus AC DONOR plant leachates. This was true for *A. frigida* and *A. laevis* shoot, root and total biomass. However, the interaction between AC and donor leachates was significant only for *A. frigida* root and total biomass, and for

A. laevis total biomass (Table 5-1). Together, these findings suggest that DONOR plant leachates (potentially adsorbed by AC) have facilitative effect on recipient plants. If DONOR plant leachates were inhibitory we would expect RECIPEINTs' biomass under minus AC condition to be smaller than under plus AC.

Effect of leachates on competition

Competition highly suppressed shoot growth for all species tested, including the grass species (*F. saximontana*), which was unresponsive to other factors (Figure 5-3; Table 5-1). Additionally, in 2 out of 3 species, competition was altered by leachates, and this is supported by the significant interaction between donor leachates and competition. In general, final shoot biomass for *A. frigida* and *A. laevis,* RECIPIENTs was significantly larger when watered with DONOR plant leachates in comparison to DONOR soil leachates indicating a facilitative effect by the former leachate type (Figure 5-3). This result further supports the findings seen above (*Effect of leachates on growth*). Interestingly, 3-way interaction between AC, donor leachate and competition was significant for *A. laevis*; shoot biomass for RECIPIENTs watered with plus AC plant leachates was 29% larger in comparison to minus AC plant leachates (Figure 5-3 panel B). *A. frigida* showed a similar pattern but the result was non-significant (Figure 5-3 panel A). This observed inhibitory effect could be due to the synergistic effect of DONOR plant leachates and the exudates of the competing RECIPIENT plants.

Effect leachates on plant evenness

DONOR leachates significantly influenced evenness (Table 5-2). On average, evenness of RECIPIENT plants watered with DONOR plant leachates was 16% higher. There was no effect of AC on evenness (Figure 5-6). However, RECIPEINTs watered with plus AC plant leachates were 17% larger than RECIPIENTs watered with minus AC plant leachates. Although the interaction between AC and donor leachates was not significant (Table 5-2), this finding suggests chemical compounds exuded by DONOR plants reduce evenness.

5.4 Discussion

Effect of leachate type on plant growth

Leachate type ('plant-cultured' versus soil) had varied effects on plant growth. Leachates from DONOR soil enhanced plant growth compared to those from DONOR plants, suggesting more nutrients were available from pots devoid of plants. It is also plausible that inhibitory chemicals in DONOR plant leachates suppressed plant shoot and total biomass production. However, the non-significant effect of AC does not support the latter hypothesis. Although there was evidence that AC may have increased nutrient availability (Figure C-1), RECIPIENT plants grown alone and watered with plus AC soil DONOR leachates were larger; this effect was not consistent across leachate type. RECIPIENTs watered with plus AC DONOR plant leachates produced similar or smaller biomass as those watered with minus AC DONOR plant leachates. Weisshuhn & Prati (2009) found that adding AC to soil increased biomass of both an invasive (*Senecio inaequidens*) and a native (*Artemisia vulgaris*) plant species when grown together with a conspecific plant. In this study, similar potential effects could not be assessed because there was no conspecific treatment and a non-traditional approach in studying chemical interactions was used. To show whether a species is allelopathic, for example, one grows the phytometer (test) plant in the presence or absence of the potentially allelopathic competitor crossed with the absence or presence of AC. In this study, however, the phytometer and the plants exuding the root chemicals of interest were not grown in the same pot eliminating the potential confounding effect of soil volume. In this experiment, if AC had a fertilizing effect (e.g. Lau *et al.* 2008; Weisshuhn & Prati 2009), the expectation would be that plus AC DONOR plant leachates in the absence of competition would also increase plant biomass as a consequence of increased nutrient availability. In contrast, final shoot biomass of plants watered with both leachate types (DONOR soil and DONOR plant) was the same for the 3 species tested, whereas final root and total biomass of RECIPIENTs watered with plus AC DONOR plant leachates were smaller. The latter finding suggests that root exudates from DONOR plants facilitate the growth of plants that were grown alone despite the RECIPIENTs not grown in the vicinity of DONOR plants.

There are examples in literature that show plants can recognize other plant neighbours and adjust their secretion profile in a species-specific fashion (Kong *et al.* 2004; Badri & Vivanco 2009; Broz *et al.* 2010). However, there are no known examples of similar responses belowground as a mechanism by which neighbours are specifically facilitated (Metlen, Aschehoug & Callaway 2009). Nonetheless, it is known that root exudates can alter soil processes and microbial populations consequently affecting neighbouring plants positively (Bais *et al.* 2006). Therefore, the observed positive effects in this scenario could be a consequential response of such a process.

Effect of leachate type on competition and evenness

Competition suppressed shoot biomass but importantly it was altered by leachate type in 2 out 3 species tested. The increase in shoot biomass for *A. frigida* and *A.laevis* RECIPIENTs watered with DONOR plant leachates is indicative of stimulatory effects from plant donors that do not share physical space with target plants. In these two species, there was evidence that leachates from DONOR plants could be inhibitory in the presence of competition but the effect of AC was not significant. Evidence on plant-plant interactions that are independent of direct competition for resources is extensive (Aphalo & Ballaré 1995; Schenk, Callaway & Mahall 1999; Callaway 2002) but these are mostly about negative consequences of such interactions. Only handful of studies show tendency of root exudates conferring benefits to plant neighbours (Biedrzycki *et al.* 2010). However, in this case, the interaction was among closely related ecotypes and competition was not directly measured. Studies on positive effects of root exudates on other plants remain limited despite its potential explanation of how co-occurring plants organize themselves (Nyanumba & Cahill 2012). In this study, non-resource effects went beyond reducing competition intensity by increasing species evenness, one of the elements that define plant community structure. The role of interference mechanisms, such as resource competition and allelopathy, on community structure and plant diversity has been well documented. Allelopathy has been identified as an example of a non-resource mechanism by which some exotic plant species competitively eliminate and exclude native neighbours (Callaway & Aschehoug 2000; Hierro & Callaway

2003) consequently reducing diversity in natural plant communities (Pimentel *et al.* 2000; Foy & Inderjit 2001). Resource competition is also believed to be one of the major forces responsible for structuring ecological communities and may help explain species distribution (e.g. Hairston, Smith & Slobodkin, (1960). Specifically, it has been shown that root competition can have an indirect role in structuring plant communities via reduction in evenness (Lamb, Kembel & Cahill 2009). The slight increase in species evenness associated with plant leachates in this study indicates a reduction in the strength of competition (Lamb & Cahill 2008) and this may affect community composition. Interactions between competition and facilitation (e.g. Callaway 1995; Brooker *et al.* 2008) has fascinated ecologists for quite some time possibly making them fail to recognize the role that root exudates may play in structuring natural plant communities (e.g. positive interactions).

In summation, findings of this study suggest that non-resource interactions among plants should not only focus on negative interactions, especially in natural systems where other factors may alter the fate of released root exudates or chemical compounds. Additionally, since the activity of chemical compounds in field situations are unlikely to be due to a single chemical (Einhellig 1995), I propose that research should be geared towards understanding collective action of chemical compound mixtures that are actively secreted or passively released by roots. These overlooked indirect positive effects may be important for structuring plant communities.

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Table 5-1. Results of mixed model testing the effects of activated carbon (AC, control), competition (competition, alone), and donor leachates (plants, soil) on RECIPIENT plants shoot biomass, root biomass and total biomass. Degrees of freedom are numerator degrees of freedom followed by denominator degrees of freedom.

		Recipient plant species					
		A. frigida		*A. laevis*		*F. saximontana*	
	Effect	**F**$_{df}$	***P***	**F**$_{df}$	***P***	**F**$_{df}$	***P***
Shoot biomass	AC	$1.321_{1, 62}$	0.255	$0.499_{1, 54}$	0.483	$1.440_{1, 56}$	0.235
	Donor leachates	$4.024_{1, 62}$	**0.049**	$0.072_{1, 54}$	0.789	$0.153_{1, 55}$	0.697
	Competition	$120.496_{1, 62}$	**<0.0001**	$169.059_{1, 54}$	**<0.0001**	$10.165_{1, 55}$	**0.002**
	AC * Donor leachates	$0.488_{1, 62}$	0.487	$0.072_{1, 54}$	0.789	$0.413_{1, 55}$	0.523
	AC * Competition	$0.773_{1, 62}$	0.383	$0.797_{1, 54}$	0.376	$0.276_{1, 55}$	0.601
	Donor leachates * Competition	$7.195_{1, 62}$	**0.009**	$5.834_{1, 54}$	**0.019**	$1.264_{1, 55}$	0.266
	AC * Donor leachates * Competition	$3.671_{1, 62}$	0.060	$3.793_{1, 54}$	**0.057**	$1.398_{1, 55}$	0.242
Root biomass	AC	$1.424_{1, 24}$	0.245	$0.010_{1, 22}$	0.923	$0.024_{1, 31}$	0.878
	Donor leachates	$0.770_{1, 24}$	0.389	$0.055_{1, 22}$	0.817	$0.064_{1, 31}$	0.803
	AC * Donor leachates	$5.971_{1, 24}$	**0.022**	$3.830_{1, 22}$	0.063	$0.184_{1, 31}$	0.671
Total biomass	AC	$0.060_{1, 30}$	0.808	$0.005_{1, 22}$	0.947	$0.046_{1, 24}$	0.832
	Donor leachates	$4.719_{1, 30}$	**0.038**	$0.089_{1, 22}$	0.769	$0.204_{1, 24}$	0.656
	AC * Donor leachates	$4.418_{1, 30}$	**0.044**	$5.493_{1, 22}$	**0.029**	$0.448_{1.24}$	0.510

Table 5-2. Results of mixed model testing the effects of activated carbon (AC, control), and donor leachates (plants, soil) on evenness (RECIPIENTs shoot biomass). Degrees of freedom are numerator degrees of freedom followed by denominator degrees of freedom.

Effect	**F_{df}**	***P***
AC	$0.386_{1, 21}$	0.541
Donor leachates	$5.969_{1, 21}$	**0.023**
AC * Donor leachates	$3.290_{1, 21}$	0.084

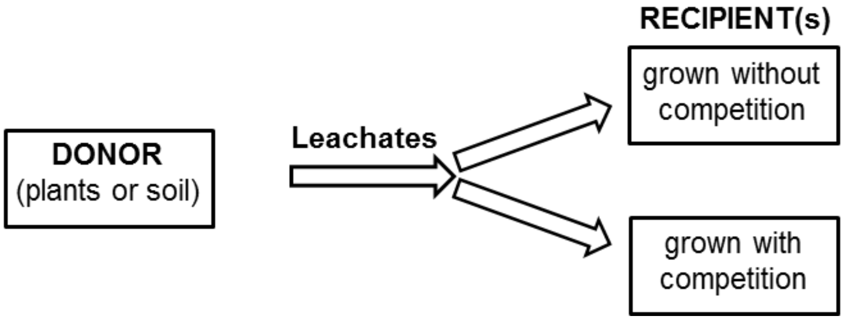

Figure 5-1. The general experimental design: leachates were obtained from plant-grown soil or soil devoid of plants, and then reapplied to recipient (target) plants grown with or without competition.

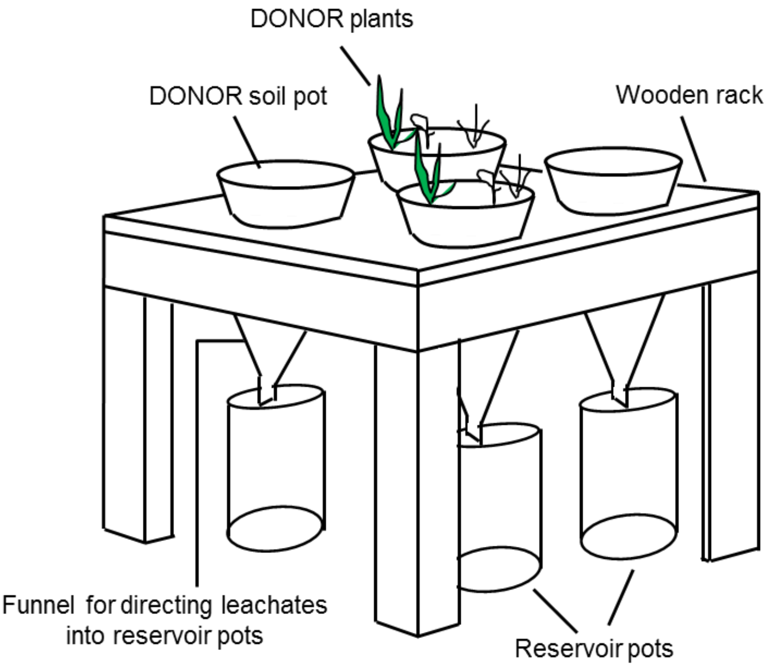

Figure 5-2. Schematic representation of experimental design. DONOR pots containing 5 plant species or only soil (control) were fitted with funnels at the bottom and placed on wooden racks above reservoir containers. Leachates collected in these reservoir containers was then used to water RECIPIENT plants.

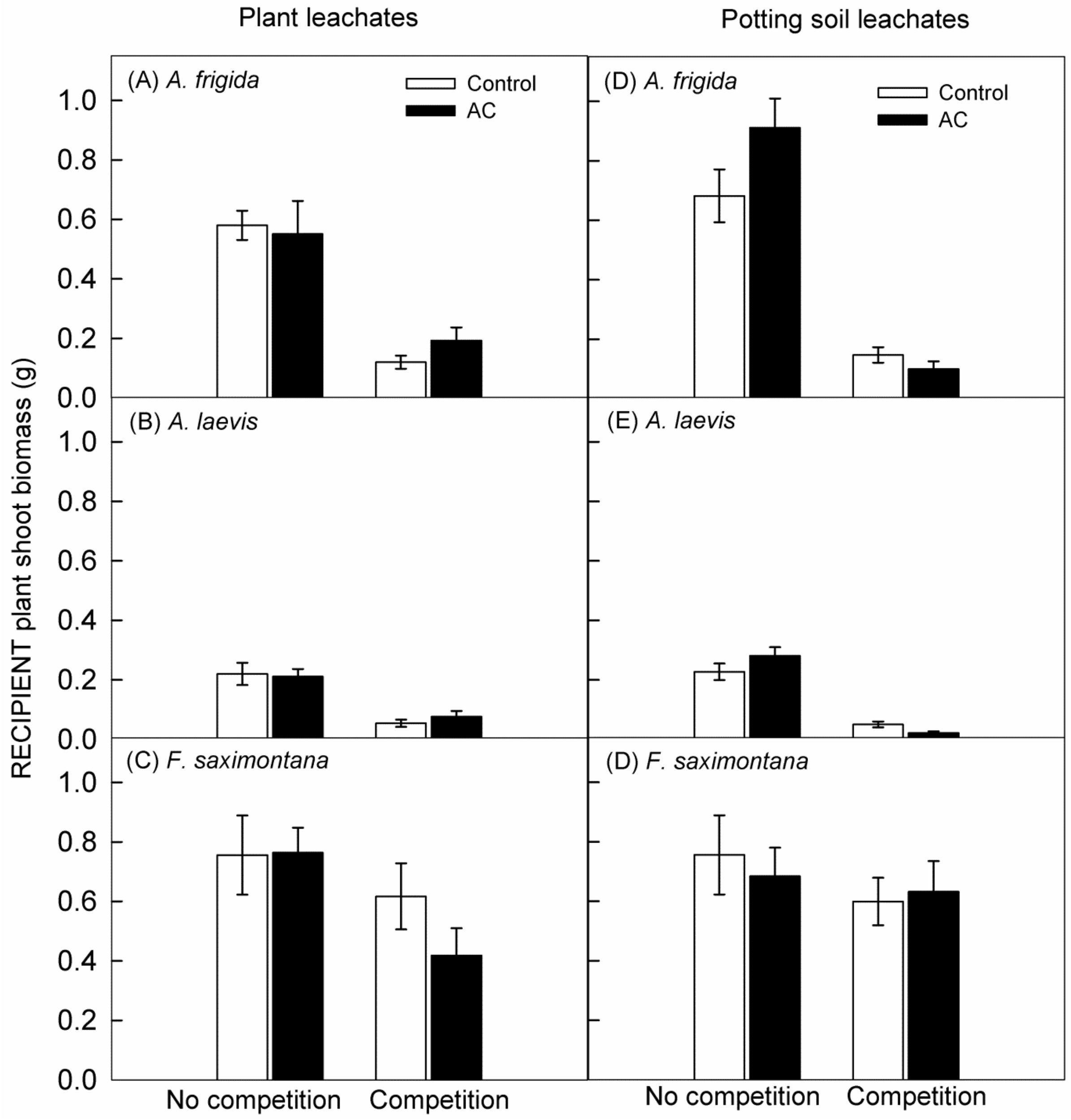

Figure 5-3. Shoot biomass (means± SE) of RECIPIENT plants grown with or without competition, and watered with plant leachates (panels A, B, C) or with soil leachates (panels D, E, F) in which DONOR soil was either treated with activated charcoal i.e. plus AC (black bars), or not i.e. minus AC (open bars).

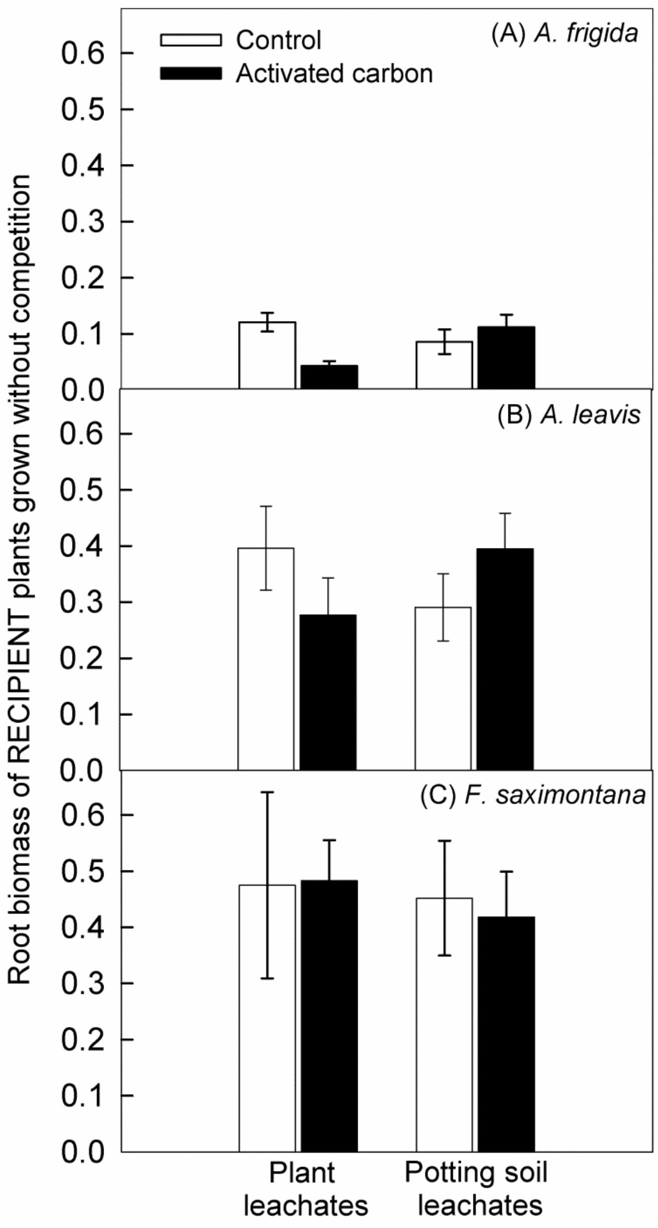

Figure 5-4. Root biomass (means± SE) of RECIPIENT plants grown without competition that were watered with plant leachates or with soil leachates in which DONOR soil was either treated with activated charcoal i.e. plus AC (black bars), or not i.e. minus AC (open bars).

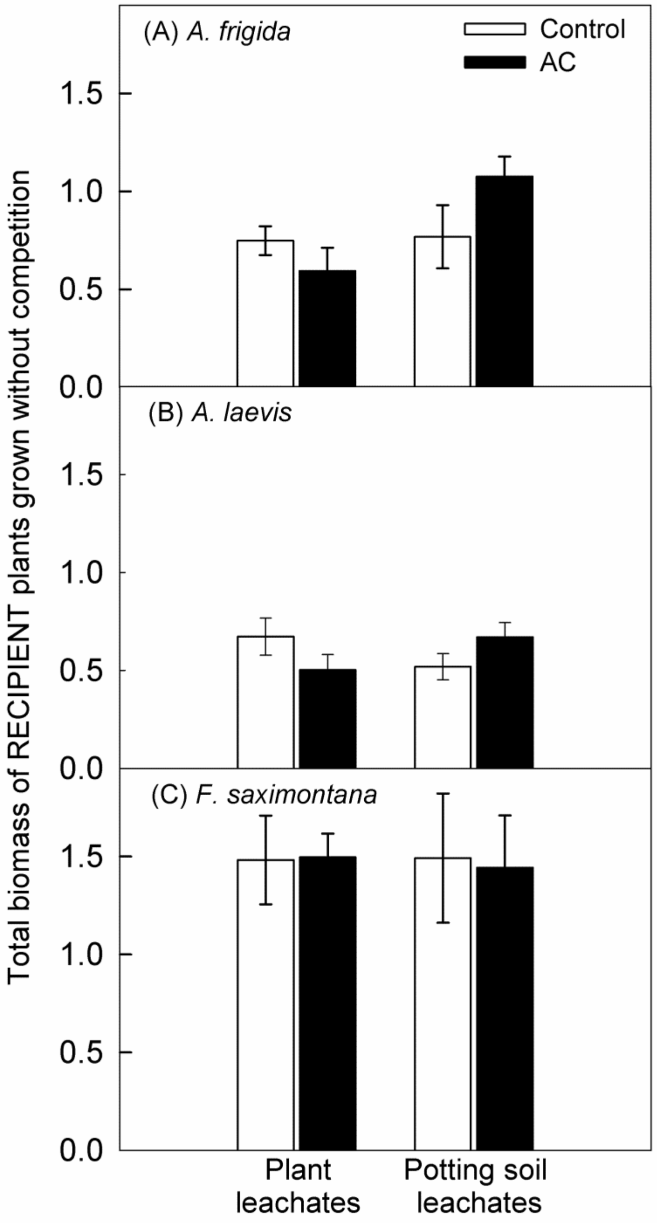

Figure 5-5. Total plant biomass production (means± SE) of RECIPIENT plants grown without competition that were watered with plant leachates or with soil leachates in which DONOR soil was either treated with activated charcoal i.e. plus AC (black bars), or not i.e. minus AC (open bars).

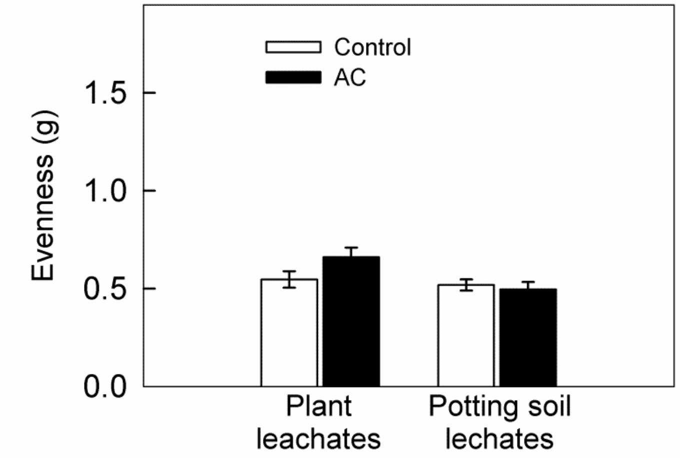

Figure 5-6. Evenness (shoot biomass means ± SE) of RECIPIENT plants that were watered with plant leachates or with soil leachates in which DONOR soil was either treated with activated charcoal i.e. plus AC (black bars), or not i.e. minus AC (open bars).

Chapter 6 General discussions and conclusions

6.1 Review and synthesis

This thesis explored somewhat varied topics: decomposition and plant-plant interactions, and the role that litter plays in these ecological aspects. Here, I present the main questions/objectives I tackled in this thesis, followed by a short synthesis of the main findings.

Research questions/questions

Chapter 2: Does litter decomposition patterns vary amongst sites, and between shoot and root litter? How are litter decomposition and the related C and N dynamics influenced by two climatic variables and land use practice (simulated grazing) across Canadian Prairie Provinces?

Chapter 3: Does litter quality (*Common site decomposition*) and interaction between litter quality and local conditions (*In situ decomposition*) regulate litter decomposition?

Chapter 4: Does the presence or absence of shoot litter alter the outcome of root competition? Does the presence or absence of shoot litter alter the outcome of belowground chemical interactions?

Chapter 5: Does leachate type alter plant growth, competition, and evenness?

I found that the sites, despite being located within the Canadian prairies, differed in their shoot and root litter decomposition patterns (Chapter 2). This finding resonates with evidence in the literature that decomposition in grasslands or terrestrial ecosystems is complex, with studies showing contrasting results (e.g. Epstein, Burke & Lauenroth 2002; Vivanco & Austin 2006; Giese *et al.* 2009). This result points to site-specific intrinsic factors or inherent interactions between factors that control litter decomposition as a cause of these differences, an element that is seldom taken into consideration in decomposition studies i.e. (e.g. Murphy *et al.* 2002; Vivanco & Austin 2006) (Chapter 3). Additionally, as expected, there were differences between shoot and root litter decomposition; k-values observed matched those found in other studies (e.g. Vivanco & Austin 2006; Giese *et al.* 2009) and were higher for root samples in this study.

In addition to these differences, the two simulated climate variables affected shoot and root decomposition differently. Increased moisture enhanced mass loss for both shoot and root litter, whereas warming retarded shoot but tended to increase root mass loss (Figure 6-1). The evidence of differences between above- and belowground litter decomposition for grassland systems is contradictory, nonetheless, considerable consequences of these differences for nutrient cycling are likely.

Conversely, direct simulated effects of clipping on litter decomposition were absent. However, there was evidence that clipping and simulated climate manipulations could influence litter decomposition indirectly through litter quality effects. Studies conducted in this experiment found alterations in plant species composition and forage quality (crude protein content) as a result of manipulated climate variables and clipping (White 2013; Figure 6-1), as well as changes in abundance and biomass of soil microarthropods (Newton 2013; Figure 6-1).

Similar alterations in litter quality have been shown to indirectly alter litter decomposition (Hobbie 1996; Cornelissen *et al.* 2007). In this study, simulated climate manipulations altered litter quality; and this change regulated decomposition. Litter pre-exposed to drought conditions had poor quality compared to ambient and the added precipitation treatments and this effect consequently hampered decomposition. I found evidence for compounding effects of climate manipulations (Chapter 3). For instance, under drought conditions, litter decomposition was further hampered when litter was placed within the plots of litter origin.

For plant-plant interaction studies, I found that aboveground litter regulated belowground interactions. The nature of the interaction switched from negative, when litter was present to positive when litter was removed (Chapter 4). Additionally, I found that root exudates enhanced plant growth, modified competition as well as evenness, one of the elements that define plant communities. Together, these results show the existence of other potential non-resource mechanisms that may play a role in the organization of natural plant communities.

6.2 Limitations, drawbacks and future directions

Climate change models and predictions are valuable for providing information and guidance for conservation of natural systems as well as formulating policies to preempt ecosystem function catastrophes. However, because processes differ from system to system as depicted by contradictory results from climate change studies, caution should be exercised when extrapolating consequences of global climate change on processes such as decomposition.

Studies considering interactive effects that include land use practices e.g. grazing on litter decomposition are rare (Semmartin *et al.* 2004; Cheng *et al.* 2010; Walter *et al.* 2013), and those that include root litter are even rarer (Silver & Miya 2001; Giese *et al.* 2009). Therefore studies, especially in grasslands, that include root samples are imperative and an understanding of how root decomposition will respond to climate change will help make proper predictions of greenhouse gases (GHGs) feedback to the atmosphere from grassland ecosystems.

Some authors have raised concerns about the difficulty of interpreting above- versus. belowground decomposition results because of the differences in initial (and subsequent) locations of these materials (Ostertag & Hobbie 1999; Gholz *et al.* 2000). Shoot/leaf litter is placed on the soil surface, whereas root litter is placed within the soil fabric, exposing it to different environmental factors and decomposer communities. This is a quagmire that will forever plague not only litter decomposition studies but plant ecology in general because of plants being simultaneously exposed to different environmental parameters. However, in some systems (e.g. grasslands), the implications of the differences between above- and belowground decomposition on nutrient cycling and C storage is significant because grasslands allocate a large proportion of their biomass production to roots (Stanton 1988).

Litter decomposition is a complex process and methods employed in studying it: mathematical models and field and laboratory experimentation have still not been able to explain the process in its entirety (McClaugherty & Berg 2008). Additionally, most studies are short-term and rely on the resulting rates to predict long-term decomposition patterns but this has proven unreliable (Prescott 2005). Negative exponential decay curve fit the first phase of decomposition but not latter phases when decomposition is slow (Aber, Melillo & McClaugherty 1990; Prescott 2005; McClaugherty & Berg 2008). It has therefore been proposed that determining the mass and nutrient content of annual litter input (e.g. Cheng *et al.* 2010), determining the maximum decomposition limit and nutrient content at that stage instead would provide reliable information on nutrient cycling and carbon storage (Prescott 2005).

I acknowledge that the use climate manipulation tools such as open-top chambers (OTCs) has its drawbacks, however, they are a low cost method to reliably manipulate temperature in the field and the use of consistent methods in similar experiments would be a step in the right direction.

Plant interactions, including belowground interactions are more complicated than in many animals, because it is hard to separate effects e.g. competition vs. allelopathy. However, advancement of technology may make this possible in some respects in the future. Pioneering work by Inderjit, von Dahl & Baldwin (2009) using transgenic, plants silenced in the production of putative chemical(s), imply that this approach can be used to achieve this end. Phenolic compounds influence the growth and development of surrounding plants and soil microorganisms (Bertin, Yang & Weston 2003) and have also been implicated to have a role in plant defense and allelopathy (Inderjit & Weiner 2001). Transgenics that do not produce these groups of compounds could be used to effectively separate resource and non-resource interactions in plants potentially replacing other tools such as the use of activated carbon in studying chemical interactions.

6.3 Conclusion

Prediction of consequences of future climate scenarios on litter decomposition is complicated by indirect effects and interactions between controlling factors of litter decomposition even within a geographic region. Nonetheless, multifactor experiments across geographical regions are needed because the use of only a few factors or only considering direct effects in predicting soil feedbacks due to climate change is inadequate (Murphy *et al.* 2002; Fraser & Hockin 2013). Interactions between climate factors e.g. warming and land use practice may have negative or positive consequences on litter decomposition because of changes in species composition (Klein, Harte & Zhao 2004) and this warrants continued investigation in the future.

From an economic perspective, climate change has the potential to negatively affect agricultural systems e.g. the Canadian Prairies, directly through changes in growing conditions (Wheaton *et al.* 2008), and indirectly through alteration of decomposition, a process that is critical for maintaining soil fertility and productivity. Little is known about climate change and land use practice effects on litter decomposition in the northern Canadian prairies. Therefore, long-term studies investigating the impact of climate change in conjunction with land use practices will increase our understanding of the response of these systems to climate change. In addition, such studies will provide vital information for mitigating potential negative impacts of such effects on biodiversity and sustainable production of grasslands.

Accurately separating mechanisms that regulate plant interactions would increase an understanding of the organization of natural plant communities. Co-occurring plant species are probably adapted to chemicals that their neighbours exude. Therefore, understanding role of these exudates on non-resource interactions could be an additional element in discussions about facilitation in natural plant communities which still focuses on resource mechanisms.

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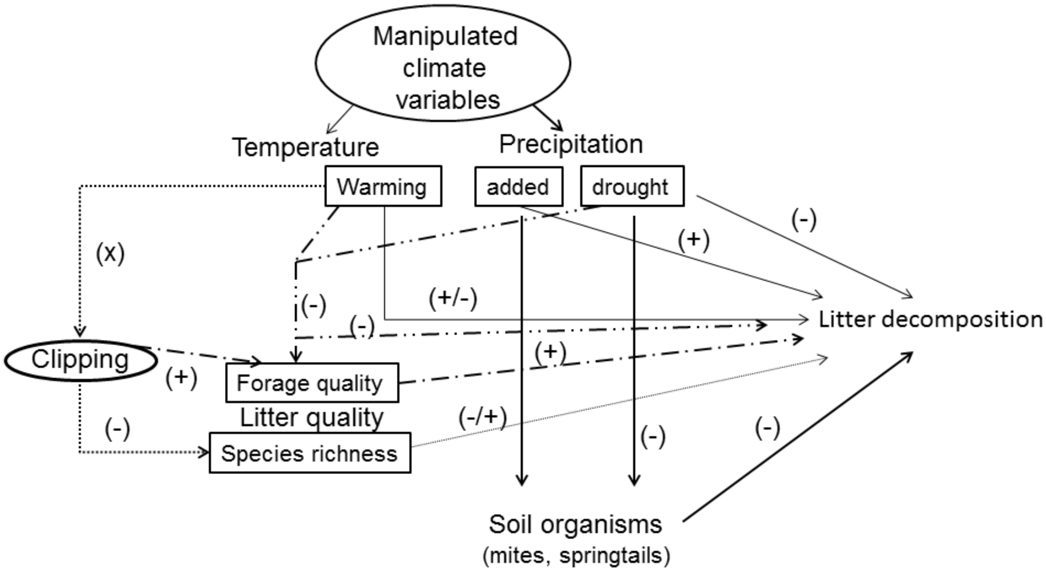

Figure 6-1. Schematic summary some of the findings in this experiment and their observed, and potential effects on litter decomposition, whereby (+), (-) and (x) means increased, reduced and interaction, respectively.

7. Appendix A: Supplemental material for Chapter 2

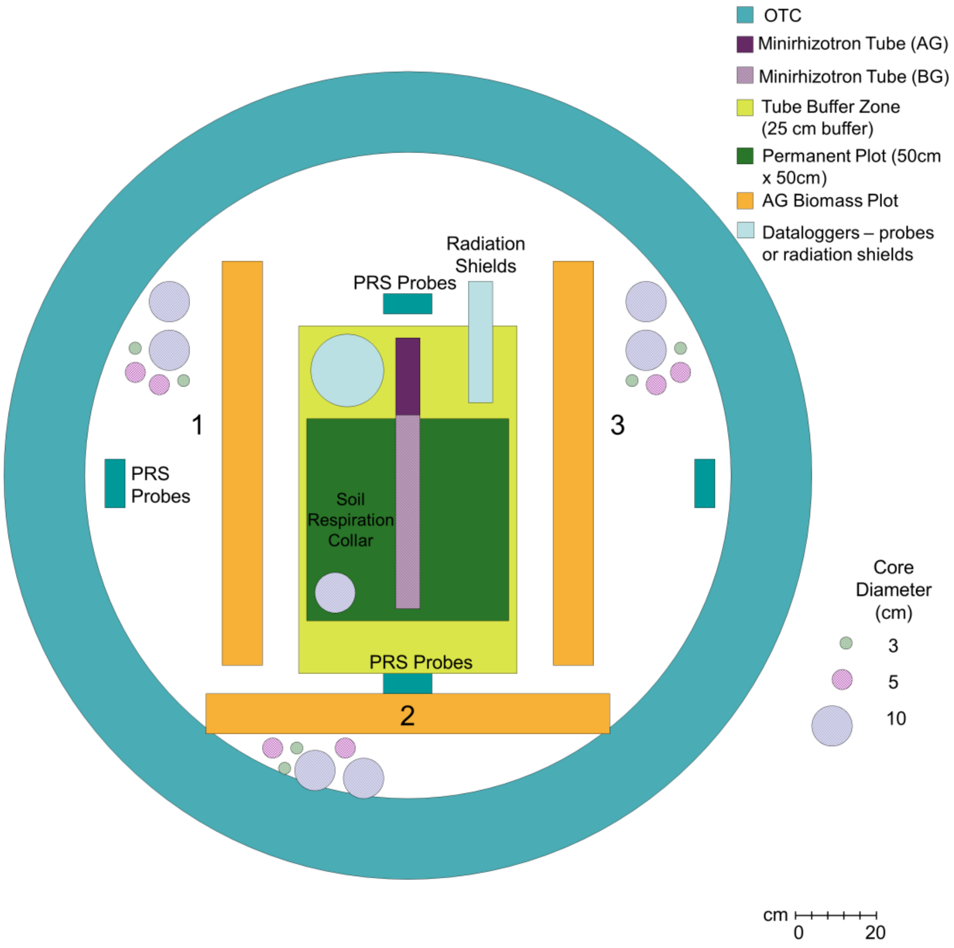

Figure A-1. Spatial plot layout and sampling diagram. The numbers represent random location for 3 year (duration of the study) rotation for destructive sampling. The intensity of activity in the experimental plots could negatively affect the outcome of responses (White et al., 2012). To minimize this risk, destructive sampling i.e. coring and clipping occurred in the same area within plots at each sampling period, reducing the overall extent of disturbance in the plots. Holes left behind were refilled with autoclaved soil from around the general area of the plots (site-specific), and their locations marked. Note: the figure shows other attributes that were measured in this study but are not discussed here.

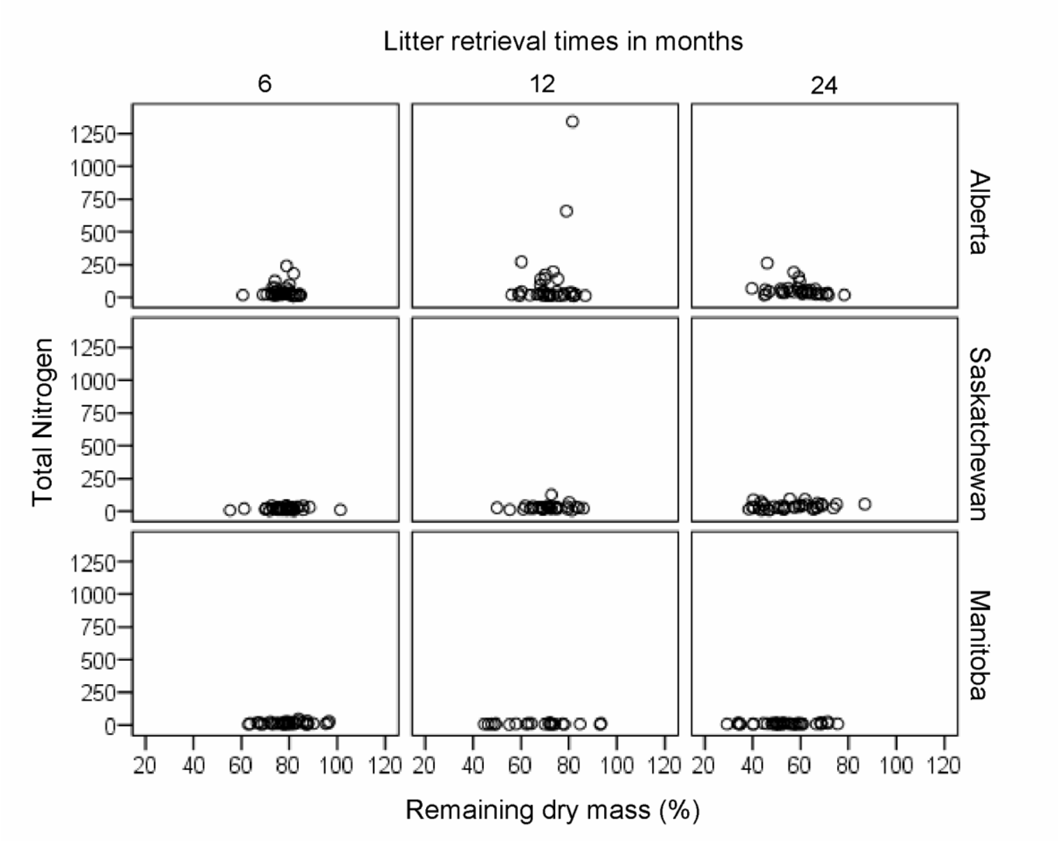

Figure A-2. Relationship between total available nitrogen and remaining shoot litter for the three litter retrieval times. Available nitrogen was measured between late June – early September and is based on total inorganic NH_4^+ and NO_3^- (mg total nitrogen/10 cm^2 ion-exchange membrane surface area/time of burial).

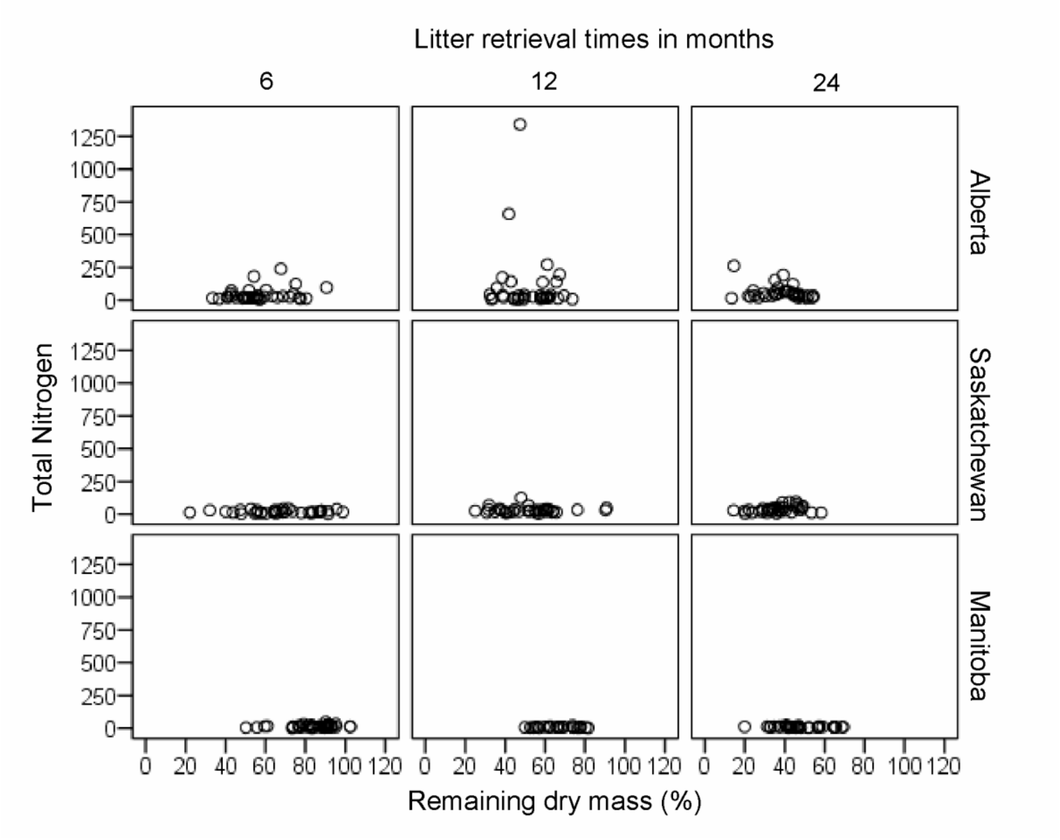

Figure A-3. Relationship between total available nitrogen and remaining root litter for the three litter retrieval times. Available nitrogen was measured between late June – early September and is based on total inorganic NH_4^+ and NO_3^- (mg total nitrogen/10 cm^2 ion-exchange membrane surface area/time of burial).

8. Appendix B: Supplemental material for Chapter 3

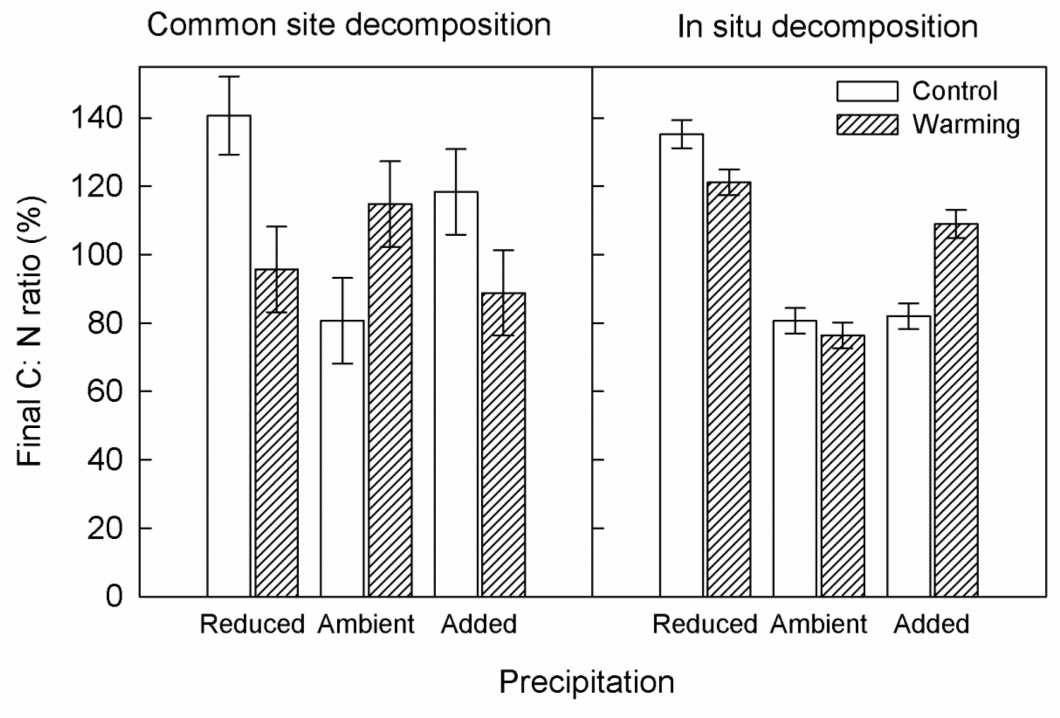

Figure B-1. Effects of litter quality (*Common site decomposition*, shoot litter of different origins were decomposed under the same site conditions/plots i.e. in control plots) and effects of local conditions and litter quality (*In situ decomposition*, shoot litter was decomposed in the same plots/sites from which they were collected) on shoot litter final C: N ratio (% mean remaining C: N ± 1 SE; n=5) after 4 months of incubation in the field.

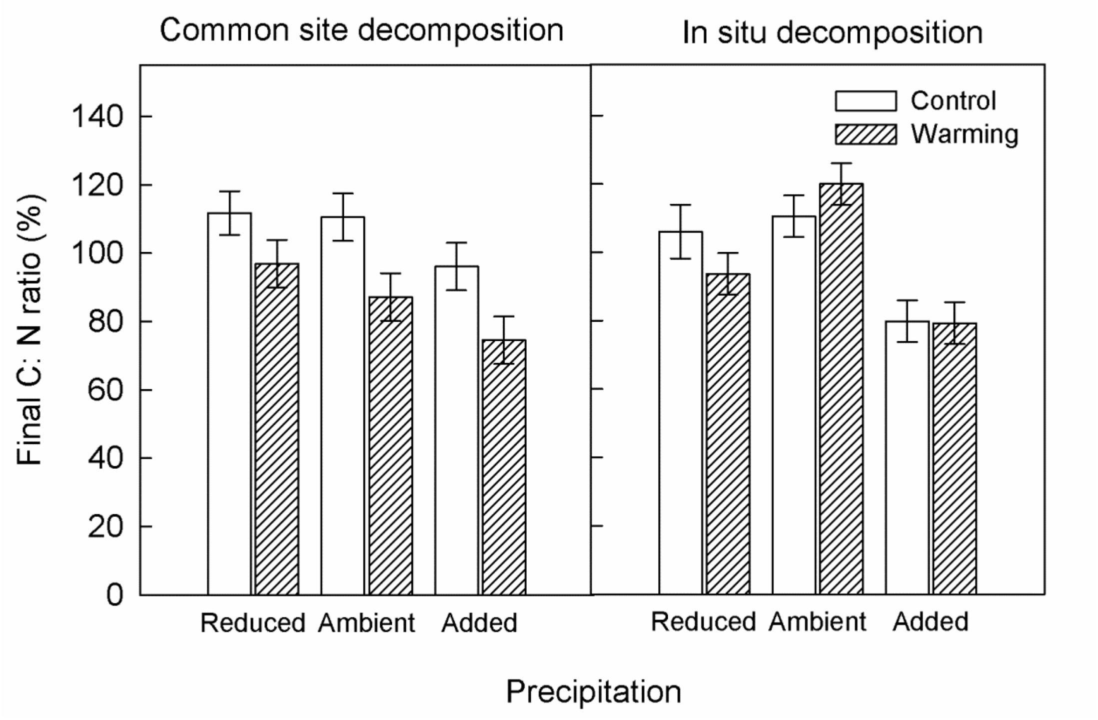

Figure B-2. Effects of litter quality (*Common site decomposition*, root litter of different origins were decomposed under the same site conditions/plots i.e. in control plots) and effects of local conditions and litter quality (*In situ decomposition*, root litter was decomposed in the same plots/sites from which they were collected) on root litter final C: N ratio (% mean remaining C: N ± 1 SE; n=5) after 4 months of incubation in the field.

Table B-1. Mixed model ANOVA results for the effects of temperature and precipitation on *common site* (litter quality) and *in situ* (litter quality and local conditions) shoot and root litter final C: N ratio

		(a) Shoot litter		(b) Root litter	
Decomposition	Effect	**F_{df}**	***P***	**F_{df}**	***P***
Common site					
	Temperature	$1.789_{1,25}$	0.193	$14.126_{1,21}$	**0.001**
	Precipitation	$1.494_{2,25}$	0.244	$4.657_{2,21}$	**0.021**
	Temperature x Precipitation	$5.753_{2,25}$	**0.009**	$0.422_{2,21}$	0.661
In situ					
	Temperature	$0.833_{1,22}$	0.371	$0.033_{1,22}$	0.857
	Precipitation	$84.460_{2,22}$	**<0.001**	$17.975_{2,22}$	**<0.001**
	Temperature x Precipitation	$14.658_{2,22}$	**<0.001**	$1.341_{2,22}$	0.282

9. Appendix C: Supplemental material for Chapter 5

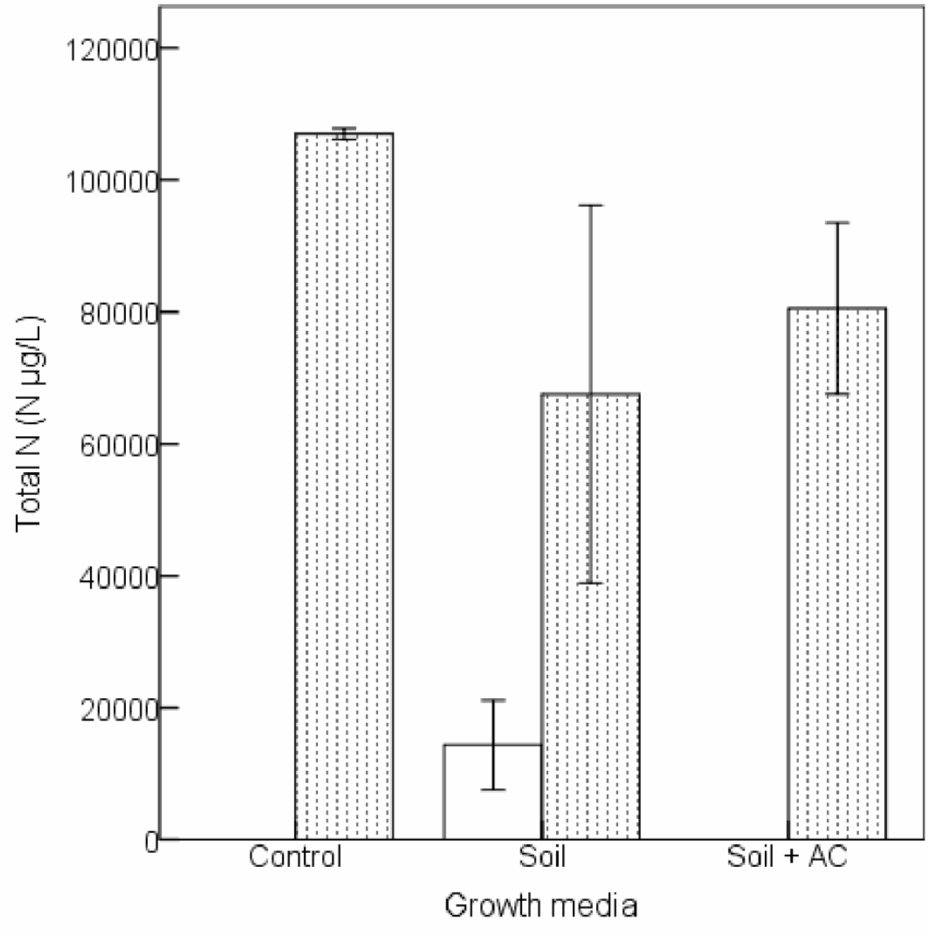

Figure C-1. Total N (mean N µg/L ± 1 SE; n=5) measured in each resultant leachate type and in control (Hoagland solution only). Watering solution was either Hoagland solution (dotted bars) or deionized water (open bar).

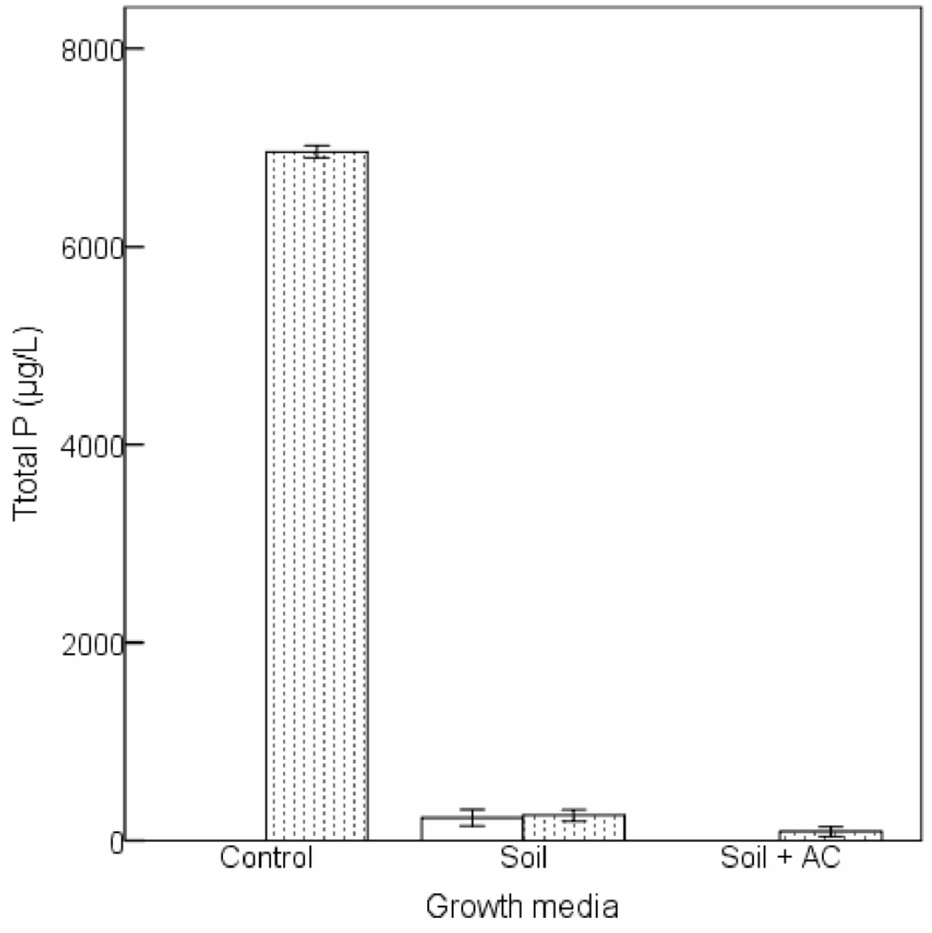

Figure C-2. Total P (mean P µg/L ± 1 SE; n=5) measured in each resultant leachate type and in control (Hoagland solution only). Watering solution was either Hoagland solution (dotted bars) or deionized water (open bar).