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Biogeochemical Cycling and Microbial Communities in Native Grasslands: Responses to Climate Change and Defoliation

by

Behnaz Attaeian

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Examining Committee

Scott X. Chang, Renewable Resources

James F. Cahill, Biological Sciences

Edward W. Bork, Agricultural, Food and Nutritional Science

Yongsheng Feng, Renewable Resources

Luo Yiqi, Botany and Microbiology, University of Oklahama

To,

My Mother, Hosnieh:

for being the flicker of a candle in dark pathways, guiding me through the right path, praying for my wishes to come true

and

in loving memory of My Father, Abbas:

who regretfully did not live to see my achievements

To,

My Husband, Mostafa:

for his endless support and constant encouragement, helping me to go through this journey,

for enormous patience, living 6215 miles far away from me,

for his constant long calls, giving me a sense of love and connection,

I am thankful every moment of my life for having him beside me

Abstract

Ongoing climate change has emerged as a major scientific challenge in the current century. Grassland ecosystems are considered net carbon (C) sinks to mitigate climate change. However, they are in turn, influenced by climate change and management practices, providing feedback to climate change via soil microbial community and biogeochemical fluxes. In this thesis, I examined the impact of warming, altered precipitation, and defoliation on soil microbial composition and function, C and N dynamics, and fluxes in soil respiration (CO_2), nitrous oxide (N_2O) and methane (CH_4), together with other belowground ecosystem functions, within two ecosites in a northern native temperate grassland in central Alberta, Canada, over a two-year period.

Fungi-to-bacteria ratio was not affected by climatic parameters or defoliation, indicating a high degree of resistance in the below ground community to the treatments imposed. However, C substrate utilization was influenced by warming and defoliation, as was soil microbial biomass. In contrast, soil respiration (or C loss) was not. Soil respiration acclimatized rather quickly to warming, and N_2O and CH_4 effluxes showed minor responses to warming at both ecosites, regardless of defoliation. These results suggest warming is unlikely to lead to positive climate change feedback due to soil-based responses, regardless of ongoing land use. However, altered precipitation (± 50%) demonstrated greater impacts on C and N fluxes relative to warming and defoliation. Increased precipitation stimulated soil C loss to the atmosphere, potentially generating positive feedback for climatic warming in this northern temperate grassland.

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Chapter 1 Introduction, background and research overview

Introduction

"With current climate change mitigation policies and related sustainable development practices, global GHG emissions will continue to grow over the next few decades. For the next two decades a warming of about 0.2°C per decade is projected for a range of SRES emissions scenarios. Continued GHG emissions at or above current rates would cause further warming and induce many changes in the global climate system during the 21st century that would very likely be larger than those observed during the 20th century."

~IPCC (2007)

While the original concerns over climate change targeted the North Atlantic, climate change has now become a global issue with increases in global average temperature evident worldwide (Biello, 2007). Associated with temperature changes are variation in precipitation pattern. Evidence for precipitation increases (eastern part of North and South America, northern Europe and northern and central Asia) and decreases (subtropical land region) are evident in various regions of the globe (IPCC, 2007). Warming may cause change in precipitation via two basic physical mechanisms: (i) warmer air is capable of holding greater amount of moisture, and (ii) warmer weather leads to greater evaporation and associated drying of the land surface (Easterling et al., 2000; Huntington, 2006).

Today, one of the main concerns is the consequence of climate change both locally and globally. To reduce the rate of CO_2 increase in the atmosphere, global efforts are underway to develop mitigation strategies to both enhance potential sinks and decrease potential sources of greenhouse gases (CO_2, N_2O, CH_4) (IPCC,

2007). One major strategy is to sequester atmospheric carbon dioxide (CO_2) into biomass and soil organic matter of terrestrial ecosystems (Izaurralde *et al.*, 2001; IPCC, 2007).

Within the biosphere, rangeland ecosystems cover up to 80% of terrestrial lands (Lund, 2004), and hence hold significant potential to sequester atmospheric CO_2. Considering the fact that 20 to 73% of global rangelands have been degraded (Lund, 2007), the current rate of C sequestration of 0.5 Pg C $year^{-1}$ (Schlesinger, 1997) might be below the maximum potential of many ecosystems. Improved management of degraded rangelands would therefore enhance the rate of C sequestration globally, and thereby mitigate the effects of climate change (Schimel *et al.*, 1990; Conant *et al.*, 2001). Despite the importance of rangelands, their role in C sequestration has been overlooked relative to studies in forested areas, leaving great uncertainty about the C storage potential in rangeland ecosystems.

Rates of soil C sequestration in rangeland ecosystems are sensitive to both climate (Conant *et al.*, 2001) and grazing management (Jones & Donnelly, 2004). As one of the most common management practices in rangeland ecosystems (Derner & Schuman, 2007), grazing is of crucial importance in C sequestration and in affecting soil C storage in such ecosystems (Bruce *et al.*, 1999). However, grazing impacts are variable among ecosystems, and thus local research is needed to understand the relationship between grazing and C budgets. Therefore, understanding the impacts of grazing strategies on the potential for (and

limitations in) C sequestration within rangeland ecosystems under the context of climate change has scientific merit.

The soil's capacity to sequester C is finite. In rangeland ecosystems, where nitrogen (N) often limits primary productivity (Derner & Schuman, 2007), N availability is one of the limitations to C sequestration (Reich *et al.*, 2006). In addition, global C and N are known to co-cycle via biomass accumulation, decomposition, and storage (Asner *et al.*, 1997). As a result, it is important to incorporate both C and N dynamics into climate change studies conducted within these ecosystems. More specifically, soil microbial processes and communities should be investigated as the soil microbial community drives biogeochemical cycles, particularly C and N cycling, as well as storage in the soil. Soil microbial properties such as size, activity and composition play key roles in nutrient cycling, carbon sequestration and in general, all biogeochemical reactions in the soil. Ultimately, the microbial community can reflect soil-plant responses to climate change and grazing management (Fig. 1-1). Consequently, predictions of global C sequestration, emerging land use policy, and routine public decisions on land management, are inextricably tied to the assemblage of both soil C and N dynamics and microbial communities found in rangeland ecosystems, as well as their response to climate change factors (e.g., warming and precipitation).

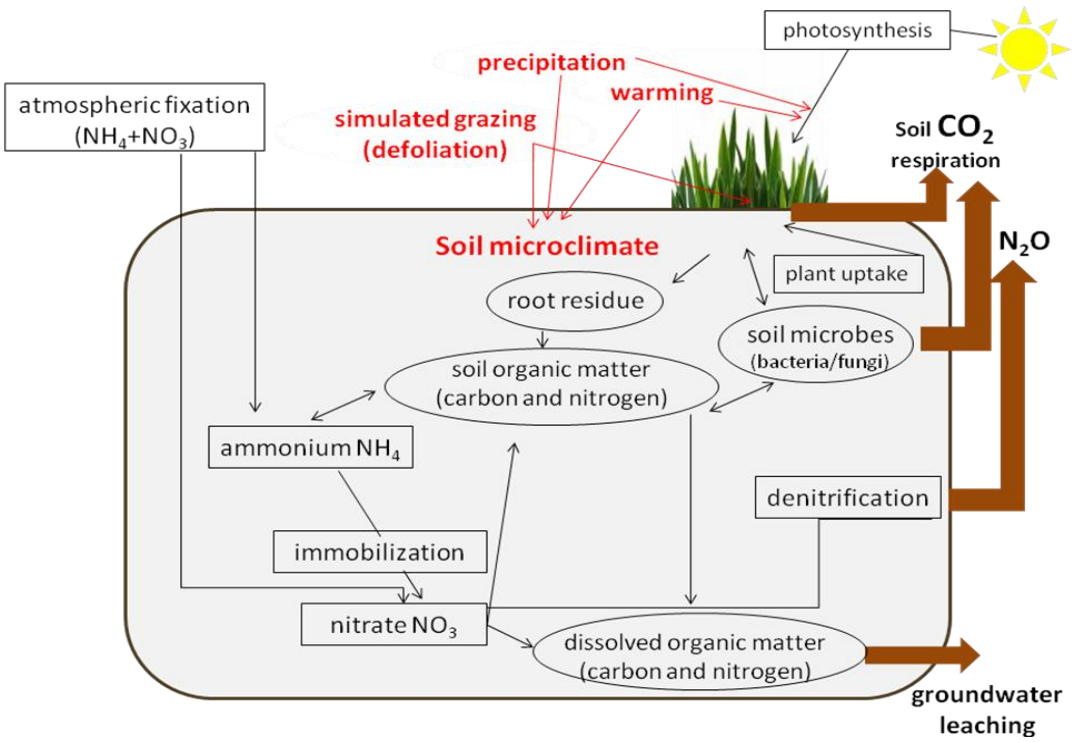

Figure 1-1. A conceptual model showing climate-grazing consequences on soil biogeochemical and microbial processes. The cause and effect relationship between soil C and N pools ⬭ and turnover/transformation processes ▭ are indicated by arrows ⟶. Red colors indicate the treatments applied and the pathways they are likely to impact.

2. Background

2.1. Linkage between climatic and biogeochemical cycles

Based on existing field-based climate change studies, soil belowground functions such as C and N dynamics may regulate global climate change via the influence of end-products of greenhouse gas (GHG) emissions, known as "feedback" (Cao & Woodward, 1998). Feedback associated from climatic parameters on GHG emissions may accelerate or dampen global warming, respectively, creating positive or negative feedbacks. Increased CO_2 concentration

or environmental warming received early attention from researchers investigating the effects of climate change in natural ecosystems (Makarov, 1959; Bazzaz, 1990). Rustad *et al.* (2001) in a meta-analysis study of 32 research sites demonstrated that 2-9 years of experimental warming with the range of 0.3-6.0°C increased soil CO_2 emission by 20% (with a 95% confidence interval of 18-22%). Beier *et al.* (2008) found that warming also increased soil CO_2 respiration in shrublands. This implies that microbial aerobic respiration may be enhanced in a warmer climate within well drained soils due to the higher level of oxygen, in turn resulting in a net loss of C via CO_2, a process potentially enhances positive feedback under climatic warming (Foley & Ramankutty, 2004; Pendall *et al.*, 2004). Changes in this large soil C flux at the global scope (e.g. 75×10^{15} gC/yr) can simultaneously affect C storage in the soil matrix (Schlesinger & Andrews, 2000), and hence the potential for C sequestration in the system.

In contrast, temperature sensitivity of soil CO_2 efflux may decline over time as observed by Luo *et al.* (2001) in a tall grass prairie and Strömgren (2001) in a boreal spruce stand. Regardless of whether it is acclimatization of soil microbial activity to climatic warming (Luo *et al.*, 2001; Strömgren, 2001) or depletion of readily decomposable substrate (Kirschbaum, 2004) that is the cause of such observations, these phenomena may weaken the positive feedback to climatic warming. Inconsistent responses have also been observed in microbial responses to warming. While soil microorganisms serve to sequester carbon in the system (Bradford *et al.*, 2002), they are in turn influenced by climatic parameters. Increased temperature may change the source of C utilization by the soil

microbial community towards either old or new carbon substrates (Zogg *et al.*, 1997; Andrews *et al.*, 2000). Furthermore, soil microbial characteristics (e.g. composition and function) also displayed contrasting responses to experimental warming (e.g., Zhang *et al.*, 2005; Rinnan *et al.*, 2007; Frey *et al.*, 2008; Rinnan *et al.*, 2009). For instance, warming shifted soil microbial community composition towards dominance by bacteria in subarctic heath (Rinnan *et al.*, 2007; Frey *et al.*, 2008) and dominance by fungi in tall grass prairie (Zhang *et al.*, 2005).

Contradictory responses of nitrogen dynamics to climatic warming have also been reported. Rustad *et al.* (2001) reported an average increase of 46% (with a 95% confidence interval of 30-64%) in net N mineralization due to 0.3-6.0°C warming during 2-9 years. . In contrast, N mineralization was relatively insensitive to experimental warming in the study by Beier *et al.* (2008). Wan *et al.* (2005) found that net N mineralization increased under experimental warming in the first year, but decreased during the second year of study. This change may be caused by differences in site characteristics such as aboveground community composition and available moisture. Moisture as well as vegetation composition can directly impact nitrogen mineralization responses to soil warming (Shaw & Harte, 2001).

Although the majority of previous global climate change studies have emphasized warming, the effects of precipitation have been the focus in more recent climate change studies (i.e., Weltzin *et al.*, 2003). Precipitation and soil water conditions influence photosynthesis, plant growth, and litter decomposition

(Coughenour & Chen, 1997), and as a result, change the potential for C sequestration in the system. Positive relationships between precipitation and soil CO_2 respiration (Wiant, 1967; Tylor *et al.*, 2004), and N_2O emission (Xu *et al.*, 2002) have been reported in several terrestrial systems. Based on this relationship, increases in the amount of precipitation may create positive feedback to global climate change via trace gas emissions. However, the prediction of feedback is difficult and dependent on the impacts of precipitation on various soil C and N pools. Precipitation regimes may influence total soil C and N pools (Walter, 2004) via changes in soil microbial biomass carbon (Singh *et al.*, 2009), soil organic C (Wichern & Joergensen, 2009), decomposer community composition (Tylor *et al.*, 2004), net N mineralization (Coughenour & Chen, 1997), and N nitrification (Wang *et al.*, 2006). A summary of other research reporting on the impacts of climate change on soil parameters is provided in Table 1-1.

Asymmetrical responses of soil C and N pools to climatic parameters provide no straightforward prediction of C sequestration in rangeland ecosystems. Despite this, the facilitation of atmospheric C sequestration in rangeland ecosystems via modified land use management has been set as a future goal (Lal, 2004; Jones & Donnelly, 2004). Moreover, the breakdown between net sources and sinks of carbon in terrestrial ecosystems remains unclear (Foley & Ramankutty, 2004). Recent reviews have recommended a need to further understand biogeochemical cycles and soil microorganisms as underlying mechanisms for the potential to influence C sources or sinks in terrestrial ecosystems (Foley & Ramankutty, 2004; Pendall *et al.*, 2004). Thus, in rangeland

ecosystems, it is fundamentally important that we understand the impacts of land use management (i.e., grazing strategies) on soil microbial communities as well as C and N dynamics under ongoing climate change.

2.2. Linkage between grazing strategies and biogeochemical cycles

Grazing strategies can influence plant communities in rangeland ecosystems. Inappropriate grazing such as high stocking rates can reduce desirable productive forage plants while increasing woody plants together with unpalatable grasses and forbs (Cingolani, 2005, Zhou *et al.,* 2006). Carbon and nitrogen dynamics in rangeland ecosystems are regulated through plant tissue quality and quantity, as well as the intensity and nature of disturbance. Disturbances such as defoliation may have importance consequences on C and N flows at the plant-soil interface (McGill *et al.,* 1986; Howe 1994), and hence determine the potential for C sequestration (Derner & Schuman, 2007). Thus far, varied effects of grazing or defoliation have been reported on microbial communities and nutrient cycling (Table 1-2).

Several studies have proposed biogeochemical cycling models with positive feedback of disturbances such as grazing in plant-soil nutrient flows in rangeland ecosystems (Wedin, 1995, 1996; Pastor & Cohen, 1997). There is some experimental evidence to support the grazing- or defoliation-positive outcomes on soil organic C (Shuman *et al.,* 2002; Pineiro *et al.,* 2009) and N availability (Seagle *et al.,* 1992) in rangeland ecosystems. In a mixed grassland ecosystem, clipping caused an increase in soil microbial C: N ratio (Harris *et al.,* 2008).

While grazing stimulated denitrification in grasslands of Yellowstone National Park (Frank *et al.,* 2000) and a semi-natural grassland (i.e. permanent pasture) in France (Le Roux *et al.,* 2003), grazing led to nitrification in various other grassland ecosystems i.e. Serengeti grassland (Seagle & McNaughton, 1993). However, Chapin *et al.* (1997) questioned the positive-feedback findings, suggesting a negative-feedback model instead. Support for the latter was provided by several studies. In tallgrass prairie, clipping suppressed soil CO_2 respiration by 16% (Zhou *et al.,* 2006). Grazing practices are also known to decrease soil organic C (Bauer *et al.,* 1987; Wright *et al.,* 2004) and inorganic-N availability in different rangeland ecosystems (Frank *et al.,* 1995; Wright *et al.,* 2004).

These divergent outcomes of modeling C and N dynamics to grazing management might be attributed to the fact that the aboveground community response to defoliation will differ as a function of the timing and intensity of disturbance. Furthermore, heterogeneity in environmental controls such as soil physical, chemical and biochemical properties on soil biogeochemical cycles contribute to the inconsistency in outcomes (Marriott *et al.,* 1997). Therefore, a better understanding of C and N dynamics across different rangeland ecosystems is essential in developing C sequestration strategies and policies (i.e. carbon credits) for rangeland ecosystems.

2.3. Interplay of grazing strategies and climatic parameters in biogeochemical cycles

Although studies focused on manipulating single factors of either climatic or land use (i.e. grazing) factors are valuable, they ignore an important component of the real world, which is the interaction between environment and disturbance. Changes in ecosystem functions are due to a combination of factors, including interactions among them. Synergetic rather than additive interactions between grazing or defoliation and climatic parameters (i.e., warming) are known to influence belowground communities within rangeland ecosystems (Zhang *et al.,* 2005; Klein *et al.,* 2007; Rinnan *et al.,* 2009). Therefore, the prediction and assessment of the potential for C sequestration in rangelands faced with climate change and human activities cannot be achieved from single-factor studies, which do not present a realistic scenario of future climate change in these systems.

3. Research Overview

3.1. Uncertainties over climate change impacts within rangeland ecosystems

> *"The pattern of future warming where land warms more than the adjacent oceans and more in northern high latitudes is seen in all scenarios."*
>
> *~IPCC (2007)*

Terrestrial ecosystems at high latitude play an important role in climate, and in turn, can be influenced by climate (Schwarz & Ross, 1990). Over the last several decades, regions in the northern high latitudes, particularly those above 50° N (Hansen *et al.,* 2006), and undergoing an increase in average temperature,

have experienced significant changes in ecosystem characteristics (e.g., latitudinal treeline advance) (Lloyd *et al.,* 2005). The prairie biome is one of the largest in North America, stretching from northwestern Canada to central Mexico (Shelford, 2008). The grassland ecosystems within this biome can be an important terrestrial C sink with relatively high susceptibility to climate change.

Over this broad region, northern grasslands within the boreal transition ecotone may be particularly susceptible to climate change. Simulation of vegetation changes in the transitional ecotone under global warming show marked areal expansion of grasslands over areas across western Canada (Bolin *et al.,* 1986), with some estimates predicting an increase in grassland of nearly 21% (Schwarz & Ross, 1990). Under this scenario, grassland and parkland may come to occupy much of the vegetation currently found in the southern boreal forest of western Canada (Schwarz & Ross, 1990). Therefore, the potential impacts of current and projected climate change on grassland ecosystems within this transitional ecotone, including potential feedbacks linked with the soil C cycling will have direct implications for the management and administration of grasslands in this region.

3.2. Structure of the research

I conducted two field experiments in a native temperate grassland ecosystem within transitional zone in Aspen Parkland ecoregion, Kinsella, Alberta, Canada (53°05 N, 111°33 W). In spring of 2006, a two-factor (i.e., warming and defoliation) manipulative experiment was established in ecosite A (Chapter 2 and

3) at the University of Alberta Research Ranch. I studied soil carbon and nitrogen dynamics in a two-year period during 2006-2007 growing seasons. The second experiment, manipulating warming, precipitation amount, and defoliation was established in ecosite B (Chapter 4 and 5) in early spring of 2007. I studied soil carbon and nitrogen dynamics over a two-year period during 2007-2008.

Warming: To simulate climatic warming, passive open-topped chambers (OTCs) were used. The OTCs enhance temperature by re-radiating incoming infrared radiation, in essence creating a greenhouse effect. The fiberglass material in the OTCs allows transmission of visible light, thereby maintaining a similar light intensity for plant growth. This method has been used worldwide (Marion, 1996), and now serves as a valuable tool to simulate climatic warming, especially in high-latitude ecosystems, while minimizing unwanted ecological side effects (Marion *et al.*, 1997).

As several environmental factors such as wind speed, solar radiation, soil texture, soil thermal conductivity, cloud cover, soil moisture, soil color and vegetation cover can affect the extent of temperature enhancement by OTCs, their performance will vary between and within study sites (Coulson et al., 1993; Marion et al., 1997; Holliste, 2003). However, an overall 1.2 - 1.8 ºC increase in mean daily temperature has been achieved from OTCs (Marion *et al.*, 1993). In this study, we used a conical shaped OTC with dimensions of 40 cm high × 2 m diameter, with a 60º side angle (Plate 1-1).

The micro-climatic conditions in OTCs determined using HOBO Pro V2 data loggers. Soil temperature and moisture (volumetric water content) were influenced by OTCs, with increases in soil temperature (≤ 1 °C) and decreases in soil moisture (Chapter 2 and 4). However, the absolute responses fluctuated over time, even during a single day (e.g., Figure 1-1).

Plate 1-1. Picture showing the set up of open-top chamber in the study site.

Precipitation: The manipulation of precipitation amount was achieved by using a transparent rainfall shelter (Plate 1-2), a modified design by Zhou *et al.*, 2006. The rainout shelter intercepted approximately 50% of ambient precipitation in the precipitation exclusion plots. In 2008, the intercepted water was channeled through a gutter and stored in a water tank to subsequently applied to the water addition plots within 24 hours of the rainfall event. By this approach, we increased and decreased the precipitation amount without altering the precipitation frequency. Both the control and water addition plots also had

shelters built overtop them, to control for any confounding effects of the structures on local climatic factors. This approach mainly depends upon the actual rainfall in the region, which is very variable during the growing seasons.

The microclimatic conditions under rainfall shelters determined using HOBO Pro V2 data loggers (Chapter 2 and 4). The results of soil moisture and temperature suggested a fluctuating pattern of these parameters even during a single day (e.g., Figure 1-2).

Plate 1-2. Picture showing the set up of transparent rainfall shelter in the study site.

Defoliation: As structure of OTCs devices was an obstacle to apply the direct livestock grazing, manual aboveground defoliation was used to simulate different grazing intensities. Aboveground vegetation within plots was defoliated in two

level of none and high defoliation intensities in ecosite A and three level of none, low, and high defoliation intensities in ecosite B. The low and high intensity defoliation treatments consisted of clipping the aboveground at a stubble height of approximately 7.5 and 2.5 cm, respectively, removing of 30% and 80% of standing current annual biomass. These levels represent the conservative and excessive use for native rangelands.

Soil microclimate i.e. soil moisture content and temperature in defoliated plots were assessed using HOBO Pro V2 data loggers (Chapter 2 and 4). Defoliation affected soil microclimate, but not uniformly (e.g., Figure 1-3).

3.3. Study Site

This study was conducted at two ecosites (A and B) located on the University of Alberta Kinsella Research Ranch at Kinsella, Alberta, Canada (53°05 N, 111°33 W). The ranch has many native rough fescue (*Festuca hallii*) grasslands, which were the predominant historical vegetation in the Aspen Parkland Ecoregion in Alberta, Canada (Lamb *et al.*, 2007) under light grazing (Natural Regions Committee, 2006). These grasslands are currently categorized as part of the northern fescue Natural Subregion, and are known to have relatively high plant diversity, which differentiates this study site from other grasslands of the region in Alberta (Natural Regions Committee, 2006).

Rough fescue grasslands occur at and around elevations of 650 m, on soils that are loamy in texture and well-drained. Parent materials include cretaceous sediments, composed of marine shales, nonmarine sandstone, and mudstones

(Natural Regions Committee, 2006). The region has a continental climate. The average long-term (1971-2000) weather condition of nearby weather station (Viking, Alberta, Canada) records the mean annual precipitation of 431.3 mm, mean annual temperature of 2.4 °C, a July mean temperature of 16.6 °C, a January mean temperature of −13.8 °C, and a mean average growing season (May-September) temperature and precipitation of13.9 °C and 312.2 mm, respectively (Environment Canada, 2009). The average annual temperature and precipitation during the study were 1.2, -1.02, and 5 °C and 297, 253.3, and 347.6 mm, respectively, in 2006, 2007, and 2008. The average temperature and precipitation of growing season (May-September) were 16.1, 13.9, and 13.9 °C and 181.2, 211.4, and 249 mm, respectively, in 2006, 2007, and 2008. The climate in this subregion (Northern Fescue Natural Subregion) represents a transition between the Dry Mixedgrass Natural Subregion and the northern Central Parkland Natural Subregion (Natural Regions Committee, 2006). Grasslands in this region were selected for testing climate change impacts for several reasons, including: (1) a wealth of background information was available as grassland in this area have been extensively studied and valuable information on plant community and site characteristics already exists, (2) this region is known to have a strong correlation between vegetation and climate. The grassland-forest boundary in this area is known to be sensitive to climate change (Vance et al. 1979), and thus, more rapid responses in soil microbial communities and nutrient cycling may be expected in response to the imposition of climatic treatments.

The two studied ecosites were 12 km away from each other and included Orthic Black-Dark Brown (ecosite A) and Brown (ecosite B) Chernozems developed under grassland vegetation (Howitt, 1988). The soil at Ecosite A is moister with the greater organic matter inputs and humus formation in comparison to ecosite B (Natural Regions Committee, 2006). In general, the two ecosites can be characterized as follows:

Ecosite A: This is a remnant native grassland ecosystem with no history of cultivation, managed with light cattle grazing during fall (Cahill, 2003a). In this soil matrix, nitrogen and water availability are the main limitation for vegetation growth (Lamb *et al.,* 2007). This ecosite includes a mixture of aspen (*Populus tremuloides* Michx.) stands and rough fescue (*Festuca hallii* (Vasey) Piper) prairie (Lamb *et al.,* 2007), and is dominated by graminoid biomass (72%) rather than forb, although the latter are a main component of plant species richness (70%) (Coupe *et al.,* 2009). Other dominant species are the graminoieds; *Stipa curtiseta, Koeleria macrantha,* and *Koeleria macrantha* (Ledeb.) J. A. Schultes f., and common forbs; *Achillea millefolium* L., *Agoseris glauca* (Pursh) Raf., *Coreopsis tinctoria* Nutt., *Helianthus petiolaris* Nutt., *Linum lewisii* Pursh, *Geum triflorum* Pursh, *Potentilla pensylvanica* L., and *Galium boreale* L. *Comandra umbellate,* and *Solidago missouriensis* (Cahill, 2003a).

Ecosite B: Similar to ecosite A, this area is a native grassland ecosystem, with light cattle grazing in the fall and no cultivation history. This ecosite contains mainly graminoids, including *Agropyron smithii, Fescue hallii, Stipa curtiseta, Bouteloua gracilis, and Koeleria macrantha.* Common forbs include *Artemisia*

frigida, *Anemone patens*, and *Commandra umbellata* (White, unpublished). The vegetation biomass is also dominated by graminoids (White, unpublished).

3.4. Objectives

The research described in this dissertation is an attempt to address the gap in our understanding of biogeochemical cycles and soil microbial communities in the native temperate grassland ecosystems found within the grassland-boreal forest ecotone. It is a matter of crucial importance that we explore both soil C and N cycling which govern ecosystem productivity and the soil microbial community that regulates soil nutrient cycling underneath the rangelands. To simulate the realistic scenario of future climate change in grazed systems such as rangelands ecosystems, we need to understand biogeochemical cycles as influenced by disturbances such as changing climate and grazing.

The central question of this thesis is how soil carbon and nitrogen dynamics and the microbial community respond to global climate change and grazing management? Thus, I focused primarily on two main aspects including (1) soil C and N cycling, and (2) soil microbial communities. My specific objectives were:

1) Investigate the responses in soil C and N dynamics to climatic parameters (i.e., warming and altered precipitation) in a northern temperate grassland ecosystem, and determine whether these responses contribute to climate change through influences on ecosystem feedback (Chapters 2 and 4).
2) Investigate the responses in soil C and N dynamics to defoliation in a northern temperate grassland ecosystem, and determine whether these

responses contribute to climate change through influences on ecosystem feedback (Chapters 2 and 4).

3) Investigate the changes in soil microbial community structure and function in relation to climatic parameters (i.e., warming and altered precipitation) in a northern temperate grassland ecosystem, and determine whether biotic and/or abiotic parameters account for the variability in soil microbial structure and function (Chapters 3 and 5).
4) Investigate the changes in soil microbial community structure and function in relation to defoliation intensities, and determine whether biotic and/or abiotic parameters account for the variability in soil microbial structure and function (Chapters 3 and 5).
5) Review the accumulative responses to multiple, interacting factors including changes in temperature, precipitation and defoliation, for this northern temperate grassland ecosystems (Chapter 6).

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Table 1-1. Summary of previous studies linking soil biogeochemical variables in response to climatic parameters across various grassland ecosystems.

Ecosystem Type	Location	Climatic parameter	Duration	Responses variables	Effect	Authors
Terrestrial ecosystem	Field Global Scale	temperature	-	soil respiration	increase	*review of* Raich and Schlesinger (1992)
Terrestrial ecosystem	Field high tundra, low tundra, grassland, and forest	warming	2-9 years	soil respiration	increase	*review of* Rustad et al. (2001)
Tallgrass prairie	Field Oklahama, USA	warming	2 years	soil respiration	no effect	Luo et al. (2001)
Tallgrass prairie	Field Oklahama, USA	warming	4 years	soil respiration	no effect	Zhang et al. (2005)
Managed fescue field	Field Oklahama, USA	warming	2 years	soil respiration	variable	Wan et al. (2007)
Grassland	Controlled chamber Antwerp, Belgium	warming	2 years	soil respiration	no effect	De Boeck et al. (2007)
Grassland	Laboratory Alberta Canada	warming	1 year	microbial respiration	no effect	Feng and Simpson (2009)
Temperate steppe	Field Inner Mongolia, China	warming	2 years	ecosystem respiration	no effect	Xia et al. (2009)
Temperate grassland	Mesocosm Scotland, UK	warming	2 years	soil respiration	no effect	Briones et al. (2009)
Temperate steppe	Field Shaanxi, China	warming	3 years	soil respiration	decrease	Liu et al. (2009)
Terrestrial ecosystem	Field high tundra, low tundra, grassland, and forest	warming	2-9 years	net N mineralization	increase	*review of* Rustad et al. (2001)

Table 1-1. *(contd)*

Ecosystem Type	Location	Climatic parameter	Duration	Responses variables	Effect	Authors
Tallgrass prairie	Field Oklahama, USA	warming	4 years	net N mineralization	no effect	Zhang et al. (2005)
Tallgrass prairie	Field Oklahama, USA	warming	1 years	net N mineralization	increase	Wan et al. (2005)
Tallgrass prairie	Field Oklahama, USA	warming	2 years	Net N mineralization	decrease	Wan et al. (2005)
Heathland	Field Kilpisjärvi, Finland	warming	10 years	NH_4^+-N concentration	decrease	Rinnan et al. (2009)
Heathland	Field Kilpisjärvi, Finland	warming	13 years	NH_4^+-N concentration	no effect	Rinnan et al. (2009)
Tallgrass prairie	Field Oklahoma, USA	warming	2.5 years	labile N	increase	Belay-Tedla et al. (2009)
Mesocosm	Greenhouse Ascot, UK	warming	9 months	microbial biomass	no effect	Bardgett et al. (1999)
Tallgrass prairie	Field Oklahama, USA	warming	4 years	microbial biomass	no effect	Zhang et al. (2005)
Grassland	Laboratory Alberta, Canada	warming	1 year	microbial biomass	no effect	Feng and Simpson (2009)
Tallgrass prairie	Field Oklahoma, USA	warming	2.5 years	microbial biomass C and N	increase	Belay-Tedla et al. (2009)
Temperate steppe	Field Shaanxi, China	warming	3 years	microbial biomass C and N	decrease	Liu et al. (2009)
Mesocosm	Greenhouse Ascot, UK	warming	9 months	relative abundance of fungi and actinomycete	no effect	Bardgett et al. (1999)
Tallgrass prairie	Field Oklahama, USA	warming	4 years	relative abundance of fungi	increase	Zhang et al. (2005)
Temperate grassland	Mesocosm Scotland, UK	warming	2 years	diversity of fungivorous mites	no effect	Briones et al. (2009)

Table 1-1. *(contd)*

Ecosystem Type	location	climatic parameter	Duration	Responses variables	Effect	Authors
Temperate grassland	Field Saskatchewan, Canada	precipitation	-	soil respiration	increase	de Jong et al. (1974)
Heathland	Field Mols, Denmark	precipitation (reduction)	2 months	soil respiration	decrease	Jensen et al. (2003)
Temperate grassland	Field Kansas, USA	precipitation (reduction)	4 years	soil respiration	decrease	Harper et al. (2005)
Temperate grassland	Field Inner Mongolia, China	precipitation (addition)	1 year	soil respiration	increase	Xiao et al. (2007)
Grassland	Mesocosm Lincoln, New Zealand	precipitation (reduction)	14 months	basal respiration	no effect	Williamson and Wardle (2007)
Temperate steppe	Field Shaanxi, China	precipitation (addition)	3 years	soil respiration	increase	Liu et al. (2009)
Heathland	Field Mols, Denmark	precipitation (reduction)	2 months	microbial biomass C and N	decrease	Jensen et al. (2003)
Temperate grassland	Field Inner Mongolia, China	precipitation (addition)	1 year	microbial biomass C	no effect	Xiao et al. (2007)
Temperate steppe	Field Shaanxi, China	precipitation (addition)	3 years	microbial biomass C and N	increase	Liu et al. (2009)
Alkaline grassland	Field Oxfordshire, UK	precipitation (addition)	3 years	gross N mineralization (in summer)	no effect	Jamieson et al. (1998)
Grassland	Mesocosm Lincoln, New Zealand	precipitation (reduction)	14 months	microbial biomass	decrease	Williamson and Wardle (2007)
Alkaline grassland	Field Oxfordshire, UK	precipitation (addition)	3 years	gross N mineralization (in fall)	decrease	Jamieson et al. (1998)
Tallgrass prairie	Field Kansas, USA	precipitation (addition)	11 years	fungal community composition	no effect	Jumpponen and Johnson (2005)

Table 1-1. *(contd)*

Ecosystem Type	location	climatic parameter	Duration	Responses variables	Effect	Authors
Sotol grassland	Field New Mexico, USA	precipitation	-	bacterial community composition	shift	Clark et al. (2009)

Table 1- 2. Summary of studies examining soil biogeochemical variables in response to grazing and defoliation strategies across various grassland ecosystems.

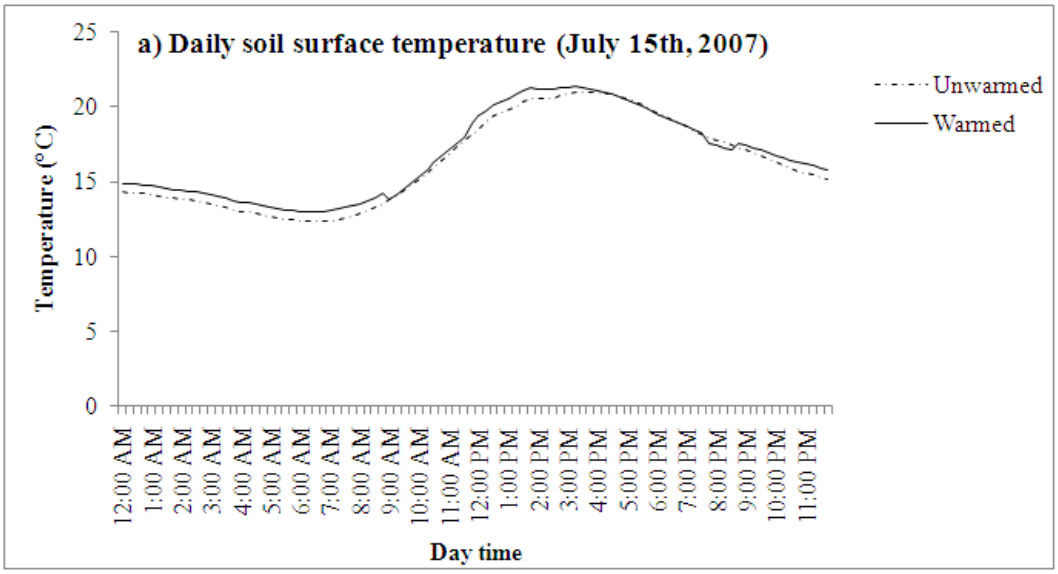
Ecosystem Type	Location	Treatment	Duration	Responses variables	Effect	Authors
Semi-natural grassland	Field Theix, France	grazing	13 years	N nitrification	increase	Le Roux et al. (2003)
Tallgrass prairie	Field Oklahama, USA	clipping	Yearly	net N mineralization	no effect	Zhang et al. (2005)
Subarctic meadow	Field Kilpisjärvi, Finland	clipping	twice in a year	NH_4^+-N concentration	no effect	Stark and Kytoviita (2006)
Subarctic heath	Field Kilpisjärvi, Finland	simulated herbivory	yearly	NH_4^+-N concentration	no effect	Rinnan et al. (2009)
Sub-arctic grassland	Greenhouse Abisko, Sweden	clipping	yearly	net N mineralization	decrease	Sorenson et al. (2008)
Pasture	Field Maaninka, Finland	grazing	3 years	inorganic-N concentration	no effect	Mikola et al. (2009)
Alpine meadow	Field Sichuan, China	grazing	-	net N mineralization	increase	Gao et al. (2009)
Mixed grassland	Field North Dakota, USA	grazing	75 years	soil C and N content	decrease	Frank et al. (1995)
Semi-natural grassland	Mesocosm Theix, France	grazing	1 year	soil organic C	decrease	Attard et al. (2008)
Steppe	Field Inner Mongolia, China	grazing	-	soil C and N content	decrease	He et al. (2008)
Tallgrass prairie	Field Oklahama, USA	clipping	yearly	soil labile C, N	decrease	Belay-Tedla et al. (2009)
Grassland	Microcosm Virginia, USA	defoliation	21 days	microbial biomass C	increase	Mawdsley and Bardgett (1997)

Table 1-2. *(contd)*

Ecosystem Type	Location	Treatment	Duration	Responses variables	Effect	Authors
Tallgrass prairie	Field Oklahama, USA	clipping	yearly	microbial biomass C and N	decrease	Zhang et al. (2005)
Subarctic meadow	Field Kilpisjärvi, Finland	clipping	twice in a year	microbial biomass C and N	no effect	Stark and Kytoviita (2006)
Grassland	Mesocosm Lincoln, New Zealand	defoliation	Every 2-3 weeks	microbial biomass	decrease	Williamson and Wardle (2007)
Mixed grassland	Field Texas, USA	clipping	yearly	microbial biomass C and N	no effect	Harris et al. (2008)
Subarctic heath	Field Kilpisjärvi, Finland	simulated herbivory	yearly	microbial biomass C and N	no effect	Rinnan et al. (2009)
Tallgrass prairie	Field Oklahama, USA	clipping	yearly	microbial biomass N	no effect	Belay-Tedla et al. (2009)
Tallgrass prairie	Field Oklahama, USA	clipping	long-term	soil respiration	no effect	Zhou et al. (2006)
Tallgrass prairie	Field Oklahama, USA	clipping	Short-term	soil respiration	decrease	Zhou et al. (2006)
Tallgrass prairie	Field Oklahama, USA	clipping	yearly	soil respiration	decrease	Zhang et al. (2005)
Pasture	Field UK	grazing	15 years	bacterial, fungal, actinomycete community profile	no effect	Clegg (2006)
Subarctic meadow	Field Kilpisjärvi, Finland	clipping	Twice in a year	microbial respiration	no effect	Stark and Kytoviita (2006)
Grassland	Mesocosm Lincoln, New Zealand	defoliation	Every 2-3 weeks	basal respiration and total MB	decrease	Williamson and Wardle (2007)
Subarctic heath	Field Kilpisjärvi, Finland	simulated herbivory	yearly	soil microbial respiration	no effect	Rinnan et al. (2009)

Table 1-2. *(contd)*

Ecosystem Type	Location	Treatment	Duration	Responses variables	Effect	Authors
Grassland	Microcosm Virginia, USA	defoliation	21 days	number of culturable bacteria	increase	Mawdsley and Bardgett (1997)
Grassland	Microcosm Virginia, USA	defoliation	21 days	number of culturable fungi	no effect	Mawdsley and Bardgett (1997)
Semi-natural grassland	Mesocosm Theix, France	grazing	1 year	bacterial community structure	shift	Attard et al. (2008)
Sub-arctic grassland	Greenhouse Abisko, Sweden	clipping	yearly	bacterial and fungal community structure	no effect	Sorenson et al. (2008)
Pasture	Field Maaninka, Finland	grazing	3 years	abundance of fungivorous nematodes	increase	Mikola et al. (2009)
Pasture	Field Virginia, USA	grazing	14 years	fungal community structure	shift	Wakelin et al. (2009)
Pasture	Field Virginia, USA	grazing	14 years	bacterial community structure	no effect	Wakelin et al. (2009)



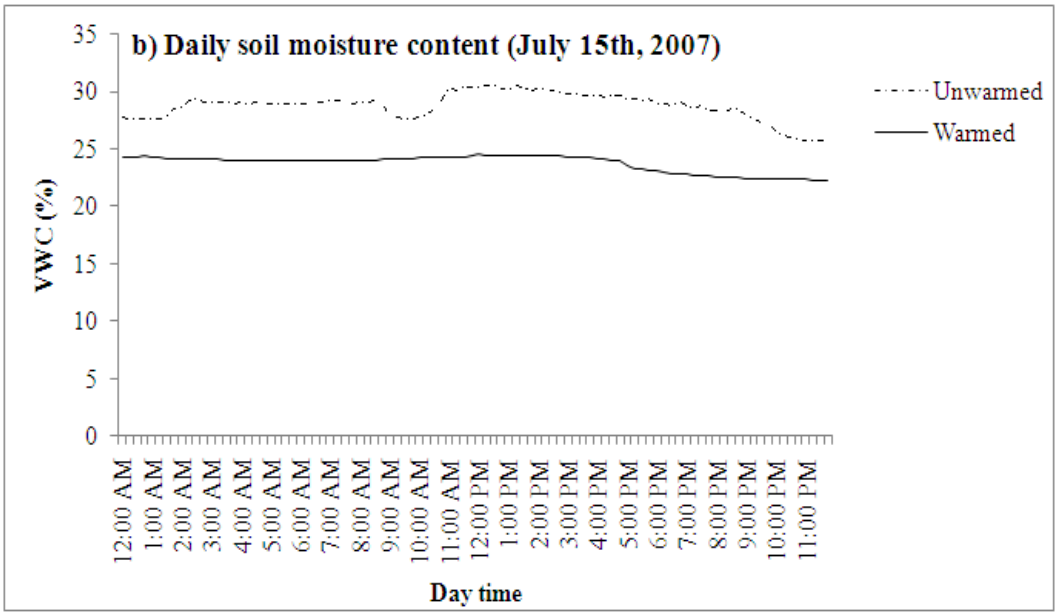


Figure 1-2. Measured daily soil (a) temperature (b) volumetric water content (VWC) at 0-5cm soil depth as influenced by warming treatment on July 15th , 2007 in ecosite B.

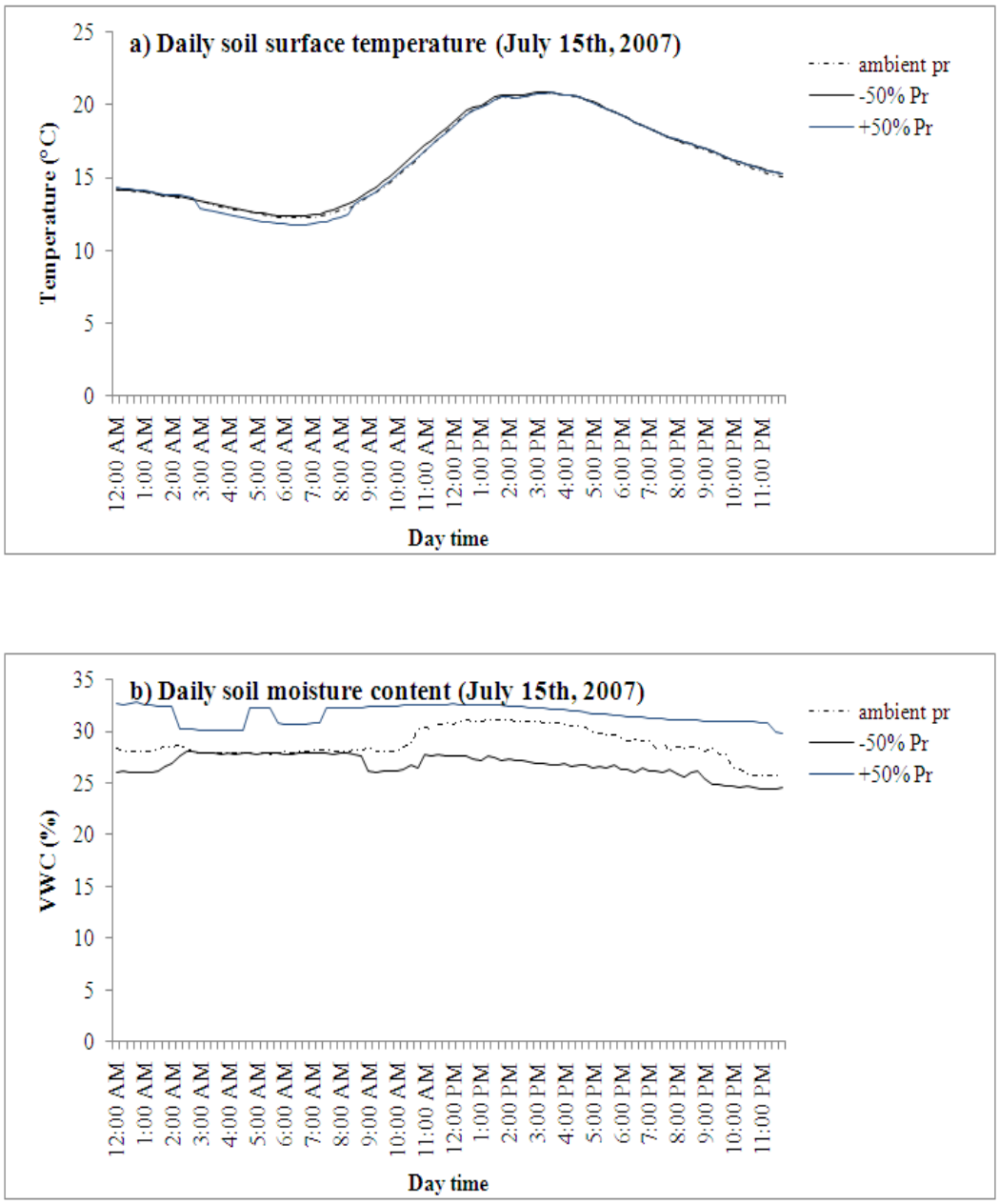


Figure 2-3. Measured daily soil (a) temperature (b) volumetric water content (VWC) at 0-5cm soil depth as influenced by precipitation treatment on July 15th , 2007 in ecosite B.

Figure 3-4. Measured daily soil (a) temperature (b) volumetric water content (VWC) at 0-5cm soil depth as influenced by defoliation treatment on July 15th , 2007 in ecosite B.

Chapter 2 Limited Impacts of Experimental Warming and Defoliation on Short-Term Soil Carbon and Nitrogen Dynamics in Native Grassland (Ecosite A)

1. Introduction

A global upward trend in average surface temperature has been evident over the last century. In the Prairie Provinces of Canada, temperatures have increased about 1.2 °C over the last 50 years (Natural Resources Canada, 2007). Atmospheric warming can substantially affect both global (Melillo *et al.*, 2002) and local (Natural Resources Canada, 2009) C cycling through changes in greenhouse gas emissions and plant productivity in terrestrial ecosystems.

Rangeland ecosystems comprise up to 80% of global land (Lund, 2007), and therefore have the potential to significantly impact, and be impacted by, global warming. Healthy rangelands can serve as C sinks, globally sequestering 0.5 Pg C $year^{-1}$ in the soil (Schlesinger, 1997). Further, C stored in rangeland ecosystems is largely allocated belowground and thus, is at a much reduced risk to release from fire (De Deyn *et al.*, 2008) compared to forest ecosystems. Moreover, effective C sequestration in rangeland ecosystems requires minimal inputs compared to other ecosystems such as croplands (Derner & Schuman, 2007). However, socio-economic factors, such as rising human and livestock populations, have degraded 20 to 73% of rangeland ecosystems globally (Lund, 2007), accelerating net C losses and decreasing C fixation through photosynthesis. An additional limitation

for C sequestration in grasslands is the reduction in N availability. Changes in N cycling could alter C storage due to their interdependency (McGuire *et al.,* 1997).

The dominant human impact on rangeland systems is livestock grazing (Lund, 2007). The effect of grazing on soil C and N dynamics and sequestration in rangeland ecosystems is still not well understood. Some studies have observed no grazing effect on soil organic C (Frank *et al.,* 1995), while others reported decreases (Bauer *et al.,* 1987; Wright *et al.,* 2004) or increases (Shuman *et al.,* 2002; Pineiro *et al.,* 2009). Similarly, there are contradictory findings for soil mineral N availability, where increased grazing intensity was associated with increases (Seagle *et al.,* 1992), decreases (Frank *et al.,* 1995; Wright *et al.,* 2004), or no changes (Bauer *et al.,* 1987) in soil N availability. These contradictory results may be due to variation in plant species composition, microbial community structure and activity, and general soil physical and chemical properties (Schuman *et al.,* 2002). As a result, the impacts of grazing on C and N dynamics will be site specific (e.g., Schuman *et al.,* 2002; Frank *et al.,* 1995; Pineiro *et al.,* 2009), and further complicated by the reality that climate in rangeland systems is dynamic.

Even though extensive studies on grazing impacts on C and N cycling have been conducted (e.g. Bauer *et al.,* 1987; Schuman *et al.,* 2002; Frank *et al.,* 1995; Wright *et al.,* 2004; Derner & Schuman, 2007; Pineiro *et al.,* 2009) only a few have focused on the impact of projected global warming on C and N dynamics in grazed ecosystems (Wan *et al.,* 2005; Zhou *et al.,* 2007; Klein *et al.,* 2007), suggesting that climatic warming along with grazing strategies might have a

synergistic effects on soil C and N fluxes. However, studies addressing the C and N cycling in a warmer world in grazed systems are still in their infancy relative to other ecosystems. This gap is important because proper grassland management strategies would enhance the potential to retain and sequester soil C.

It is well established that grazing and warming can alter C and N dynamics by changing the quality, quantity and timing of organic C input into soil (Schimel, 1986; Ingram *et al.*, 2008). Interactions between warming and grazing could make the direction and magnitude of their impact unpredictable (Klein *et al.*, 2007). More importantly, grazing may affect regional atmospheric circulation and climate patterns by altering hydrologic cycles (Eastman *et al.*, 2001). Moreover, with a short growing season, and despite a history of climate change (Vance 1979), northern temperate grasslands may be particularly susceptible to global warming. Therefore, a better understanding of the impact of warming and grazing management on C and N dynamics in northern temperate grasslands is necessary to predict changes in C under projected global warming and various grazing management strategies.

The overall objective of this research was to understand the effects of defoliation and warming on short-term *in situ* C and N dynamics, microbial biomass and gas fluxes in a northern temperate grassland in central Alberta, Canada.

Materials and Methods

2.1. Site Description

The research was conducted over 2 growing seasons (2006-07) in a native grassland at the University of Alberta Research Ranch near Kinsella, Alberta, Canada (53°05 N, 111°33 W). The site is within the Aspen Parkland Natural Subregion on the ecotone between prairie (at the northern extent of the Great Plains) and forest (at the southern edge of the boreal forest) ecosystems. Referred to as an ecological 'tension zone' (Bird, 1961), this region is known to be susceptible to climatic fluctuations (Vance *et al.*, 1979). The site is dominated by rough fescue [*Festuca hallii* (Vasey) Piper], *Aster falcatus*, *Artemisia ludoviciana*, and *Commandra umbellata.* The soil is an Orthic Dark Brown Chernozem (Howitt, 1988) with a 15 cm Ah horizon of loamy texture (clay: 21%, silt: 33%, sand: 45%) and a pH ($CaCl_2$) of 5.7. The study area has a continental climate with the long-term (1971-2000) annual average precipitation of 431.3 mm and mean annual temperature of 2.8 °C and average long-term (1971-2000) growing seasons (May- September) was 13.9 °C and 312.2 mm, respectively. Average precipitation and temperature during the growing seasons of the years of study, respectively, were 181.2 mm and 16.1 °C in 2006 and 211.4 mm and 13.9 °C in 2007 growing seasons. Precipitation was greater and mean daily temperature lower during 2007 compared to the 2006 growing season. However, maximum daytime temperatures peaked around 32 ºC in July of each year.

2.2. Experimental Design

In May 2006, twenty 2×2 m plots were established in a completely randomized block (RCB) design to evaluate the effects of warming and grazing

on soil C and N dynamics (reported in this paper), and plant community structure and rangeland productivity (Bork *et al.* 2008). Each block (n=5) consisted of four treatments: control (C), warming (W), defoliation (D), and warming plus defoliation (WD). Warming was achieved through circular open-top chambers (OTCs, 2 m diameter × 0.4 m height) placed at the centre of the plot. Defoliation was done once per year by manually clipping vegetation to 5 cm height. Previous studies with OTCs have shown an increase of ~1-2 °C in daily maximum temperature (Marion *et al.*, 1997; Rozema *et al.*, 2009). Although manual defoliation differs from cattle grazing (i.e., lack of plant selectivity and hence changes in plant composition and C and N partitioning, soil trampling, urine input and hence nutrient recycling), our study was limited by the use of the OTCs and small plot size, which precluded the use of grazing animals in our experiment. Defoliation occurred in early June of 2006, consistent with grazing practices in the region. Defoliation was not applied in 2007 to allow us to observe the post-treatment effect on rangeland ecosystem properties and processes.

Air temperatures at the soil surface were monitored every 10 min during the growing season using HOBO (HOBO® H8 *Pro Series*) temperature probes and data loggers (Onset Computer Co., Pocasset, MA. USA). Average daily soil surface temperature and average daytime soil surface temperature increased about 1 and 1.6 °C, respectively, within the warmed treatment during the two growing seasons. This increase is equivalent to the rise in temperature over the last 50 years in the prairie provinces of Canada (Natural Resources Canada, 2007).

2.3. Soil Sampling and Biochemical Analysis

Each plot was sub-divided into two areas for destructive soil sampling in the two years. Intact soil cores 5 cm in diameter were collected monthly during two growing seasons (2006 and 2007) at two depths (0-5 cm and 5-15 cm). Soil cores were kept in a cooler, transported to the laboratory, and stored at 4 °C until further processed. Samples were sieved to pass through a 4 mm mesh, and 10 g sub-samples dried overnight at 105 ºC to determine gravimetric soil moisture content.

The chloroform fumigation-extraction method (CFE) was used to determine soil microbial biomass C (MBC) and N (MBN) (Brookes *et al.*, 1985). Two 20 g (oven dry weight equivalent) fresh subsamples were extracted by 0.5 mol L^{-1} K_2SO_4 (soil: solution ratio was 1:5) before and after chloroform fumigation to measure extractable organic C and N concentrations using a Shimadzu TOC-V CSH/CSN Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). *In situ* net N mineralization was measured monthly using the buried-bag method (Eno, 1960) during each growing season. Total inorganic N [nitrate (NO_3^-) and ammonium (NH_4^+)] concentrations were determined from one of the two cores as the initial inorganic N concentration by 2 mol L^{-1} KCl extraction, with the other core incubated *in situ* for 30 days, then retrieved and used to determine inorganic N concentrations. Ammonium and nitrate concentrations in the extract were determined using Indophenol blue (Page *et al.*, 1982) and the one reagent colorimetric method of Doane & Horwath (2003), respectively.

Soil N supply rate was assessed monthly in the second growing season (May-August 2007) using Plant Root Simulator (PRS™) probes (Western Ag

Innovations Inc.). PRS™ probes were inserted in the soil for 30 days, retrieved and sent to Western Ag Innovations Inc. for analysis of ammonium and nitrate supply rates. Such rates are expressed as mg of N absorbed per 10 cm^2 of surface area of the probe per burial period.

2.4. Soil Greenhouse Gas Emissions

PVC collars (132.7 cm^2 in cross sectional area and 3 cm in height) were permanently installed 3 cm into the soil to monitor *in situ* soil greenhouse gas (CO_2, N_2O and CH_4) emission rates using static gas chambers as outlined in Hutchinson and Mosier (1981). Gas samples were collected monthly during the growing seasons of 2006 (June-September) and 2007 (May-August) between noon and 14:30 hours. During each sampling, gas samples were taken through the rubber septum using a gas-tight 20 mL syringe (Norm-Ject, Henke Sass Wolf, Tuttlingen, Germany) at 0, 5, and 15 min after the chamber was placed on top of the PVC collar and stored in evacuated 10 mL soda glass Isomass Exetainers® (Labco Limited, Buckinghamshire, UK). Samples were analyzed using a Varian CP-3800 gas chromatograph (Varian Canada, Mississauga, Canada). Greenhouse gas emission rates were calculated based on gas concentration changes (slope of the regression line) in the chamber headspace over 15 min and the result expressed in mg m^{-2} day^{-1}.

2.5. Statistical Analysis

Prior to analysis, all data were tested for normality and homogeneity of variance, with a square root [microbial biomass C (5-15 cm), rates of nitrification (0-5 cm) and mineralization (0-5 cm) and CO_2 efflux] or logarithmic [rate of ammonification (0-5 cm), total N supply rate and ammonium supply rate] transforms performed where necessary.

To assess treatment effects, analysis of variance (ANOVA) was performed using mixed models, with warming and defoliation as fixed effects, and block as a random effect. To examine temporal variation in treatment responses within growing seasons, data were analyzed with sampling date as repeated measures.

We used the average temperature during the gas sampling hours from 10:00 to 15:00 hours, and soil moisture content, microbial biomass C and N at each sampling at both soil depths to examine the relationship between greenhouse gas fluxes and the above variables, using stepwise multiple regressions. Because of the limited number of replicates available in this study, we set the alpha value at 0.1 (Scheiner & Gurevitch, 2001). All analysis was performed using SAS statistical software (version 9.1, SAS Institute Inc.).

Results

3.1. Soil Temperature and Gravimetric Moisture Content

The use of OTCs enhanced ($p \leq 0.0001$) mean daily soil temperature in the top 5 cm of soil during 2006 and 2007 by 0.78 and 0.6 ºC, respectively. Additionally, warming interacted with defoliation in both years ($p \leq 0.04$). In the absence of defoliation, warming led to minor increases in soil temperature of 0.54

and 0.4 ºC in 2006 and 2007, respectively (Fig. 2-1). However, larger increases in temperature were evident when warming was accompanied by defoliation, with temperatures rising 1.02 and 0.78 ºC during 2006 and 2007 (Fig. 2-1). Soil temperatures also varied markedly throughout both growing seasons (Fig. 2-2).

In 2006, changes in soil moisture content were only evident in the deeper soil layer (5-15 cm) with respect to the treatments: defoliation decreased moisture content by 2% ($p \leq 0.02$). In 2007, moisture content in both soil depth was affected by a warming x defoliation interaction ($p \leq 0.07$). Under warmed conditions, the additional presence of defoliation decreased soil moisture content by 2% relative to plots without defoliation while in unwarmed conditions defoliation caused 1.3% increase in soil moisture content. Strong fluctuations in soil moisture content were evident in both years, peaking early in the year, declining to lows in midsummer, and rebounding by late summer (Fig. 2-2).

3.2. Microbial Biomass Carbon and Nitrogen

Microbial biomass carbon (MBC) averaged 2625.5±98 and 2678.2±75 (0-5 cm), and 3102.7±114 and 2646.5±99 (5-15 cm) kg ha^{-1} in 2007 and 2008, respectively. During 2006, soil MBC was unaffected by warming ($p > 0.10$) but had a defoliation x date interaction ($p \leq 0.09$) at both soil depths. These results indicated that defoliation decreased MBC by 546.3 and 781.2 kg ha^{-1} at the 0-5 and 5-15 cm soil depths, respectively, but only during the month in which defoliation treatments were applied (i.e. July 2006). In 2007, soil MBC responded to warming ($p = 0.06$) and defoliation ($p = 0.08$) in the 0-5 cm soil depth: MBC at

this depth declined by 182.1 and 192.8 kg ha^{-1} due to defoliation and warming, respectively. In addition, soil MBC at the 5-15 cm depth had a warming x defoliation interaction ($p = 0.05$) in 2007, whereby MBC was lowest in untreated plots as well as those with warming and defoliation (Table 2-1). Soil MBC at both soil depths varied within and between-growing seasons, reaching a peak in August and a low in July (Table 2-2).

Mean soil microbial biomass nitrogen (MBN) was 291.3±15 and 389.5±14 (0-5 cm), and 272.2±13 and 517.8±18 (5-15 cm) kg ha^{-1} in 2006 and 2007, respectively. In 2006, MBN in the 0-5 cm soil depth was affected by a warming x sampling date interaction ($p=0.07$), with warming increasing MBN by 106.3 kg ha^{-1} in the first month after OTC installation in June 2006. At deeper soil layer (5-15 cm), warming led to a general increase in MBN ($p=0.06$) of 33.3 kg ha^{-1} during 2006. In 2007, although there was no response in MBN in the 0-5 cm soil depth, MBN at the 5-15 cm soil depth was affected by a warming x defoliation interaction. Similar to MBC, MBN peaked in untreated plots, as well as those warmed and defoliated (Table 2-1). Soil MBN exhibited within and between-growing season variability (Table 2-2) with minimum MBN in July at either soil depth in both years.

3.3. Net N Mineralization Rates

Net ammonification, nitrification and N mineralization rates were 0.001±0.006, 4.2±0.26, 4.2±0.25 (2006) and 0.01±0.02, 1.3±0.18, 1.5±0.19 (2007) kg^{-1} ha^{-1} day^{-1}, in the top 5 cm of soil, At the 5-15 cm soil depth, these

same responses were 0.001±0.006, 0.3±0.02, 0.3±0.02 (2006), and 0.16±0.02, 0.16±0.01, 0.20±0.02 (2007) kg^{-1} ha^{-1} day^{-1}. Neither the main effects of warming nor defoliation effected net N mineralization in either soil depth during the study ($p > 0.10$). When the effect of warming was significant at the 0-5 cm soil depth, it interacted with sampling date in 2007. Warming x date influenced net N nitrification and mineralization in 2007 within the 0-5 cm soil depth ($p \leq 0.01$), with warming increasing nitrification and mineralization rates by up to 54 and 52%, respectively, in May. Furthermore, warming and defoliation interacted to influence net N nitrification and mineralization in the 5-15 cm soil depth in 2006 (Table 2-1). At this depth, warming and defoliation alone increased N nitrification and mineralization, while the combination of the two reduced these parameters to values similar to those of the untreated plots (Table 2-1). Marked variation was also evident in net N ammonification, nitrification and consequently mineralization rates within and between-growing seasons at each soil depth in both years (Table 2-2).

3.4. Soil N Supply Rate

Total soil inorganic-N supply rate (8.2 µg N 10 cm^{-2} 30 day^{-1}) in 2007 was comprised approximately equally of nitrate (4.1 µg N 10 cm^{-2} 30 day^{-1}) and ammonium (4.2 µg N 10 cm^{-2} 30 day^{-1}). Total inorganic-N and NO_3^- supply rates were not affected by warming or defoliation ($p \geq 0.17$). However, soil NH_4^+ supply rate was affected by a three-way interaction of warming, defoliation and sampling date ($p \leq 0.07$): warming changed soil NH_4^+ supply rates by +98% and -

23%, respectively, in non-defoliated and defoliated plots, shortly after spring snowmelt in May 2007. Unlike other soil variables, total inorganic-N, nitrate and ammonium supply rates did not vary throughout the growing season ($p \geq 0.14$).

3.5 Soil Greenhouse Gas Emission

The average rate of C respiration in the form of CO_2 was 51.2±4.4 and 82.2±6.4 mg C m^{-2} hr^{-1}), respectively, in 2006 and 2007. Soil CO_2 efflux was influenced by warming x defoliation x sampling date in 2006 ($p = 0.02$), with no significant responses in CO_2 in 2007 ($p \geq 0.10$). During 2006, warming-induced changes largely in June and July (Fig. 2-3a). In June, warming tended to change CO_2 efflux by +35% and -46% in the absence and presence of defoliation. In July, the effect of warming was only evident at defoliated plots, showing a sharp increase of 148% in CO_2 efflux. Nevertheless, only 18% of variation in CO_2 could be explained by the combination of soil surface temperature, gravimetric moisture content and MBC and MBN in the 0-5 cm soil depth. Soil respiration varied within and between growing seasons (Table 2-3).

The grassland examined here was a net methane (CH_4) sink (Fig. 2-3b), with an average CH_4 uptake rate of -0.020±0.002 and -0.024±0.001 mg C m^{-2} hr^{-1}, respectively, in 2006 and 2007. CH_4 uptake showed no significant response to either warming or defoliation ($p \geq 0.11$). Mean daytime soil surface temperature, soil moisture content, MBC and MBN at the 5-15 cm soil depth, and MBN at the 0-5 cm soil depth, collectively explained only 5% of CH_4 efflux. Strong temporal variability in CH_4 efflux was evident in both growing seasons (Table 2-3),

reaching a minimum CH_4 consumption at the end of each growing season (Fig. 2-3b).

On average, the grassland soils examined here were a net N_2O source in 2006 and a net sink in 2007, with an average rate of 0.0017±0.0008 and -0.0014±0.002 mg N m^{-2} hr^{-1}, respectively. Soil N_2O emission did exhibit a warming (in 2006) and warming x defoliation interaction (in 2007, Table 2-1). Warming in 2006 changed plots from an N_2O source (0.003 mg N m^{-2} hr^{-1}) to a sink (-0.0002 mg N m^{-2} hr^{-1}). A similar trend was evident in the absence of defoliation in 2007 (Table 2-1), but the addition of defoliation to warming turned plots into a source of N_2O. Mean daytime soil surface temperature, soil moisture content, MBC and MBN in the 0-5 and 5-15 cm soil depths, explained only 2% of the variation in soil N_2O emissions. No clear seasonal trend was observed in N_2O emission throughout the study (Table 2-3).

Discussion

During the two-year study, warming and defoliation had limited impacts on C and N dynamics in this temperate grassland ecosystem. However, some important transient effects on microbial biomass and carbon dioxide evolution occurred soon after the treatments were applied, suggesting that the belowground function in this ecosystem has the potential for rapid recovery and acclimatization to defoliation and warming. As acclimatization happen soon after the treatment application in 2006 and continued in 2007 (lower average temperature and higher average precipitation than 2006), the acclimatization in this system occurred

regardless of the average weather conditions during the years. While the limited impacts of defoliation and warming on belowground C and N dynamics was inconsistent with our hypothesis and previous findings (Wan *et al.,* 2005; Klein *et al.,* 2007), this is not surprising given the limited responses in aboveground community i.e., productivity and diversity (Bork *et al.,* 2008). A number of factors could have caused this rapid acclimatization, possibly driven by substrate depletion, changes in enzymatic activity and belowground community structure (Rinnan et al., 2007) or high plant biodiversity at this site (Lamb, 2008) and associated microbial biodiversity (Garbeva *et al.,* 2006). However, shifts in belowground community structure can not be a reason in this study, showing no significant responses to warming and defoliation (Chapter 3). The fact acclimatization suggest the proposed positive feedback between soil C release and global warming in climate change models could be dampened due to rapid acclimatization of grassland ecosystems. However, warming also facilitated N_2O loss, particularly in the presence of defoliation, suggesting N_2O could be the main contributor to greenhouse gas emissions under improper grazing strategies in this temperate grassland.

4.1. Warming and Defoliation Effects on MBC and MBN

Changes in soil MB were influenced by warming and defoliation. The induce changes of warming x defoliation was dependent on soil microclimate: soil moisture ($p = 0.07$), soil temperature ($p = 0.04$). This suggests the importance of both direct and indirect effect of warming and defoliation on soil microbial

biomass. While many previous studies suggest that microbial biomass is relatively unaffected by experimental warming in various ecosystems (Jonasson *et al.*, 1999) the interactive effects found here of warming and defoliation on microbial biomass are consistent with those found in grassland ecosystems elsewhere (Zhang *et al.*, 2005). One critical aspect of our study was the contradictory responses of soil MBC and MBN to the main effect of warming. While the positive response of soil MBN to warming could be explained by direct or indirect effects of temperature (i.e., on substrate quality; see Zhang *et al.*, 2005), the decrease in soil MBC was unexpected. Considering the response of soil CO_2 respiration to warming x defoliation x date in this site during July and August 2006 (Table 2-1), the decrease in soil MBC may reflect the loss of soil organic C as CO_2 or in other forms such as labile soil C. Alternatively, given that the effect of warming in the following year depended on the presence of defoliation, other mechanisms may account for these results. For example, direct and indirect effects may be occurring following defoliation such as changes in root exudates (Holland, 1995), litter quantity and quality (Park & Matzner, 2003), or soil microclimate (Sayer, 2006), all of which may either offset or encourage the effect of warming in this soil system. No changes in litter quantity due to the main effect of warming in this study (Bork *et al.*, 2008) may simply discard the hypothesis of litter quantity effect on soil microbial biomass in this study. Overall, the net effect of warming and defoliation on soil microbial biomass may depend on how the biological and chemical processes involved in soil microbial growth respond and offset one another (Wardle, 1992). Furthermore, the transient effect of warming

and defoliation (20% decrease) during the first year, and smaller magnitude of MB changes (≈ 7%) in the following year suggests that microbial biomass could quickly adapt to the warmer conditions associated with aboveground biomass removal, in turn leading to stability within the active C pools of this grassland.

Seasonal changes in soil microbial biomass were expected through direct effects of soil moisture content and temperature, and indirect effects of changing plant productivity over a season (Bristowa & Jarvis, 1991; Wardle, 1992). When plants are growing, nutrient uptake is faster (Barbour & Billings, 2000), and thus, high competition occurs between plants and soil microbes. In this study, high N competition between vegetation and microbes appeared evident through the larger reduction in soil MBN but not MBC, in July 2006 and 2007 (Table 2-2), during peak vegetation growth.

1.2. Warming and Defoliation Effects on N Dynamics

The effects of warming and defoliation on nitrogen dynamics were mainly evident in the first year, when their interaction caused a marked change in N nitrification and mineralization rates by up to 36% in the deeper soil depth; however, this response disappeared the following year. The effect of warming x defoliation was dependent of soil moisture ($p = 0.07$) and temperature ($p = 0.04$) as discussed above. Following changes in net N mineralization, plant N content was also influenced by warming x defoliation in the first year but not in the following year (Bork *et al.*, 2008). No lasting effects of defoliation were expected in this grassland ecosystem. Hamilton and Franks (2001) reported short-term

effects of defoliation on soil N availability in a northern mixed grassland that lasted less than a week. However, net N responses in grasslands can also be influenced by edaphic characteristics and defoliation intensity (Seagle *et al.*, 1992). Site-specific characteristics might also explain why warming had limited impacts on N mineralization in this study. Experimental warming of 0.3 to 6 °C has been found to increase net N mineralization by 46% on average in terrestrial ecosystems (Rustad *et al.*, 2001). Considering the lack of response in N mineralization in the second year, N dynamics in this northern temperate grassland may have a high potential to acclimatize to warming and recover from aboveground biomass removal in the short-term. Conversely, larger responses might occur following a time lag, and could therefore only be observed in long-term studies. This acclimatization, together with the fact that N is a major limitation to growth in terrestrial lands (Hungate *et al.*, 2003), and the positive correlation between N availability and plant productivity (Harner & Harper, 1973) may weaken any positive effects of higher temperature and/or CO_2 enrichment on net primary productivity. This also suggests that earlier climate change models (i.e., Rastetter *et al.*, 1997) may have overestimated the potential of the biosphere to sequester C through photosynthetic pathways.

Seasonal changes in soil N mineralization, including ammonification and nitrification, are largely controlled by plant characteristics (Antil *et al.*, 2001) and the soil environment such as moisture availability (Piccolo *et al.*, 1994). While soil moisture content explained only 32 % (0-5 cm) and 15 % (5-15 cm) of the total variability in N mineralization (data not shown), other abiotic and biotic

characteristics (e.g., plant phenology) might also mediate N mineralization. In contrast, inorganic-N supply rate showed no seasonality (Table 2-2). One potential explanation could be that N is relatively deficient in this ecosystem. Under these conditions, high N competition in the plant-soil microbe system could have caused PRS^{TM} probes to act as a dynamic N exchanger with other N reservoirs instead of solely as a N sorption system (Meason & Idol, 2008), thereby desorping the N already absorbed in the resin membrane. To decrease the probability of desorption phenomena, it is recommended to isolate the PRS^{TM} probes from plant roots by using PVC collars (Western Ag Innovations Inc.). However, in this study we were unable to use collars due to the restricted plot size employed.

4.3. Warming and Defoliation Effects on Greenhouse Gas Emissions

Temporal variation in soil CO_2 respiration was expected with varying plant phenology and physiology (Davidson & Holbrook, 2009) and changing climatic conditions. Significant responses to warming and defoliation were limited to early in the trial near the date of defoliation, with no changes in soil respiration in August 2006 and the following year. The effects of warming on soil respiration was not influenced by warming-induce changes on soil moisture ($p= 0.58$). Considering no differences in root biomass due to our treatments (measured in August 2006; Bork *et al.,* 2008), the transient response of soil CO_2 efflux to warming and defoliation, and the weak correlation between CO_2 efflux and soil temperature, we conclude that the overall sensitivity of soil autotrophic and

heterotrophic respiration to temperature is low, consistent with previous studies (Johnson *et al.*, 2000; Thornley & Cannell, 2001; Luo *et al.*, 2001). Soil respiration appears to rapidly acclimatize to warming and promptly recover from defoliation in this temperate grassland. Acclimatization could offset the additional soil C release to the atmosphere in a warmer climate, at least for a short term, suggesting that earlier climate-carbon models may overestimate the positive feedback effect of global warming on the global C cycle. Another source of uncertainty in C budgeting is the amount of CH_4 consumption/release.

Similar to previous findings (Mosier *et al.*, 1991) but unlike the observations of Jones *et al.* (2005) in managed grassland, the northern temperate grassland studied here appeared to be a CH_4 sink of between -0.05 and 0 mg C m^{-2} hr^{-1} (Fig. 2-3b). Thus, the potential of this grassland ecosystem to serve as a CH_4 sink was not affected by warming or defoliation. Moreover, overall C release to the atmosphere at this site in the form of CO_2 and CH_4 were not stimulated by warming under defoliation, which is in agreement with several previous studies (e.g., Christensen *et al.*, 1997; Briones *et al.*, 2004). However, the fairly stable C balance in this temperate grassland also contrasts other investigations (e.g., Ruess *et al.*, 1999).

While soil N_2O efflux remained stable seasonally, it was found to be sensitive to warming and dependant on soil temperature ($p < 0.0001$), which is consistent with the notion that soil temperature can both directly and indirectly influence soil N_2O emission (Conrad, 1996). However, N_2O sensitivity to soil warming in the second year of our study also depended on the presence and absence of

defoliation. Warming suppressed N_2O emissions in the absence of defoliation while encouraging it following defoliation. This suggests the importance of defoliation-induced changes on plant community rhizodeposition and soil microclimate with warming changed the ecosystem from a sink to a source of N_2O. The negative effect of warming on soil N_2O efflux was surprising and contrasted previous findings in teresteerial ecosystems (e.g., Bijoor *et al.,* 2008). Nitrous oxide is a product in different dissimilatory pathways, such as the sequential reduction of nitrate by denitrifying bacteria and nitrite reduction by nitrifying bacteria (Conrad, 1996), processes that change the proportion and consumption of gaseous end products (Davidson, 1991). As net effluxes of N_2O depend on the tradeoff between all these processes in the soil, they therefore remain unpredictable.

A more important question here perhaps is the extent to which warming could encourage N_2O emission in this defoliated temperate grassland system? While this grassland soil acted as an N_2O source, the rate of N_2O emission was approximately 0.003 mg m^{-2} hr^{-1}. Considering the typical range of N_2O production in rangeland ecosystems is from 0.001 to 0.01 mg m^{-2} hr^{-1} (Burke *et al.,* 2002), and the reality that N_2O contributions to global warming are nearly 310 times greater than that of CO_2 (IPCC, 2001), N_2O in this grassland could be a small contributor potential global warming, even considering its CO_2 equivalent (0.93 mg CO_2 m^{-2} hr^{-1}).

Conclusions

Experimental warming and defoliation had limited impacts on the grassland ecosystem examined over the two-year study period. As a result, this northern temperate grassland appears resistant to climate change in the short term. However, the underlying mechanisms for such fast acclimatization are not well understood. We hypothesize that the high plant biodiversity and productivity of the grassland studied may be one of the main reasons for its resistance to climate change in the short term and/or rapid acclimatization. Acclimatization of ecosystem processes has the potential to offset the negative effect of warming by weakening any positive feedback of C and N loss from the ecosystem on the climate system. Although positive feedback could happen in temperate grasslands between warming and global C cycling, this process does not appear to occur as rapidly as expected or to the magnitude anticipated from current predictions of climate-carbon coupling models. Finally, long-term responses need to be studied and will be of value to further our understanding of warming and grazing management interactions in northern temperate grassland ecosystems.

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Table 2- 1. Summary of ANOVA analysis results for soil C and N fluxes in the deep soil layer (5-15 cm) during the 2006 and 2007 growing seasons. Data are means ± 1 standard error.

Source	Year	Pr > F	Non-defoliated		Defoliated	
			Unwarmed	Warmed	Unwarmed	Warmed
MBC	2006	0.18	3017±242	3365±241	3071±234	2955±200
	2007	**0.05**	2545±217 [b]	2764±181 [a]	2795±237 [a]	2481±158 [b]
MBN	2006	0.46	258± 24	304± 27	252± 29	273± 24
	2007	**0.001**	483± 41 [b]	554± 37 [a]	546± 40 [a]	486± 30 [b]
NAR	2006	0.89	0.001± 0.0013	0.006±0.0014	-0.004± 0.0012	0.002± 0.0014
	2007	0.85	0.16± 0.03	0.19± 0.04	0.15± 0.04	0.16± 0.06
NNR	2006	**0.08**	0.32± 0.07 [b]	0.42± 0.04 [a]	0.45± 0.04 [a]	0.35± 0.06 [b]
	2007	0.66	0.16±0.02	0.17± 0.03	0.17± 0.02	0.16± 0.04
NMR	2006	**0.07**	0.30± 0.07 [b]	0.41± 0.04 [a]	0.40± 0.04 [a]	0.33± 0.06 [b]
	2007	0.60	0.19± 0.04	0.21± 0.04	0.21± 0.04	0.20± 0.05
CO_2	2006	0.83	48± 8.7	52± 8.5	51± 10	51± 8.4
	2007	0.10	89± 14	74± 12	81± 14	83± 11
CH_4	2006	0.11	-0.016± 0.0038	-0.02± 0.0051	-0.026± 0.0039	-0.018± 0.0046
	2007	0.84	-0.024± 0.003	-0.021± 0.0037	-0.027± 0.0036	-0.022± 0.0032
N_2O	2006	0.37	0.002± 0.002	0.0004± 0.001	0.004± 0.0012	-0.0008± 0.001
	2007	**0.02**	0.003± 0.0019 [a]	-0.003± 0.002 [b]	-0.009± 0.007 [b]	0.004± 0.003 [a]

MBC = Microbial Biomass C, MBN = Microbial Biomass N, NAR = Ammonification Rate, NNR = Nitrification Rate, NMR = Net Mineralization Rate

Table 2- 2. Summary of ANOVA analysis results for temporal changes in soil C and N fluxes throughout the 2006 and 2007 growing seasons at both the 0-5 and 5-15 cm soil depth. Data are means ± 1 standard error.

Variables	Pr > F	Year	May	June	July	August
<u>0-5 cm Soil Depth</u>						
MBC (kg ha^{-1})	0.67	2006	-	2616±154	2543±196	2716±164
	≤.0001	2007	2932±143 ^{a}	2419±124 ^{b}	2305±122 ^{b}	3055±147 ^{a}
MBN (kg ha^{-1})	**0.0001**	2006	-	350±23 ^{a}	174±12 ^{b}	348±24 ^{a}
	0.04	2007	446±27 ^{a}	359±32 ^{bc}	340±26 ^{c}	411±25 ^{ab}
Ammonification	**0.0001**	2006	-	0.02±0.01 ^{a}	0.03±0.01 ^{a}	-0.04±0.006 ^{b}
	0.0001	2007	0.33±0.04 ^{a}	0.09±0.02 ^{b}	0.07±0.02 ^{b}	-
Nitrification	**0.0001**	2006	-	4.6±0.31 ^{b}	2.0±0.17 ^{c}	5.9±0.32 ^{a}
	0.0001	2007	2.7±0.34 ^{a}	1.3±0.1 ^{b}	0.09±0.007 ^{c}	-
Mineralization	**0.0001**	2006	-	4.6±0.31 ^{b}	2.1±0.17 ^{c}	5.8±0.32 ^{a}
	0.0001	2007	3.0±0.35 ^{a}	1.4±0.1 ^{b}	0.17±0.02 ^{c}	-
<u>5-15 cm Soil Depth</u>						
MBC (kg ha^{-1})	**0.0002**	2006	-	2575±163 ^{b}	3467±218 ^{a}	3265±155 ^{a}
	0.0001	2007	2460±143 ^{b}	2329±161 ^{b}	2270±106 ^{b}	3525±229 ^{a}
MBN (kg ha^{-1})	**0.0001**	2006	-	300±19 ^{b}	167±14 ^{c}	348±12 ^{a}
	0.0001	2007	542±23 ^{b}	566±27 ^{b}	316±16 ^{c}	645±35 ^{a}
Ammonification	**0.01**	2006	-	0.02±0.01^{a}	0.03±0.01^{a}	-0.04±0.006 ^{b}
	≤.0001	2007	0.33±0.04 ^{a}	0.1±0.02 ^{b}	0.07±0.02 ^{b}	-
Nitrification	**0.0001**	2006	-	0.41±0.04 ^{b}	0.21±0.03 ^{c}	0.51±0.04 ^{a}
	0.0001	2007	0.29±0.02 ^{a}	0.14±0.01 ^{b}	0.07±0.007 ^{c}	-
Mineralization	**0.0001**	2006	-	0.39±0.04 ^{a}	0.20±0.03 ^{b}	0.49±0.05 ^{a}
	0.0001	2007	0.38±0.03 ^{a}	0.16±0.02 ^{b}	0.05±0.01^{c}	-

MBC = Microbial Biomass C, MBN = Microbial Biomass N

Table 2- 3. Summary of ANOVA analysis results for soil greenhouse gas effluxes during the 2006 and 2007 growing seasons. Data are means ± 1 standard error.

Source	Pr > F	Year	May	June	July	August	September
CO_2	**0.0001**	2006	-	67±6.7 [b]	30±4.2 [c]	92±7.8 [a]	14±1.3 [d]
	0.0001	2007	112±4.6 [b]	147±10.9 [a]	42±5.6 [c]	28±2.5 [d]	-
CH_4	**0.0001**	2006	-	-0.03±0.005 [a]	-0.03±0.004 [a]	-0.017±0.002 [b]	-0.004±0.002 [c]
	0.0001	2007	-0.03±0.003 [a]	-0.02±0.003 [b]	-0.02±0.003 [b]	-0.01±0.002 [c]	-
N_2O	0.56	2006	-	0.0036±0.002	0.0006±0.001	0.0014±0.001	0.0013±0.002
	0.58	2007	0.0008±0.003	0.002±0.002	-0.006±0.008	-0.002±0.002	-

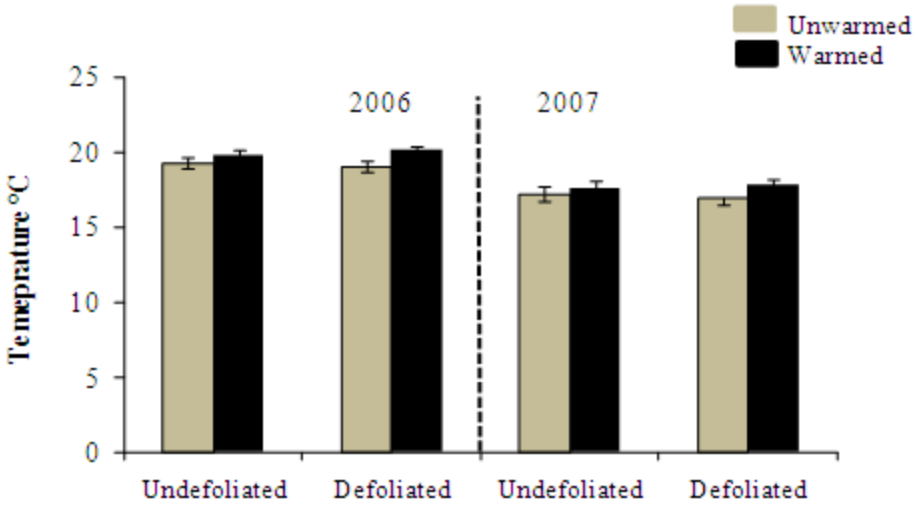

Figure 2- 1. Defoliation and warming effects on average daily soil surface temperature for the 2006 (left) and 2007 (right) growing seasons. Error bars are1 SE.

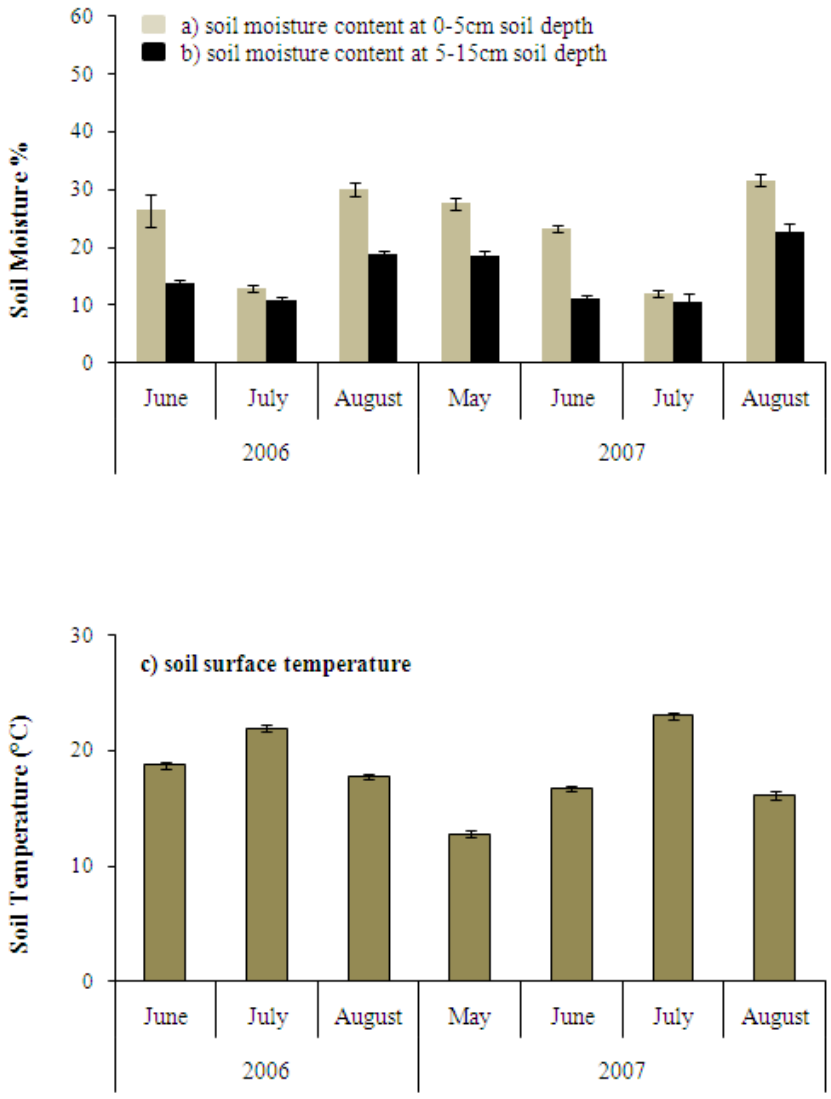

Figure 2- 2. Defoliation and warming effects on monthly soil gravimetric moisture content in the (a) 0-5 cm depth, and (b) the 5-15 cm soil depth and (c) soil temperature at 0 cm soil depth for the 2006 and 2007 growing seasons. Error bars are 1 SE.

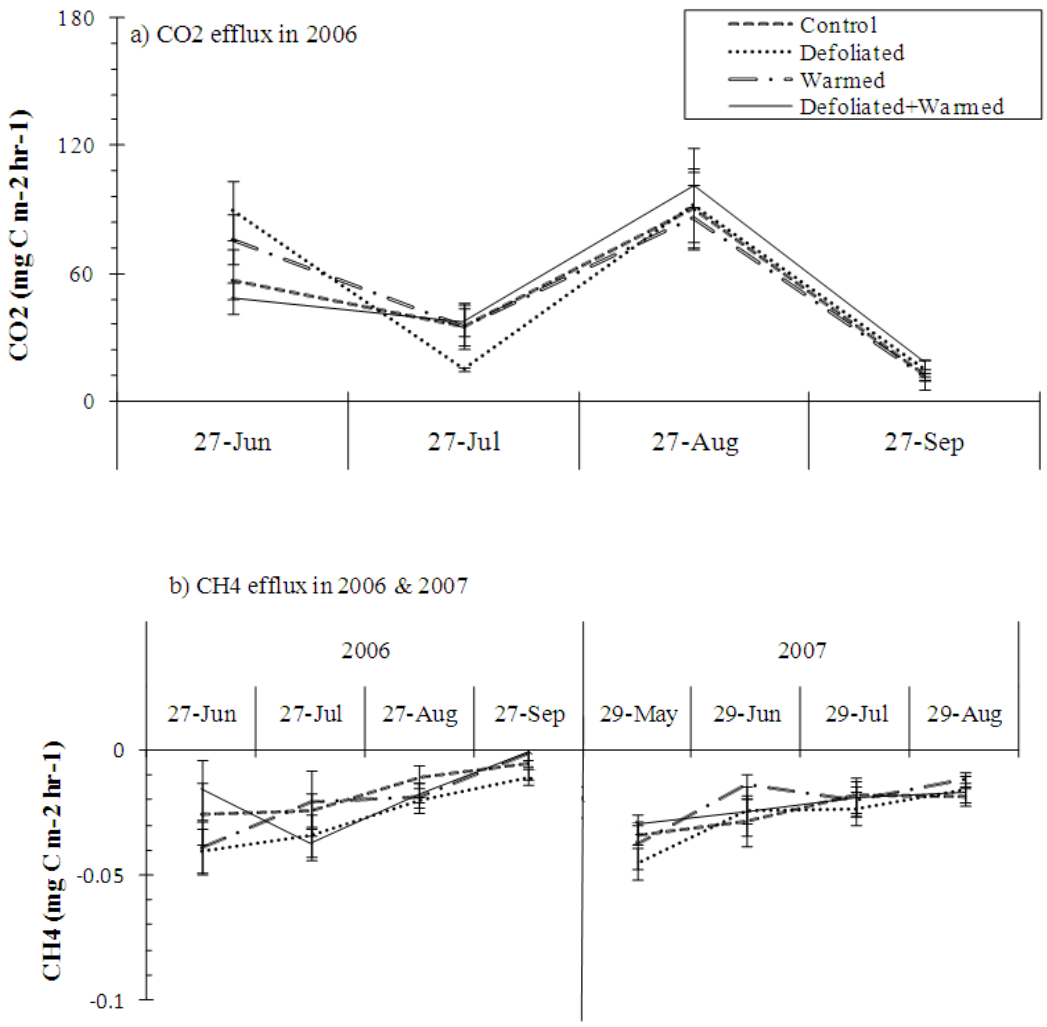

Figure 2- 3. Defoliation and warming effects on monthly soil greenhouse gas emissions of (a) carbon dioxide (CO_2) in 2006 and (b) methane (CH_4) in both the 2006 and 2007 growing seasons. Error bars are 1 SE.

Chapter 3 Stability of Soil Microbial Community Composition in Response to Warming and Defoliation in a Native Grassland (Ecosite A)

Introduction

Understand and adapting to ongoing global warming is a major scientific challenge for scientists and land managers. Both experimental and theoretical evidence suggest that feedback in the carbon cycle can amplify global warming (Woodwell & Mackenzie, 1995; Cox *et al.*, 2000). Fundamental knowledge of specific biological mechanisms regulating carbon (C) cycling is of key importance in adapting to the global impacts of anthropogenic warming (Heimann & Reichstein, 2008). Among biomes, grasslands comprise up to 80% of terrestrial lands, and represent an important piece of the global carbon cycling puzzle (Lund, 2007). Furthermore, with considerable phytomass allocated to roots (Chen *et al.*, 2006), grasslands provide large C storage belowground (Briones *et al.*, 2009). Coupled with a relatively slow decomposition rate and reduced risk of fire, grasslands are a significant global carbon sink (Hunt *et al.*, 2002).

Representing the single largest pool of living organic carbon belowground, soil microorganisms are essential to C dynamics (i.e., organic matter decomposition) in grassland ecosystems (Coleman *et al.*, 2004). Additionally, the high diversity of soil microorganisms (i.e., functional diversity) within grassland soils (Clegg & Murray, 2002), particularly in temperate grasslands (Curry, 1994), may help stabilize ecosystem function and resist the influence of environmental stress including a changing environment (Rooney *et al.*, 2006). Consequently,

there is concern over how changes in soil microorganisms as influenced by global warming and management practices (e.g., grazing) in grassland ecosystems may change ecosystem stability and function, including the potential for C sequestration (Klumpp *et al.*, 2009).

There have been several studies addressing the impacts of climatic warming and grazing on soil biota. However, results of experimental warming (e.g., Zhang *et al.*, 2005; Rinnan *et al.*, 2007; Frey *et al.*, 2008; Rinnan *et al.*, 2009) and grazing (Zhang *et al.*, 2005; Klumpp *et al.*, 2009; Rinnan *et al.*, 2009) have not showed consistent impacts on soil biota. For instance, Zhang *et al.* (2005) reported an increase in the fungi-to-bacteria ratio in a tallgrass prairie on the U.S. Great plains, while Rinnan *et al.* (2007) observed a decrease in the relative abundance of soil fungi in subarctic heath region, Finland. Similarly, grazing led to shifts in soil microbial community composition (Zhang *et al.*, 2005; Klumpp *et al.*, 2009) and function (Rinnan *et al.*, 2009), but left no detectable changes on the soil microbial community (Denef *et al.*, 2009; Rinnan *et al.*, 2009) of various grassland ecosystems.

One plausible explanation of such variable responses could be the effects of site characteristics. It is apparent that natural heterogeneity in terrestrial ecosystems can influence belowground community responses to climate change and grazing management. Although natural variability has often been considered as random effects hindering attempts to draw general conclusions, it also is known to have a predictable pattern (Ettema & Wardle, 2002). As a result, studying soil microbial communities across different ecological scales is

suggested as a step forward to ensure the accurate prediction of heterogeneity in ecological structure and function of both aboveground and belowground communities (Ettema & Wardle, 2002). Furthermore, understanding soil microbial structure and function at relevant spatial scales is the key in reducing uncertainties regarding the projection of future climate change impacts (Pendeall *et al.,* 2008). Nevertheless, soil studies conducted at the local level are an initial step for the prediction of soil microbial structure and function under grazing practices in a warmer world.

Studies conducted to date on soil microbial community responses, to both climatic warming and grazing are rare and produce inconsistent results, the impacts of both climatic warming and grazing strategies on soil microorganisms and consequently possible climate-microbe feedback remain a major source of uncertainty in the global C budget. More detailed studies of the impacts of warming and grazing on soil microbial community are needed across different grassland ecosystems.

The goal of this research was to understand how warming and grazing influence the soil microbial community in northern temperate grassland ecosystems. The main objective was to investigate the response of soil microbial communities, including structure, composition and function, to warming and defoliation in a northern temperate grassland ecosystem in Alberta, Canada over a two-year period, and to determine whether the responses have any influence on ecosystem feedback to climate change.

Materials and Methods

2.1. Site Description

The two-year study was conducted in a native plains rough fescue (*Festuca hallii*) grassland at the University of Alberta Research Station near Kinsella, Alberta, Canada (53°05 N, 111°33 W) beginning in June 2006. The site has a continental climate with the long-term (1971-2000) average precipitation of 431.3 mm and temperature of 2.8 °C and average long-term (1971-2000) growing seasons (May- September) temperature and precipitation were 13.9 °C and 312.2 mm, respectively. Average precipitation and temperature during the growing seasons of the years of study were 181.2 mm and 16.1 °C in 2006 and 211.4 mm and 13.9 °C in 2007 growing seasons. The average temperature and precipitation was different at the time of soil sampling (July) in 2006 and 2007 with the cooler (by 1.3 °C) and drier (by 16.8 mm) condition in July 2007 than July of 2006.

The soil on the study site was an Orthic Dark Brown Chernozem (Howitt, 1988) with a 15 cm Ah horizon, loamy texture (clay: 21.8%, silt: 33%, sand: 45.2%) and pH (in 0.01 mol L^{-1} $CaCl_2$) of 5.7. The study site was selected for three main reasons: (1) High plant diversity is known to occur on the site (Coupe, 2003), and hence should lead to relatively high soil microbial diversity (Porazinska *et al.*, 2003). Vegetation is primarily *Festuca hallii* and *Stipa curtiseta* as dominants, but up to 53 vascular species have been observed in the immediate area (Coupe, 2003); (2) The site is at the grassland-forest boundary and susceptible to climatic fluctuations (Vance *et al.*, 1979), so changes in plant community diversity and composition could be expected under warmer

conditions; (3) The northern temperate grasslands as a component in northern hemisphere in temperate latitude are generally considered a net C sink (Ciais *et al.*, 1995; Pacala *et al.*, 2001). Thus, even small changes in belowground community function in this grassland ecosystem may have important implications for global C sinks.

2.2. Experimental Design

Twenty plots, each 2 m× 2 m in size, were arranged in a randomized block design (n=5 blocks) to evaluate the effects of warming and defoliation on the soil microbial community in 2006- 2007. Each block included four treatments: the untreated control (C), warming (W), defoliation (D) and the combination of warming and defoliation (WD). Warming was achieved through circular open-top chambers (OTCs, 2 m diameter × 0.4 m height). Defoliation was applied manually by clipping vegetation to 5 cm stubble height in mid June of 2006. Plots were not defoliated in 2007 but rather left to examine the residual impact of defoliation from the previous year. Methodological details on treatment application and soil microclimate are discussed in Chapter 2. In brief, OTCs successfully increased the mean daily soil surface temperature by +0.54 and +0.4 ºC in 2006 and 2007, respectively, in the absence of defoliation, and by +1.02 and +0.78 ºC in 2006 and 2007, respectively, with defoliation.

2.3. Soil Sampling

Composite soil samples of three soil cores (5cm diameter × 10 cm) were obtained from the 0-10 cm soil depth.. Soil sampling was done on 27 July 2006 and 29 July 2007 at approximately peak vegetation growth. Fresh soil samples were then promptly cooled, transported to the lab, and sieved through a 4-mm mesh, with all visible plant/root material removed. Two soil subsamples for the assessment of soil microbial structure (PLFA analysis) and physiological function (Biolog™ analysis) were then removed, and kept at -20 and 4°C, respectively, for further analysis.

2.4. Soil Microbial Community Structure

To study soil microbial structure, PLFA profiles were obtained from 10 g of freeze-dried soil following the method described by Bossio *et al.* (1998). To identify PLFAs, the standard protocol of Sherlock Microbial Identification System $V_{3.1}$ (MIDI, 1995) using a Gas Chromatograph (Hewlett Packard 5890 A, Hewlett-Packard Co., Avondale, PA, USA) was followed. Fatty acid nomenclature followed that described by Bossio *et al.* (1998).

For a given sample, the abundance of PLFA was expressed as relative abundance (mol % of the total PLFAs) and categorized to the following taxonomic groups: bacteria, fungi, actinomycete, gram positive bacteria, gram negative bacteria, and arbuscular mycorrhizae fungi (AM) as described in Table 3-1.

2.5. Soil Microbial Community Function

The physiological function of soil bacteria and fungi was determined by assessing the community-level physiological profile (CLPP) based on the utilization of arrays of carbon substrate, using Biolog™ microplates (Biolog Inc., Hayward CA., USA): ECO and SF-N2 for bacteria (2006 & 2007) and fungi (2007), respectively. Methodological details are provided in Chapter 4. In brief, the area under the curve of color development in each well (C substrate) was measured over a 7-day period, thereby providing an estimate of the utilization of each designated C substrate. In addition, the average well color development (AWCD) of all available C substrate in each Biolog microplate was calculated and expressed as the overall metabolic potential in a microplate (Garland & Mills, 1991). To test the effects of warming and defoliation on organic compounds, the C substrate available within Biolog™ microplates was grouped into the major substrate types for ECO and SF-N2. The ECO plate consisted of carbohydrate, polymer, carboxylic acid, phosphorylated chemical, amino acid, amine, and ester compounds (Jena *et al.*, 2006), while the SF-N2 plates were grouped into carbohydrate, polymer, carboxylic acid, phosphorylated chemical, amino acid, amine, ester, alcohol, and nucleoside compounds (Petrucci *et al.*, 2002).

2.6. Statistical Analysis

Prior to analysis, measures of Shannon diversity index (*H*) were computed for each sample according to the following equation:

$$H = -\sum p_i \ln p_i,$$

where p_i is the proportion represented by each PLFA relative abundance or C substrate utilization relative to their totals.

All univariate analyses were performed using SAS v 9.1 (SAS Institute Inc., 2003). The effects of warming and defoliation on Shannon diversity index, physiological function, metabolic potential (AWCD), relative abundance of microbial taxonomic groups, and the ratio of fungi-to-bacteria were analyzed using the GLIMMIX macro, with normal distribution and identity link-function (Shannon diversity index, physiological function, AWCD), or gamma distribution and log link-function (relative abundance of microbial taxonomic groups, ratio of fungi-to-bacteria).

The existence of corresponding biotic (soil microbial structure and function) and abiotic (i.e. environmental) variables from the same soil samples and plots allowed us to explore the relationships among these factors. Shannon diversity index values were subjected to multiple regression analysis against explanatory variables including: microbial biomass carbon (MBC) and nitrogen (MBN), as well as soil temperature and moisture content (Chapter 2), using the stepwise procedure in SAS with a minimum alpha value for entry of $p<0.10$. To control for the effect of year, the CLPP dataset for ECO microplates was analyzed separately by years. An alpha value of 0.1 was again used for significance due to low replication (Scheiner & Gurevitch, 2001).

The effects of warming and defoliation on C substrate utilization and microbial structure patterns in soil bacteria and fungi communities, based on the PLFA and CLPP datasets, were analyzed with multivariate analysis using PC-

ORD version 5.10 (McCune & Mefford, 2006). Relationships between the soil microbial community under warming or defoliation in ordination space were assessed using non-metric multidimensional scaling (NMS) (Kruskal, 1964). The NMS autopilot was applied on the PLFA and CLPP datasets using the Sørensen distance metric. Two matrices were used in soil microbial community structure analysis: the main matrix (soil samples × PLFAs relative abundance) and a secondary matrix (soil sample × explanatory variables and microbial taxonomic groups). Similarly, during microbial physiological function analysis, two matrices were used consisting of: the main matrix (soil samples × color development for individual C substrates) and a secondary matrix (soil samples × explanatory variables and substrate types). Relationship between plots and associated explanatory variables in ordination space were examined by overlaying variables as joint plots, as determined by Pearson and Kendall correlations of explanatory variables with the ordination axes. Multi-Response Permutation Procedures (MRPP) using Euclidian distance measures were used to test for differences in soil microbial community structure and functional diversity with respect to the initial treatments.

Results

3.1. Soil Microbial Community Function

The metabolic potential (AWCD) of soil bacteria was not affected by either main treatment in 2006 and 2007 ($p \geq 0.16$). However, bacterial utilization of polymers ($p = 0.03$) and amino acids ($p = 0.07$) in 2006, and carbohydrates ($p =$

0.03) in 2007, was affected by an interaction of warming x defoliation (Fig. 3-1). In the case of polymers and carbohydrates, warming suppressed microbial C utilization , but only within defoliated plots. For amino acids, warming increased microbial activity, but only in the absence of defoliation (Fig. 3-1).

NMS ordination (two-dimensional solution) explained 91% of the total variance in bacterial carbon substrate utilization in 2006 and 2007. There was no clear separation however, in the data with respect to warming in 2006 (MRPP, p = 0.32, A=0.0007) or 2007 (MRPP, p = 0.42, A= -0.001), nor with respect to defoliation in 2006 (MRPP, p = 1.00, A= -0.01) or 2007 (MRPP, p = 0.38, A= -0.0003). During 2006, a positive correlation was found between carboxylic acid utilization by bacteria and axis 1 from the associated NMS. One year later in 2007, axes 1(negatively) and 2 (positively) were correlated with the utilization of polymers and carboxylic acid, respectively (Table 3-2).

The metabolic potential (AWCD) and substrate utilization by soil fungi was affected by a warming x defoliation interaction (Table 3-3). Where the effect was significant, substrate utilization was generally highest within plots treated with either warming or defoliation, but tended to decrease in plots exposed to both warming and defoliation (Fig. 3-2; Table 3-3). The leading NMS ordination solution was a one-axis configuration with a stress value of 0.00001, which explained 81% of the variation in the substrate utilization data. However, no significant separation was evident in fungal substrate utilization due to warming (MRPP: p = 0.89, A= -0.009) or defoliation (MRPP: p = 1.00, A= -0.01) based on the ordination results. Pearson correlation analyses indicated that axis 1 was

negatively correlated with the utilization of amine, amino acid, phosphorylated chemicals, and nucleosides (Table 3-2).

Total microbial functional diversity of both bacteria and fungi did not vary among the treatments (Table 3-4), although bacterial and fungal functional diversity were respectively correlated with soil temperature and soil microbial biomass (Table 3-5).

3.2. Soil Microbial Community Structure

Non-metric multidimensional scaling (final stress = 7.49, final instability = 0.000), explained 95% of the total variances in microbial PLFAs signature, revealed no shift in soil microbial structure due to warming (MRPP: $p = 1.00$, A= -0.012), and defoliation (MRPP: $p = 0.40$, A= -0.001). A strong negative correlation was observed in the mol% PLFA of bacteria with axis 1 and gram-positive bacteria with axis 2, as indicated by Pearson correlation (r) analysis (Table 3-2). Neither the relative abundance of individual taxonomic groups, nor the ratio of fungi to bacteria showed any direct response to defoliation or warming ($p \geq 0.13$), with the exception of the actinomycete group ($p = 0.0008$), which decreased by 18% in response to warming. Total microbial diversity at the community level was increased by defoliation (Table 3-4). This response was mainly caused by greater evenness in soil bacteria across defoliated plots (with the evenness values of 0.85 and 0.88, respectively, in non-defoliated and defoliated plots). Multiple regression analysis indicated that microbial biomass

nitrogen and carbon were most strongly correlated with microbial diversity, explaining a combined 25% of variation in the latter (Table 3-5).

Discussion

Simultaneous applications of defoliation and warming had limited impacts on soil microbial community diversity, structure and composition in this study, as it was also reported for plant diversity in this study (Bork *et al.,* 2008). However, stronger impacts of treatments were observed on substrate utilization, particularly by soil fungi. As substrate utilization by bacteria and fungi varied with the interaction of warming and defoliation, these results suggest that the consequences of global warming on soils in this temperate grassland depend on grazing activities, consistent with previous findings (Zhang *et al.*, 2005; Rinnan *et al.*, 2009). However, based on the stability in soil microbial composition, specifically fungi:bacteria ratio and microbial functional diversity, these results suggest that this grassland soil is quite resistant to climatic warming as well as grazing.

4.1. Soil Microbial Physiological Function

Soil is a dynamic habitat, resulting from the flow of organic compounds and activities among soil biota (i.e. organic C decomposition). Soil organic C pools, ranging from fast-cycling (i.e. carbohydrates) to slow-cycling (i.e., cellulose) pools, are of special interest because small changes in C fluxes out of this large soil C pool (e.g. 2344 Gt at the top 3m; Jobbagy & Jackson, 2000) can amplify

global warming via feedback in the carbon-cycle. Of key concern is how the activity of bacteria and fungi on organic C substrate in the soil matrix will be influenced by the impacts of warming and grazing, considering their effects on root C exudation (Pendeall *et al.*, 2008 and Craine *et al.*, 1999) and soil temperature.

In this grassland, warming increased the potential for C utilization in the absence of defoliation, while decreasing it in the presence of defoliation. The effects of warming x defoliation was dependant on the induce-changes on soil temperature ($p = 0.04$) and moisture content ($p = 0.07$) (Chapter 2). This suggests the importance of both direct and indirect effect of warming x defoliation on soil microbial C utilization. Part of the rational for the interplay between warming and defoliation impacts on the potential of organic C utilization, despite no shift in soil microbial composition, may arise from changes in most labile C (Rinnan *et al.*, 2009), microbial functional group abundance (Garrett, 1951), and plant species composition (Grayston *et al.*, 1998b). However, the lack of a pronounce response in plant species composition (Bork *et al.*, 2008) and microbial functional diversity (Table 3-4) in this study does not support the latter two hypotheses. Instead, changes in organic substrate properties such as the quality of root exudates and litter may be the more important factor in this grassland. An indirect reason could be changes in forage quality induced by warming x defoliation effects on standing biomass at the end of the 2006 growing season (Bork *et al.*, 2008), which may affect the substrate quality and hence, soil microbial activity (Bossuyt *et al.*, 2001) in the following year (2007).

Considering the end products of C utilization (i.e., assimilation by soil microbes into microbial biomass C and associated CO_2 fluxes), changes in C substrate utilization by soil fungi and bacteria (with the stronger response in soil fungi) may have implications for soil organic C transformation and microbe-climate feedback in this grassland. The patterns of change in C substrate utilization found here parallel differences in soil microbial biomass carbon found earlier in relation to the treatments (Chapter 2), but not associated plant growth and N content (Bork *et al.,* 2008) and soil CO_2 fluxes (Chapter 2). The significantly higher C substrates utilized by soil bacteria and fungi (in response to warming in non-defoliated plots) did not appear to be respired out from the system. Once again, this tends to support the notion that climatic warming and microbes in this grassland soil will not create positive feedback to climate change, even if utilization of the soil organic C pool is increased. Instead, the stimulation of microbial biomass C may affect organic C transformation, with the larger active fraction of organic C pool in the form of microbial biomass in this grassland. Second, the suppressive effect of warming on C substrate utilization in defoliated plots may create a negative climate-microbe feedback that may deplete the potential for sustained C sequestration in this system.

Decreased soil microbial C utilization with reduced microbial populations may suppress important biogeochemical process (Wood, 1995) such as N supply rates, which in turn constrain plant growth (Díaz *et al.,* 1993; Bardgett *et al.,* 2008) with the consequence of potentially altering C sequestration. However, because plant growth and N content (Bork *et al.,* 2008) as well as soil N supply

rates (Chapter 2) did not decrease under warming regardless of defoliation in this study, the negative climate-microbe feedback may not occur in this temperate grassland ecosystem. Focusing at smaller scales, one potential explanation for the lack of feedback between climate and microbes, is that even if C utilization had changed, soil bacteria may exhibit functional redundancy (Mukerji *et al.*, 2006). Support for this hypothesis stems from the lack of significant changes in bacterial functional diversity, and the increase in bacterial diversity associated with increased evenness among bacteria functional groups (Table 3-4). In addition, overall changes in organic C consumption by bacteria were relatively small, indicating soil bacteria may be able to replace one another in the C cycle should a species not function well under environmental stress. In this grassland, redundancy in soil bacteria could ensure stability in overall soil function, at least with regards to C cycling.

4.2. Soil Microbial Community Structure

Given the general concept of soil habitat influences on soil microorganisms (e.g., Coleman *et al.*, 2004, Chapter 2), the lack of change in soil microbial composition (i.e. in fungi:bacteria ratio) found here with respect to warming and defoliation was surprising. These results suggest soil bacteria and fungal populations may have relatively high resilience to changes in soil temperature and aboveground phytomass removal in this ecosystem, findings that contrast with earlier studies in grassland (Zhang *et al.*, 2005). There are many potential reasons why soil microbial structure may show contrasting responses between grasslands,

including both biotic and abiotic properties of the ecosystem. One key characteristic is soil organic matter content, which holds the organic C and supplies nutrients (i.e., nitrogen) and energy for soil biota through decomposition (Violante, 2002). In our study area, the relatively high soil organic matter content ($\geq$ 2%) (Natural Regions Committee, 2006) may provide a reliable energy source for soil biota and lead to enhanced stability in soil microbial composition, at least for a short-term. In contrast, the study by Zhang *et al.* (2005) was done on a soil, (Nash-Lucien complex), with low organic matter (less than 0.1%) (Elshahed *et al.*, 2008). Therefore, we may postulate that soil microbial communities with high organic carbon and nitrogen resources are more resistant and adaptable to climatic warming and defoliation, at least for a short-term.

As fungal and bacterial energy flows largely through the decomposition of recalcitrant and labile C, respectively (Schröter *et al.*, 2003), the stability of soil microbial composition could maintain one of the widely discussed contributions of soil microbes to global warming: decomposition processes and associated C fluxes (Bardgett *et al.*, 2008). A parallel study to the one conducted here found that the rate of soil CO_2 efflux was not sensitive to defoliation and acclimated to experimental warming quite quickly (Chapter 2). This finding provides an optimistic picture of global warming in this grassland ecosystem, in which warming does not appear to accelerate net soil C transfer to the atmosphere, even under simultaneous defoliation. This may explain why the microbial community in this grassland soil is resistant to climatic warming and does not appear to

amplify global climate change via climate-microbe feedback, at least in the short term.

Consistent with the diversity-stability hypothesis, the resilience and resistance of an ecosystem may also be influenced by biodiversity (Elton, 1958). Microbial diversity indices (i.e. Shannon diversity) can be used as a bio-indicator of community stability, especially when environmental stress occurs (Atlas, 1984; Mills & Wassel, 1980). At the microbial community structure level, diversity showed similar values in warmed and unwarmed plots, and greater values (particularly evenness) in defoliated plots relative to non-defoliated plots. While such observations could occur via defoliation effects on the plant community, rhizodeposition (Wardle, 2002), or the disintegration of soil microhabitats and more even distribution of soil C substrate (Torsvik *et al.*, 2002), the ability of this grassland soil to withstand disturbances such as climatic warming and defoliation may depend in part, on sustained microbial diversity in the ecosystem.

Overall, the structure of belowground communities in this temperate grassland appeared to be robust to disturbances such as defoliation, even in the face of environmental stresses such as climatic warming. High resistant of the belowground community to defoliation and 1°C warming might occur due to the fact that this temperate grassland historically experienced such stresses e.g., cattle grazing. This resistance could help maintain the stability of soil microbial communities in this grassland soil under a warmer climate, and minimize contributions to global warming through carbon-cycling feedback.

Conclusions

Two years of warming and defoliation demonstrated limited impacts on soil microbial community composition and structure in this northern temperate grassland, but affected C utilization, particularly by soil fungi. Despite high stability in microbial diversity, overall organic C utilization, and hence C transformation in soil under warmer conditions may depend further on grazing management activities. Changes in organic C utilization did not create any climate-microbe feedbacks in this temperate grassland ecosystem. Furthermore, stability of the microbial communities to warming was high within the temperate grasslands examined here, regardless of the additive stress of defoliation. This included the relative abundance of fungi vs. bacteria, and individual bacterial and fungal functional diversity. Of these, overall results suggest that the microbial community in these will be resistant to regional temperature increases over the short-term. However, understanding the effects of warming and grazing still must be jointly considered across various grassland ecosystems to fully understand the impact of these stresses on soil function and associated C cycling atthe regional scales in order to move forward towards a proper ecological approach for adaptation and mitigation strategies in grassland ecosystems.

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Table 3- 1. Summary of signature microbial PLFA indicators examined.

Sources	PLFA indicator	References
Bacteria	2OH-10:0, 2-OH 11:0, a12:0, a13:0, i14:0, 14:0, i15:1c, i15:0, a15:0, 15:0, i16:1, 16:0 N alcohol, i16:0, a16:0, 2-OH 16:1, 3-OH 16:0, 16:1ω7c alcohol, i17:0, 17:1ω7c, a17:0, 17:0 cy, 17:0, 17:1ω8c, 17:1ω6c, 18:1ω7c, 18:1ω6c, 18:1ω5c, 18:0, 2OH-18:1, 3OH-18:0, 19:0 cy	Ratledge and Wilkinson (1988), O'Leary and Wilkinson (1988), Bowman *et al.*(1991); Genter and Lehman (2000), Kourtev *et al.* (2003), Hamer and Makeschin (2003), Oravecz *et al.* (2004), Zhang *et al.* (2005), Kao-Kniffin and Balser (2007), Amir *et al.* (2008), Rahman *et al.* (2008), Aira *et al.* (2009), Denef *et al.* (2009)
Gram negative bacteria	2OH-10:0, 2-OH 11:0, 2-OH 16:1, 3-OH 16:0, 17:1ω7c, 17:0 cy, 18:1ω7c, 2OH-18:1, 3OH-18:0, 19:0 cy	Kourtev *et al.* (2003), Oravecz *et al.*(2004), Kao-Kniffin and Balser (2007), Amir *et al.* (2008), Aira *et al.*(2009)
Gram positive bacteria	a12:0, a13:0, i14:0, i15:1c, i15:0, a15:0, i16:0, a16:0; a17:0; 17:1ω8c	O'Leary and Wilkinson (1988), Genter and Lehman (2000), Hamer and Makeschin (2003), Kourtev *et al.* (2003), Zhang *et al.* (2005)
Actinomycete	10 Me16, 10 Me17, 10 Me18, 10 Me19	McKinley *et al.* (2005), Joergensen and Wichern (2008)
Fungi	16:1ω5c, 16:1ω7c +15i 2OH, 18:3ω 3.6.9, 18:2ω 6.9c, 18:1ω9c	Kourtev *et al.* (2003), Evgrafova *et al.* (2008), Denef *et al.* (2009), Rajaniemi and Allison (2009)
Arbuscular mycorrhizae fungi	16:1ω5c	Rahman (2008)

Table 3- 2. Pearson correlation (r) results between the relative abundance of microbes (PLFAs), fungi or bacteria, with various microbial taxonomic groups and soil or plant properties as determined by the NMS ordination in 2008.

Sources	PLFA		Sources	Fungi	Sources	Bacteria	
	NMS Axis 1	NMS Axis 2		NMS Axis 1		NMS Axis 1	NMS Axis 2
Bacteria	-0.58	-0.70	Carbohydrate	0.11	Carbohydrate	-0.53	0.82
Fungi	-0.54	0.17	Polymer	0.10	Polymer	-0.68	0.87
gram+bacteria	-0.45	-0.92	Carboxylic acid	0.12	Carboxylic acid	-0.37	0.91
gram–bacteria	-0.41	-0.07	Phospho chemical	-1.00	Phospho chemical	-0.07	0.58
Mycorrhizae	-0.44	-0.78	Amino Acid	-1.00	Amino Acid	-0.61	0.73
Actinomycete	-0.57	-0.84	Amine	-1.00	Amine	-0.22	0.35
F: B	0.46	0.69	Ester	0.15	Ester	-0.66	0.70
MSC	0.50	0.68	Alcohol	-0.99	Moisture	-0.14	0.26
Moisture	0.12	0.003	Nucleoside	-1.00	Temperature	0.19	0.06
Temperature	0.01	0.07	Moisture	0.08	MBC	-0.10	0.06
MBC	-0.15	0.08	Temperature	-0.17	MBN	-0.03	0.01
MBN	-0.28	-0.21	MBC	-0.5	NH_4-N	-0.02	0.18
NH_4-N	0.43	0.18	MBN	0.38	NO_3-N	0.04	0.12
NO_3-N	0.20	0.08	NH_4-N	-0.11			
			NO_3-N	0.13			

F: B = the ratio of fungi-to-bacteria, MSC = Sum of all fatty acids not associated with any particular microbial group, MBC = microbial biomass carbon, MBN = microbial biomass nitrogen.

Table 3- 3. Summary of ANOVA analysis results for metabolic potential (AWCD) and substrate utilization by soil fungi in 2007. Data are means ± 1 standard error.

Sources	Pr > F	Non-defoliated		Defoliated	
		Unwarmed	Warmed	Unwarmed	Warmed
AWCD	**0.02**	42.3±4 [b]	52.6±4 [a]	50.0±4 [ab]	43.8±4 [ab]
Carbohydrate	**0.07**	47.2±2 [b]	53.1±5 [a]	53.7±3 [a]	47.0±3 [b]
Polymers	**0.09**	45.2±2 [b]	51.7±6 [ab]	51.1±2 [a]	45.0±3 [b]
Carboxylic acids	**0.03**	43.6± 3 [b]	53.3±4 [a]	50.2±3 [ab]	44.2±4 [b]
Phospho Chemicals	0.61	37.7±12 [ns]	48.2±15 [ns]	45.9±14 [ns]	37.0±12 [ns]
Amino acids	**0.02**	41.2±2 [b]	53.4±5 [a]	47.9±4 [ab]	42.2±3 [b]
Amines	**0.06**	36.8±2 [b]	47.02±5 [a]	42.2±4 [ab]	37.8±3 [b]
Alcohol	**0.06**	44.4±2 [ab]	51.5±5 [ab]	50.1±3 [a]	43.3±2 [b]
Ester	0.10	43.9±3 [ns]	50.1±5 [ns]	50.7±3 [ns]	44.1±3 [ns]
Nucleoside	**0.05**	39.8±2 [b]	51.4±5 [a]	45.2±4 [ab]	41.2±3 [b]

[1] Within a row, means with different letters differ, $p<0.1$.

Table 3- 4. Summary ANOVA results for the evaluation of microbial structural and functional diversity based on Shannon diversity indices for each of 3 assessment methods.

Sources	Year	PLFAs			ECO Plates			SF-N2 Plates		
		[1]*df*	F	p	*df*	F	p	*df*	F	p
Warming (W)	2006	-	-	-	1/32	0.93	0.34	-	-	-
	2007	1/32	0.51	0.47	1/36	0.51	0.47	1/32	1.18	0.28
Defoliation (D)	2006	-	-	-	1/32	0.09	0.76	-	-	-
	2007	2/32	3.94	**0.05**	1/36	0.18	0.67	1/32	0.89	0.35
W × D	2006	-	-	-	1/32	0.63	0.43	-	-	-
	2007	2/32	0.17	0.68	1/36	2.85	0.10	1/32	1.01	0.32

[1] Degrees freedom are numerator/denominator, respectively.

Table 3- 5. Multiple regression analysis of microbial structural and functional diversity responses in relation to various soil properties.

Dependant Variable	Year	Independent Variables	Parameter Estimate	Partial R^2	Model R^2	F	p
Microbial structural diversity (PLFAs)	2007	MBN (kg/ha)	0.0009	0.14	0.14	6.42	0.01
		MBC (kg/ha)	-0.0001	0.11	0.25	5.57	0.00
Bacterial functional diversity	2006	Temperature ºC	0.02	0.20	0.20	9.76	0.003
		NO_3-N (kg/ha)	0.004	0.10	0.30	5.39	0.02
	2007	Temperature ºC	0.009	0.07	0.07	3.17	0.08
Fungal functional diversity	2007	MBN (kg/ha)	0.0008	0.15	0.15	6.77	0.01
		MBN (kg/ha)	-0.0001	0.21	0.36	12.3	0.001
		Moisture content (%)	0.005	0.06	0.42	4.12	0.04

MBC = microbial biomass carbon, MBN = microbial biomass nitrogen

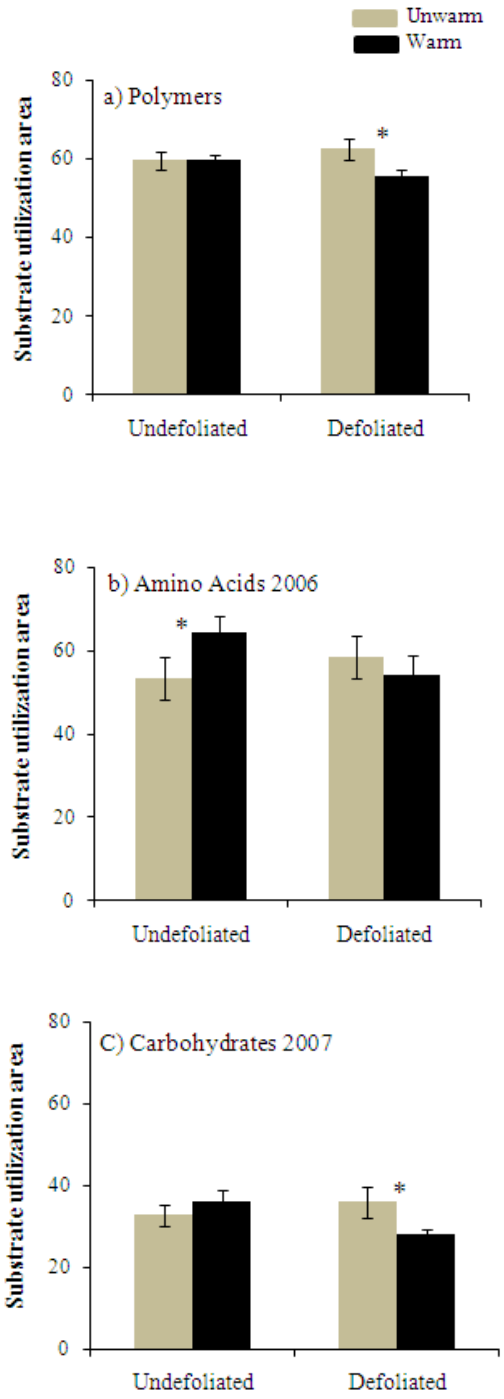

Figure 3- 1. Warming and defoliation effects on bacterial physiological function on (a) polymers in 2006 (b) amino acids in 2006, and (c) carbohydrates in 2007. Error bars represents 1 SE.

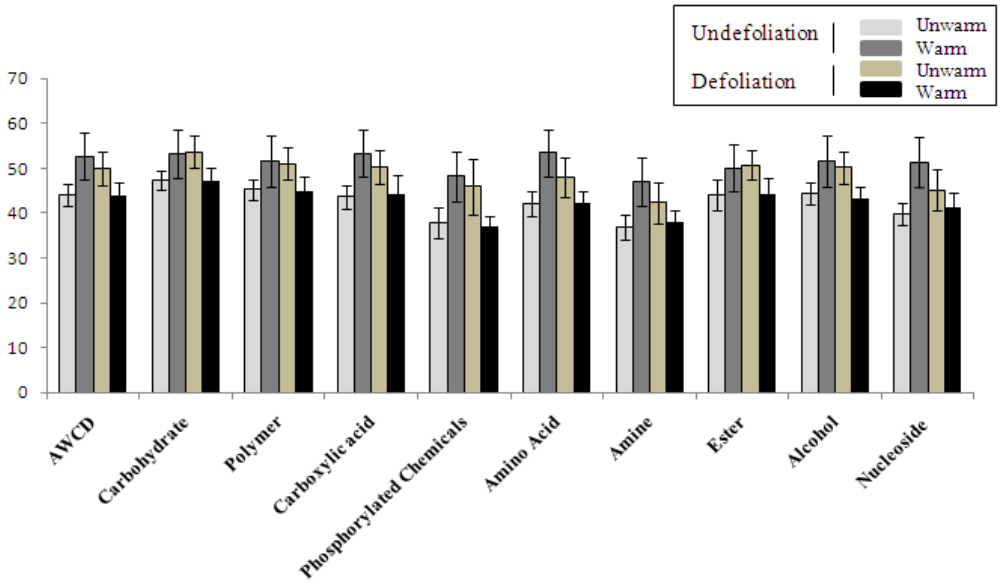

Figure 3- 2. Warming and defoliation effects on fungal physiological function across various substrate types and metabolic potential (AWCD). Error bars represents 1 SE.

Chapter 4 Greenhouse Gases Emissions, Dissolved and Microbial Carbon and Nitrogen Pools Subjected to Warming, Precipitation, and Defoliation in a Native Grassland (Ecosite B)

Introduction

Annual anthropogenic greenhouse gas (GHG) emissions increased by 70% between 1970 and 2004, leading to changes in global climate (IPCC, 2007). Air temperatures have been rising worldwide, particularly in northern latitudes (Hansen *et al.,* 2006). Associated with changes in temperatures has been substantial variation in precipitation, with increased rainfall in some areas (eastern part of North and South America, northern Europe and northern and central Asia), and reduced precipitation in others (subtropical land region) (IPCC, 2007). To mitigate some of these climatic shifts, the terrestrial biosphere has been considered one of the main reservoirs to sequester atmospheric carbon (C) (IPCC, 2007) through photosynthesis.

The potential of forest ecosystems to sequester C has been extensively addressed (e.g., Yang & Guan, 2008); however, there is a growing concern that their potential for increased storage is decreasing (Gough *et al.,* 2008). In contrast, grasslands are more widespread than forests (Lund, 2007), and their potential to increase C sequestration has been comparatively overlooked. To understand the potential value of rangelands as C sinks, it is critical that we understand the factors that influence carbon balance in these systems.

The potential amount of C sequestration that could occur in rangelands depends upon nitrogen availability in the soil (Reich *et al.,* 2006) as well as many other parameters (i.e., soil water content). For example, high levels of atmospheric N-deposition reduced CO_2 release to the atmosphere from European grassland soils (Fleischer & Bouse, 2008). Because of the coupling of C and N cycles, investigation of C sequestration potential requires detailed measures of N cycling. Both C and N fluxes are likely to vary with climate change and as a function of different land management practices.

Grazing, a common management practice in rangeland ecosystems, can mediate soil C and N cycling through defoliation, fecal returns, physical disturbance, and changing the availability of C substrate (Hamilton *et al.,* 2008). Within studies assessing grazing, there is great variability results. For example, aboveground plant removal may increase (Stark, 2002 and Cheng *et al.,* 2007) or decrease (Zhou *et al.,* 2007; and de Klein *et al.,* 2006) C and N fluxes in grass-dominated ecosystems. Similar variability has been found in response to climatic factors, where soil C and N effluxes may increase (e.g., Jalota *et al.,* 2007 and Keeney *et al.,* 1979) or decrease (e.g., Wan *et al.,* 2007 and Jamieson *et al.,* 1998) in response to experimental warming. Some of this variability is likely due to local conditions and habitat variation. Further progress could be achieved through larger multi-factor experiments that are able to manipulation both grazing and changes to climatic factors. To our knowledge, no studies have previously focused on the response of C and N fluxes to the combined effects of grazing, precipitation and warming.

We conducted a field experiment to investigate the interactive effects of precipitation, warming and defoliation on carbon and nitrogen fluxes in a northern temperate grassland in Alberta, Canada. The specific objective in this study was to quantify the impacts of different precipitation regimes and experimental warming on soil microbial C and N, as well as soil carbon and nitrogen fluxes under defoliation (different defoliation intensity levels) in a native grassland ecosystem.

Material and Methods

2.1. Site Description

The research was conducted during two growing seasons (2007-2008) at the University of Alberta Research Ranch in Kinsella, Alberta, Canada (53°05 N, 111°33 W). The site is within the Aspen Parkland ecoregion; a savanna-type habitat that consists of fescue grassland (*Festuca hallii*) interspersed among aspen stands (*Populus tremuloides*). The Aspen Parkland is a transition zone between the dry mixed prairies to the south, and the boreal forest to the north. This region has historically been sensitive to climatic changes (Vance, 1979). The experiment was established within grasslands of this region, in a 0.5 ha region dominated by *Festuca hallii*, *Artemisia frigida*, *Elymus trachycaulus*, *Pulsatilla patens*, *Koeleria macrantha, Oxytropis campestris,* and *Stipa curtiseta*. The soil is an Orthic Dark Brown Chernozem (Howitt, 1988) with a thick Ah horizon. Soil texture was sandy loam at both 0-5 cm (clay: 6%, silt: 31%, sand: 63%) and 5-15 cm (clay: 11%, silt: 27%, sand: 62%) sampling depths. The long-term (1971-2000) mean

annual and growing season precipitation and temperature are 431.3 and 312.2 mm, and 2.4 and 13.9 °C, respectively. During the 2007 and 2008 growing seasons (May-September), average temperatures and precipitation were 13.9 °C, 211 mm and 13.9 °C, 249 mm, respectively.

2.2. Experimental Design

In early May 2007, we established a completely randomized block experiment with five blocks and 90 plots (2× 2 m^2). Each block included one replicate of 18 unique treatment combinations, including all factorial combinations of warming (ambient and warming), defoliation (none, low intensity and high intensity) and precipitation manipulation (-50%, ambient, +50%).

The warming treatment was applied using circular open-top chambers (OTCs) 2 m diameter × 0.4 m height and 60° side angle), made of flexible fiberglass material (Solar Components Corporation, Manchester, NH, USA), a method common to climate change studies (Marion *et al.,* 1997). As the OTCs were an obstacle for direct livestock grazing in the plots, manual defoliation was used for simulating the grazing treatment. Defoliation occurred through mowing the aboveground vegetation to a stubble height of 7 cm for the low intensity and 3 cm for the high intensity treatment, once per year in June. This timing and intensity of defoliation are similar to those used by local producers.

Manipulation of precipitation was achieved through the use of transparent rainout shelters designed to intercept approximately 50% of ambient precipitation in the precipitation exclusion plots. In 2008, the intercepted water was channeled

through a gutter and stored in a water tank. Captured water was subsequently applied to the water addition plots within 24 hours of the rainfall event. This approach allowed us to increase and decrease the magnitude of precipitation without altering precipitation frequency. Both the control and water addition plots also had shelters built overtop them to control for any confounding effects of the structures on local climatic factors. Rainfall was permitted through these sham structures by cutting a large number of holes into the plastic cover. The exclusion treatment and sham controls were established in 2007, which continued through 2008. The rainfall addition treatment occurred only in 2008.

PRS™ probes were used to monitor changes in soil inorganic-N supply rate during the growing season in 2007 and in July 2008. HOBO Pro V2 data loggers were used to monitor soil temperature and soil volumetric water content at two different soil depths (0-5 and 5-20 cm) every 15 min during the growing seasons. Data loggers were installed in only two of the five blocks (36 plots) due to financial constraints.

2.3. Soil Sampling and Microbial Analysis

To minimize physical disturbance in the plots, soil samples were collected once during each growing season on July 22, at two soil depths: 0-5 (shallow soil) and 5-15 (deep soil) cm. The soil cores from 0-5 cm depth were considered as rhizosphere because grass roots were extensively visible throughout the cores (Kennedy *et al.*, 2005). Upon sampling, soil cores were promptly cooled on ice to transport and stored at 4 °C in the laboratory within 72 hours. Samples were

sieved through a 4 mm sieve and visible roots removed by hand. All soil samples were processed for gravimetric moisture content, soil microbial biomass carbon (MBC) and nitrogen (MBN), and dissolved organic carbon (DOC) and nitrogen (DON) measurements within a week of collection. Soil gravimetric moisture content was determined after drying 10-g subsamples at 105 ºC in an oven for 24 hours.

Microbial biomass carbon and nitrogen were measured using a chloroform fumigation- extraction method (CFE) (Brookes *et al.*, 1985). In brief, two 20-g dry weight subsamples were extracted with 0.5 mol L^{-1} K_2SO_4 (the ratio of soil: solution was 1:4 weight: volume) before and after a 24-hour fumigation with alcohol-free chloroform in the dark. Soil extractable organic carbon and nitrogen contents were determined using a Shimadzu TOC-V CSH/CSN Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan). Subtraction of soil organic carbon and nitrogen after and before the fumigation was multiplied by the corresponding efficiency coefficient (k_{EC}= 0.25, k_{EN}=0.18) to calculate MBC and MBN, respectively (Voroney *et al.,* 1993). To measure dissolved organic carbon and nitrogen, a 20-g dry weight subsample was extracted with 25 ºC distilled deionised water (the ratio of soil: water was 1:4 weight: volume), shaken for 1 hour at low speed on reciprocal shaker (Reciprocal Shaker 6000, Eberbach Co., USA), and then centrifuged for 30 minutes at 3000 rpm and average G force of 661. The supernatant was then filtered through a 0.45 µm Whatman micro-filter, and total organic carbon and nitrogen contents analyzed using a Shimadzu TOC-

V CSH/CSN Total Organic Carbon Analyzer (Shimadzu Corporation, Kyoto, Japan).

2.4. Soil Greenhouse Gas Emission

Soil CO_2, CH_4 and N_2O emissions were measured in 2007 and 2008 using the static gas chamber method (as described by Hutchinson & Mosier 1981). To ensure reliable sealing of the gas chamber, PVC soil collars (132.73 cm^2 in area and 3 cm in height) were permanently inserted into the soil (June 2007). Sampling occurred between 10 am and 2 pm in clear days to avoid any confounding effect of weather and diurnal temperature fluctuation. Gas samples were collected by inserting the needle of a 20 mL gas-tight syringe (Norm-Ject, Henke Sass Wolf, Tuttlingen, Germany) through a septum into the chamber after 0, 5, and 15 minutes of the chamber's placement. Samples were then stored in evacuated 10-mL soda glass Isomass Exetainers® (Labco Limited, Buckinghamshire, UK) and analyzed on a Varian CP-3800 gas chromatograph (GC, Varian Canada, Mississauga, Canada). The linearity of gas concentrations in 15 minute time intervals showed that gas emissions did not overpressure and suppress during sampling (Nakayama, 1990). Gas effluxes were calculated as the increase of gas concentration over time, following an adjustment for temperature effects on the molecular volume of gas (temperature data came from the averaged readings of the previously described dataloggers), the height of the chamber, and the time interval of sampling (Nakayama, 1990).

2.5. Statistical Analysis

To determine soil C and N fluxes in response to our treatments, a series of repeated generalized linear mixed models were used in SAS v 9.1 (SAS Institute Inc.), using the Proc Mixed procedure. Defoliation, precipitation, and warming served as fixed factors and block a random effect. To reduce the complexity of the statistical model, separate analyses were conducted for each soil depth. Because of the low replication necessitated by logistical constraints imposed by a large multi-way design, we used an alpha value of 0.1 as an indicator of significance (Scheiner & Gurevitch, 2001).

The number of biotic and abiotic variables measured in the two datalogged blocks allowed us to explore the relationships among a number of factors using multiple regression analysis. In separate analyses, greenhouse gas emissions served as the response variables, with explanatory parameters including the average of soil temperature and soil moisture content during the gas sampling hours (0-5 and 5-20 cm soil depths), total C and N content in soil and decomposed shoots (after 6 and 12 months corresponding to 2007 and 2008, respectively) (Nyanumba, unpublished data), the average biomass of vegetation (forbs, grass, moss) and litter coverage (expressed as percentage and g/m^2) (White, unpublished data), and soil inorganic-N supply rate. Multiple regression analyses were performed using the *proc stepwise* procedure with the alpha value adjustment using slentry = 0.1.

Prior to all analyses, data were tested for normality and homogeneity of variance. Logarithmic transformations (CO_2 emission, DOC at 5-15 cm, DON at

5-15cm) were performed as needed. As the level of precipitation addition treatment was added during the 2008 growing season, years were not repeated factors and thus were analyzed separately.

Results

3.1. Soil Moisture Content and Temperature

The OTCs were generally successful in increasing soil temperature (Table 4-1), with an overall average increase of 0.44 and 0.87 °C in shallow soils and 0.49 and 0.73 °C in deep soils in 2007 and 2008. The exact warming that occurred in a given plot was a function the combination of defoliation x warming x precipitation treatments it received (Fig 4-1; Table 4-1). Average soil moisture content varied with precipitation at both soil depths in 2008 (Fig 4-2; Table 4-1), and was influenced by warming and defoliation intensity and date of sampling (Table 4-1). There were fewer effects on soil moisture content in 2007, when the water addition treatment had not yet been initiated.

3.2. Soil Greenhouse Gas Efflux

Average soil CO_2 emission was 76.9 and 93.8 mg C m^{-2} hr^{-1}, respectively, in 2007 and 2008. Soil carbon dioxide efflux varied as a function of the warming and precipitation treatments (Table 4-2), and defoliation x date in 2007 (Table 4-2). A transient effect of defoliation stimulated CO_2 efflux on July 20, right after the defoliation application, by more than 242 %. The lowest rates of CO_2 efflux occurred when precipitation was excluded, in either the presence or absence of

warming (Fig. 4-3). Responses of CO_2 efflux in 2008 were simpler, influenced only by the precipitation treatment (Table 4-2). In 2008, CO_2 respiration was lowest with water exclusion and highest with water addition. Multiple regression analysis indicated that soil respiration was most strongly correlated with litter quantity in 2007 and 2008 (Table 4-3). In both years, these correlations were negative, such that the highest respiration occurred in plots with the lowest plant litter (Fig. 4-4).

In contrast to CO_2, this system was consistently a methane sink across all treatments by an average of -0.021 and -0.024 mg C m^{-2} hr^{-1} in CH_4 uptake, respectively, in 2007 and 2008. Soil CH_4 uptake was influenced by combinations of warming, precipitation, and defoliation treatments in both years (Fig. 4-5; Table 4-2), but was much stronger in 2008, when precipitation was added to the system. When results were significant, warming suppressed the CH_4 uptake at low intensity defoliation (2007-2008) and increased CH_4 uptake at non-defoliation (2008) across ambient precipitation plots. Interestingly, no significant response was observed in CH_4 uptake when precipitation was added. The variability of soil methane uptake was poorly explained in our multiple regressions, with no correlations in 2007, and a weak positive correlation with moss coverage in 2008 (Table 4-3).

Average soil N_2O emission was 0.0012 and 0.0028 mg N m^{-2} hr^{-1}, respectively, in 2007 and 2008. Soil nitrous oxide efflux was maintained at fairly constant levels throughout the growing seasons, as indicated by a lack of a sampling date effect (Table 4-2). However, N_2O levels did vary in response to

different combinations of the treatments in 2007 and 2008 (Table 4-2). N_2O efflux was a function of precipitation x warming and precipitation x defoliation at the first sampling date on July 20, 2007, and the warming x defoliation x precipitation x sampling date in the following growing season (2008). N_2O efflux showed an overall complexity with regard to sampling dates during both years. For example, the non-defoliated plots with ambient precipitation and increased warming had decreased N_2O efflux on June 20, but increased efflux on August 6 in 2008. Moreover, multiple regression analysis showed that the explanatory parameters were not consistent between the years (Table 4-3).

3.3. Soil Microbial Biomass Carbon and Nitrogen

Soil microbial biomass was more sensitive to defoliation and precipitation treatments than warming (Table 4-4). Although warming increased MBC and MBN by over 40% in the shallow soils in 2007, these effects were absent in 2008. In contrast, the main and interactive effects of precipitation on soil MB appeared stronger in 2008 (Table 4-4) when the precipitation addition treatment occured. In general, when the effect was significant, microbial biomass was greatest in precipitation addition and lowest in precipitation exclusion plots (Fig. 4-6). Defoliation and defoliation x warming caused significant changes in soil microbial biomass (Table 4-4). High intensity defoliation resulted in a reduction ($\leq$ 27%) in MBC and MBN in shallow soil, and in MBC (2007: 39 %), in the absence of warming at deep soil.

3.4. Soil Dissolved Organic Carbon and Nitrogen

Overall, dissolved organic materials (DOM) were fairly insensitive to warming, defoliation and precipitation (Table 4-4). The only significant effect on dissolved organic material was a function of precipitation and defoliation at the shallow soil depth in 2008. Surprisingly, any changes in ambient precipitation, regardless if additive or reductive, resulted in a lower dissolved organic material in low intensity defoliated.

Discussion

The two-year manipulation of climate and defoliation demonstrates the prevailing effect of precipitation regimes on C and N fluxes in this system. Considering significant responses in soil active (microbial biomass) and labile C and N (dissolved organic matter) pools, plus soil CO_2 evolution, our observations suggest a relatively higher C loss from this grassland soil and greater N assimilation in soil microbes in wetter conditions. In the case of N_2O evolution, the response was more complex under interactions of treatments and inter-seasonal variability. This grassland soil was an overall small N_2O source. Given the small magnitude of N_2O emission (the CO_2 equivalent) and the potential of CH_4 sink in this grassland soil, CO_2 would be the main contributor to greenhouse gas (GHG) effects in this temperate grassland ecosystem if precipitation increased.

4.1. Fluxes of Soil Carbon

In North American grasslands, there are strong positive relationships between precipitation and ecosystem C fluxes (e.g., Risch & Frank, 2007). Consistent with these findings, precipitation, more than warming or defoliation, influenced soil CO_2 evolution, MBC, DOC, but not CH_4.

A positive feedback of precipitation on soil MBC (Singh *et al.,* 2009; Singh *et al.,* 2009), DOC (Jobbágy & Jackson, 2000), and CO_2 evolution (Wiant, 1967; Tylor *et al.,* 2004) was expected. The expression of that positive potential is apparently contingent on the direct effects of soil moisture content (Table 4-1) on MBC (Singh *et al.,* 2009), CO_2 efflux (Wiant, 1967), DOC (Borken *et al.,* 1999), and/or its indirect effects on MBC (i.e., soil organic C; Wichern & Joergensen, 2009), CO_2 efflux (i.e., decomposer community composition; Tylor *et al.,* 2004), and DOC (i.e., soil microbial C assimilation; Hullar *et al.,* 1996). Furthermore, precipitation-induce changes on soil temperature (Table 4-1) may partially explain the impacts of precipitation amount in this study site.

Large amount CO_2 recorded to increases ($\leq$ 61%) in the precipitation addition and decreases ($\leq$ 51%) in the precipitation exclusion suggest the highest potential of C release wet conditions. In addition, significant increases ($\leq$ 15%) and decreases ($\leq$ 37%) of soil MBC, respectively, in the precipitation addition and exclusion treatments, in conjunction with decreased DOC ($\leq$ 68%) in both precipitation regimes at low intensity defoliation area were wvidant in this study at shallow depth (2008). The reduction of DOC might be a result of lower mobilization and degradation rates of dissolved organic matter in drier conditions (Borken *et al.,* 1999) and leaching (Jobbágy & Jackson, 2000), and vertical

mixing by soil organisms (Jobbágy & Jackson, 2000) in wetter condition. Overall, given the magnitudes of change in active (MBC) (Hu *et al.,* 1995), labile (DOC) C pools and C evolution, these processes may result in high potential C loss with the projection of increased precipitation in this grassland ecosystem.

Release of C in the form of CO_2 was also a function of litter amount. A negative correlation with litter mass (g/m^2) in 2007 and litter cover (%) in 2008 was evident in this study. Notably, our result differ from earlier findings, where litter respiration positively contributes to soil CO_2 evolution (Redmann & Abouguendia, 1978). The quality of litter may contribute to our result. For example, Tewary *et al.,* (1982) found a lower soil respiration rate beneath conifer than broad-leaved trees, and attributed this to the low nitrogen and high lignin content in the forest floor of conifer stands. Another example is changes in soil pH due to litter quality; litter can potentially alter the soil pH (Liu *et al.,* 2009), and consequently soil respiration (Lee & Jose, 2003). Therefore, we postulate that suppression of soil respiration with litter accumulation might be a result of litter quality and hence changes in the soil properties. As studies on litter quality and soil respiration in grassland ecosystems are scarce, further investigation is needed on the mechanisms of litter respiration. In any case the results may suggest that litter accumulation can mitigate precipitation-induce increase in CO_2 release from the soil matrix.

Soil C release in the form of CH_4 was mainly a function of defoliation , suggesting a role of the plant as substrate supplier for methanogenic bacteria (Cheng *et al.,* 2007) and the importance of indirect effect of defoliation on soil

temperature (Table 4-1) in this system. Although, our result suggests a better CH_4 sink in a warmer climate at lightly grazed area, the more important question is to what extent defoliation could influence CH_4 efflux? To answer this question, we note that this grassland was generally a small sink of methane across all plots. This temperate grassland soil is a small sink for CH_4, which was maintained even in warmer, drier, and wetter conditions.

Despite the significant impacts of both precipitation amounts and defoliation on aboveground community composition and productivity (Bork *et al.*, 2009), the belowground C balance was mainly influenced by precipitation. Overall, precipitation regime is likely to be one of the important abiotic factors influencing the C balance in this temperate grassland ecosystem. One plausible explanation for the dominant effect of precipitation is the high summer moisture index (SMI) in this subregion. The approximate SMI value of 5.2 in this northern fescue subregion indicates a dry climatic condition with the moisture deficiency during the growing season (Natural Regions Committee, 2006). Furthermore, both years of study (2007-2008) were relatively dry years considering the average precipitation during growing seasons of 2007 (211 mm) and 2008 (249 mm) compare to the long-term average precipitation (312.2 mm) in this area. Thus, the overall dry climatic condition in the subregion along with the low precipitation amount in the years of study can mainly explain the strong and positive effects of precipitation amount in the study site.

4.2. Fluxes of Soil Nitrogen

Significant contributions of precipitation to soil MBN and DON under low intensity defoliation suggests controlling of soil moisture content at the sampling time (July 20th , 2008) ($p = 0.08$) over nitrogen utilization by soil microorganisms in temperate grassland ecosystems in consistent with previous finding (Burke *et al.*, 2002). The positive effect of precipitation on soil MBN and DON was independent of changes in soil temperature ($p = 0.67$). Therefore, as expected and in concordance with earlier studies, soil MBN was increased as much as 44% due to precipitation addition and decreased by less than 43% in the precipitation exclusion treatments. Soil DON decreased ($\leq 40\%$) in both precipitation addition and exclusion at low intensity defoliation, similarly to DOC. Given the review of mechanisms regulating soil DON concentration in terrestrial ecosystems, we hypothesize that declines in DON concentration might be a result of leaching (Jobbágy & Jackson, 2000), plant DON uptake (Neff *et al.*, 2003), and faster DON mineralization (Scharenbroch & Lloyd, 2004) under wetter conditions, or slower organic matter degradation (Borken *et al.*, 1999) and microbial turnover (Seely & Lajtha, 1997) in drier soils. However, the lack of response in soil DON in the deeper soil layer (5-15 cm) (Table 4-4) does not support the first hypothesis of the DON leaching to deeper layer and instead encourage the chance of microbial and plant N uptake in the system. However, the low chance of leaching in this study is not surprising given that overall dry climatic condition in this area along with the drier climatic condition in the years of study as discussed above. Should this be the case, reduction of DON, together with the fact that microbial biomass conserves soil N in grassland ecosystems (Garcia & Rice, 1994) may

suggest a more efficient N conservation in this temperate grassland soil due to changes in precipitation.

Contrary to the above prevailing effect of precipitation on MBN and DON, N_2O evolution was a function of defoliation, warming, precipitation and inter-seasonal variability. The impacts of defoliation, warming, and precipitation on soil N pools were independent of soil moisture and temperature (Table 4-1), suggesting the importance of indirect effect of the soil temperature and moisture on soil N_2O (i.e. through aboveground community) rather than the direct effect on soil microclimate. According to Flechard *et al.,* (2007), soil N_2O production was expected to be vary in response to our treatments; however, the interplay of treatments and inter-seasonal variability caused an overall complexity on N_2O production, resulting in no clear pattern of N_2O efflux with respect to treatments and seasonality. As the natural production of N_2O is a result of nitrification, denitrification, and the proportion and consumption of their gaseous end products (Davidson, 1991), the net products of these processes are unpredictable and depend on the tradeoff between all these processes in the soil matrix. Combined with the N partitioning the between soil microbes and plants (Jaeger *et al.,* 1999), seasonality in plant N uptake (Jackson *et al.,* 1988), and intense plant-soil microbe competition for N in grassland ecosystems (Reich *et al.,* 2006) could result in the observed inter-seasonal complexity in N_2O production within this temperate grassland ecosystem.

Direct evidence of such complexity was that this temperate grassland acted as both source and sink of N_2O with respect to time and treatment interactions.

Despite all the complexity in the act of this temperate grassland as N_2O source or sink, this soil remains an overall small source of N_2O. According to Burke *et al.*, (2002), rates of N_2O production in rangelands may typically vary between 0.001 to 0.01 mg m^{-2} hr^{-1}. Therefore, considering the maximum and minimum N_2O efflux in this soil matrix, 0.003 and -0.002 mg m^{-2} hr^{-1}, and their equivalent to CO_2 (0.93 and -0.62 mg C m^{-2} hr^{-1}, respectively; Grover, 2004), respectively, in overall this temperate grassland is not a major N_2O contributor to greenhouse gas effects in future climate change scenario.

4.3. Relationships between Soil Carbon and Nitrogen Fluxes

In terrestrial ecosystems, there is mounting evidence suggesting that coupling of carbon and nitrogen cycles may have some consequences on the potential impact of changing climate and its possible feedback to soil C and N pools (Asner *et al.*, 1997; Reich *et al.*, 2006). In agreement with earlier findings, carbon and nitrogen fluxes showed correlation with soil C and N content in this grassland soil, however the correlations were not strong or constant. Soil CO_2 emission was weakly correlated with soil N content only in 2008 (partial R^2 = 0.05). Similarly, correlation between soil N_2O emission and C and N content in 2007 (partial R^2 equal to 0.14 and 0.07, respectively) was relatively weak and inconsistant. Given that measured soil respiration consisted of both autotrophic and heterotrophic respiration, and most of the phytomass (87%) allocated belowground in this temperate grassland (Coupe, 2003), the weak correlation may suggest that autotrophic respiration was the main contributor to total soil

respiration rather than soil microbial activity on soil organic matter. Therefore, C allocated to plant shoots and roots could be the main component of the C budget in this system rather than soil C content. Alternatively, higher C: N ratio in vegetation compare to soil may suggest that plant N uptake is dominant over soil microorganisms (Asner *et al.,* 1997), resulting in N deficiency in this soil to play a controlling role on C fluxes in the system. However, the lack of studies on C and N coupling in grassland ecosystems emerges further investigation.

Conclusions

The two-year projection of climate change and defoliation demonstrate that precipitation regimes is of importance for soil C and N fluxes in the studied temperate grassland ecosystem, which significantly contributes to C loss to GHG emissions in the system. The effect of precipitation is strong on soil C loss and might lead the system to a greater CO_2 source. With estimates of other GHG emissions, this system does not appear the major contributor of N_2O and CH_4 to greenhouse gas effects even under the projection of climate change. However, we could just speculate about biotic and abiotic parameters underlying our observations. From a practical point of view, accurate accounting of soil C and N fluxes in this study had some limitation (i.e., the lack of direct livestock grazing, site replication, and frequent soil sampling). Furthermore, the short timescale of treatment application (two years) might be a limitation to detect differences in soil biogeochemical processes with respect to climate change and defoliation in this system. As such, these deficiencies may limit our ability to observe significant

differences in soil C and N fluxes in this temperate grassland ecosystem, which reader should be aware when interpreting the results. Further research with longer timescales and direct livestock grazing is recommended in different types of grassland ecosystems.

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Table 4- 1. Repeated measure analysis for soil temperature (T °C) and volumetric water content (VWC%). Bold numbers represent statistically significant changes in soil temperature and VWC.

Sources	VWC_{Sh}		T_{Sh}		VWC_{D}		T_{D}	
	Pr> F		Pr> F		Pr> F		Pr> F	
	[1]*df* (num,den)		*df* (num,den)		*df* (num,den)		*df* (num,den)	
	F		F		F		F	
	2007	2008	2007	2008	2007	2008	2007	2008
Warming(W)	0.26	**0.01**	**<.00**	**<.00**	0.11	0.83	**0.00**	**<.00**
	1,37.1	1,111	1,37	1,111	1,37	1,86	1,37	1,93
	1.26	7.17	25.2	61.43	2.67	0.05	7.88	64.38
Defoliation(D)	0.11	0.75	**0.00**	**<.00**	0.39	0.98	**0.00**	**<.00**
	2,37.2	2,111	2,37	2,111	2,37	2,86	2,37	2,93
	2.28	0.28	6.30	25.15	0.96	0.02	8.80	28.82
Precipitation(P)	**0.10**	**0.01**	0.4	**<.00**	0.21	**0.01**	0.18	**0.07**
	2,37.6	2,111	2,37	2,111	2,37	2,86	2,37	2,93
	2.47	5.57	0.93	10.93	1.71	5.87	1.76	2,60
W × D	0.00	0.1	**0.01**	**<.00**	**0.07**	0.54	0.21	**0.00**
	2,37.3	2,111	2,37	2,111	2,37	2,86	2,37	2,93
	5.43	2.71	4.75	17.69	2.80	0.63	1.61	8.36
W × Pr	0.95	0.12	0.52	**0.09**	0.18	0.7	0.14	**<.00**
	2,34.3	2,111	2,37	2,111	2,37	2,86	2,37	2,93
	0.04	2.31	0.67	2.36	1.76	0.36	2.03	20.93
D × Pr	0.24	0.21	0.52	**0.00**	0.1	0.27	0.4	**0.07**
	4,37.4	4,111	4,37	4,111	4,37	4,86	4,37	4,93
	1.43	1.63	0.81	3.64	2.17	1.43	1.04	2.25
W × D × Pr	0.76	0.94	0.15	**<.00**	0.16	0.36	**0.03**	**<.00**
	4,37.3	4,111	4,37	4,111	4,37	4,86	4,37	4,93
	0.46	0.17	1.79	7.18	1.71	1.17	2.88	7.09
Date	**<.00**	**<.00**	**<.00**	**<.00**	0.21	**<.00**	**<.00**	**<.00**
	2,37	6,111	2,37	6,111	2,37	6,86	2,37	6,93
	12.25	30.78	1438	386.9	1.62	63.67	1014	308.3
W × Date	0.74	0.94	0.63	0.39	0.93	0.95	0.92	**0.02**
	2,37	6,111	2,37	6,111	2,37	6,86	2,37	6,93
	0.30	0.27	0.46	1.05	0.07	0.26	0.08	2.58
D × Date	0.55	0.32	0.18	0.14	0.99	0.8	0.82	0.24
	4,37	12,111	4,37	12,111	4,37	12,86	4,37	12,93
	0.77	1.16	1.64	1.48	0.01	0.63	0.37	1.28
Pr × Date	0.2	0.15	0.45	0.16	0.95	**0.00**	0.99	0.49
	4,37	12,111	4,37	12,111	4,37	12,86	4,37	12,93
	1.55	1.46	0.93	1.43	0.17	3.33	0.06	0.96
W × D × Date	0.76	**0.04**	0.18	0.28	0.99	0.95	0.94	0.56
	4,37	12,111	4,37	12,111	4,37	12,86	4,37	12,93
	0.45	1.87	1.62	1.22	0.02	0.40	0.18	0.89

Table 4-1. *(contd)*

Sources	VWC_{Sh}		T_{Sh}		VWC_{D}		T_{D}	
	Pr> F		Pr> F		Pr> F		Pr> F	
	df (num,den)		*df* (num,den)		*df* (num,den)		*df* (num,den)	
	F		F		F		F	
	2007	2008	2007	2008	2007	2008	2007	2008
W × Pr × Date	0.99	0.44	0.91	0.99	0.99	**0.00**	0.79	0.98
	4,37	12,111	4,37	12,111	4,37	12,86	4,37	12,93
	0.06	1.01	0.23	0.28	0.01	3.32	0.42	0.31
D × Pr × Date	0.9	0.54	0.75	0.99	1.0	**0.02**	0.99	0.99
	8,37	24,111	8,37	24,111	8,37	24,86	8,37	24,93
	0.42	0.94	0.63	0.39	0.03	1.89	0.08	0.26
W × D × Pr × Date	0.92	0.78	0.99	0.79	1.0	0.42	0.91	0.99
	8,37	24,111	8,37	24,111	8,37	16,86	8,37	24,93
	0.39	0.75	0.19	0.75	0.02	11.85	0.39	0.36

The subscript for soil temperature (T) and volumetric water content (VWC) represent the corresponding depth; Sh for shallow depth (0-5cm) and D for deep soil (5-15cm). [1]Degrees freedoms are numerator/denominator, respectively.

Table 4- 2. Repeated measure analysis of soil greenhouse gas emission.

Bold numbers represent statistically significant changes in soil greenhouse gas emissions.

Sources	CO_2 [1]		N_2O [1]		CH_4 [1]	
	Pr> F		Pr> F		Pr> F	
	[2]*df* (num,den)		*df* (num,den)		*df* (num,den)	
	F		F		F	
	2007	2008	2007	2008	2007	2008
Warming(W)	**0.01**	0.13	0.17	**0.08**	**0.00**	0.44
	1,212	1,500	1,212	1,500	1,209	1,498
	6.47	2.22	1.86	3.14	8.09	0.58
Defoliation(D)	0.87	0.29	0.78	0.64	0.48	**0.07**
	2,212	2,500	2,212	2,500	2,209	2,498
	0.13	1.23	0.24	0.44	0.73	2.63
Precipitation(P)	**<.00**	**<.00**	**0.08**	0.1	0.36	**<.00**
	2,212	2,500	2,212	2,500	2,209	2,498
	66.54	336.92	2.44	2.30	1.03	18.10
W × D	0.23	0.41	0.76	0.33	0.23	0.2
	2,212	2,500	2,212	2,500	2,209	2,498
	1.46	0.88	0.26	1.10	1.45	1.60
W × Pr	**0.01**	0.13	**0.01**	0.6	0.52	0.21
	2,212	2,500	2,212	2,500	2,209	2,498
	4.25	2.05	4.32	0.50	0.66	1.52
D × Pr	0.93	0.47	0.18	0.66	0.91	0.18
	4,212	4,500	4,212	4,500	4,209	4,498
	0.21	0.88	1.57	0.59	0.23	1.56
W × D × Pr	0.1	0.17	0.8	0.62	**0.08**	**0.02**
	4,212	4,500	4,212	4,500	4,209	4,498
	1.97	1.59	0.40	0.66	2.09	2.75
Date	**<.00**	**<.00**	0.96	0.24	**<.00**	**<.00**
	2,212	6,500	2,212	6,500	2,209	6,498
	127.2	78.03	0.03	1.33	35.14	15.42
W × Date	0.13	0.28	**0.07**	0.21	**0.04**	**0.01**
	2,212	6,500	2,212	6,500	2,209	6,498
	2.01	1.25	2.63	1.39	3.19	2.58
D × Date	**0.00**	0.75	0.93	0.69	**0.03**	0.62
	4,212	12,500	4,212	12,500	4,209	12,498
	4.33	0.70	0.21	0.76	2.68	0.83
Pr × Date	**0.00**	**0.00**	0.32	0.18	0.81	**<.00**
	4,212	12,500	4,212	12,500	4,209	12,498
	5.27	2.75	1.17	1.35	0.40	8.66
W × D × Date	0.19	0.82	0.97	0.74	0.17	0.92
	4,212	12,500	4,212	12,500	4,209	12,498
	1.54	0.62	0.12	0.71	1.61	0.51
W × Pr × Date	0.5	0.75	**0.00**	0.69	0.71	0.82
	4,212	12,500	4,212	12,500	4,209	12,498
	0.84	0.70	4.65	0.75	0.52	0.62

Table 4-2. *(contd)*

Sources	CO_2		N_2O		CH_4	
	Pr>F		Pr>F		Pr>F	
	df (num,den)		*df* (num,den)		*df* (num,den)	
	F		F		F	
	2007	2008	2007	2008	2007	2008
D × Pr × Date	0.83	0.45	**0.06**	0.98	0.84	0.98
	8,212	24,500	8,212	24,500	8,209	24,498
	0.52	1.00	1.91	0.45	0.51	0.45
W × D × Pr ×	0.92	0.89	0.94	**0.05**	0.35	0.74
Date	8,212	24,500	8,212	24,500	8,209	24,498
	0.39	0.66	0.34	1.53	1.12	0.79

[1] unit of measurement: mg m^{-2} hr^{-1}. [2] Degrees freedoms are numerator/denominator, respectively.

Table 4- 3. Multiple regression analysis for soil greenhouse gas emissions with soil and plant properties.

Study Variable	Year	Parameters	Parameter Estimate	Model R-Square	F value	Pr >F
CO_2	2007	Litter(g m^{-2})	-0.16	0.18	7.47	0.01
	2008	Litter (%)	-2.2	0.50	31.25	<.0001
		Forbs Biomass (g/m^2)	0.47	0.60	7.99	0.008
		Soil nitrogen content (0-5 cm) (%)	41.03	0.66	4.62	0.04
N_2O	2007	Soil carbon content (5-15 cm) (%)	-0.02	0.14	5.71	0.02
		Soil nitrogen content (5-15 cm) (%)	0.21	0.22	3.18	0.08
	2008	Soil moisture content (5-20 cm) (%)	0.0001	0.09	3.27	0.08
CH_4	2007	-	-	-	-	-
	2008	Moss (%)	0.0006	0.11	4.15	0.05

Table 4- 4. Repeated measure analysis for microbial biomass and dissolved organic materials. Bold numbers represent statistically significant changes in microbial biomass and dissolved organic material.

Sources	Year	Warming Pr> F *df* (num,den) F	Defoliation Pr> F *df* (num,den) F	Precipitation Pr> F *df* (num,den) F	W× D Pr> F *df* (num,den) F	W×Pr Pr> F *df* (num,den) F	D×Pr Pr> F *df* (num,den) F	W×D×Pr Pr> F *df* (num,den) F
0-5 cm								
MBC [a]	2007	**0.00** 1,44 11.16	**0.09** 2,44 2.53	0.88 1,44 0.02	0.34 2,44 1.08	0.79 1,44 0.07	0.1 2,44 2.36	0.68 2,44 0.39
	2008	0.97 1,66.1 0.00	**0.01** 2,66.1 4.61	**<.00** 2,66.1 17.21	0.9 2,66.1 0.1	**0.08** 2,66.1 2.57	0.33 4,66.1 1.17	0.2 4,66.1 1.52
MBN [b]	2007	**0.06** 1,44 3.52	0.14 2,44 2.00	**0.06** 1,44 3.64	0.2 2,44 1.56	0.3 1,44 1.09	0.6 2,44 0.51	0.23 2,44 1.50
	2008	0.99 1,65.2 0.00	**0.01** 2,65.3 4.79	**<.00** 2,65.3 32.29	0.99 2,65.4 0.01	**0.05** 2,65.4 2.99	**0.08** 4,65.3 2.16	0.39 4,65.3 1.04
DOC [c]	2007	0.32 1,44 1.00	0.73 2,44 0.31	0.52 1,44 0.42	0.32 2,44 1.15	0.55 1,44 0.36	0.26 2,44 1.37	0.35 2,44 1.05
	2008	0.48 1,72 0.50	0.45 2,72 0.81	0.54 2,72 0.62	0.2 2,72 1.67	0.24 2,72 1.47	**0.01** 4.72 3.27	0.42 4,72 1.00
DON [d]	2007	0.22 1,44 1.49	0.62 2,44 0.47	0.63 1,44 0.23	**0.04** 2,44 3.30	0.24 1,44 1.39	0.59 2,44 0.53	0.55 2,44 0.59
	2008	0.61 1,68 0.26	0.38 2,68 0.98	0.57 2,68 0.57	0.22 2,68 1.51	0.21 2,68 1.58	**0.02** 4,68 3.12	0.45 4,68 0.92
5-15cm								
MBC	2007	0.44 1,48 0.58	**0.01** 2,48 4.60	**0.00** 1,48 8.99	**0.01** 2,48 4.70	0.71 1,48 0.13	0.13 2,48 2.11	0.23 2,48 1.49
	2008	0.9 1,67 0.01	0.49 2,67 0.72	**0.05** 2,67 3.00	0.48 2,67 0.73	0.71 2,67 0.34	0.46 4,67 0.91	0.38 4,67 1.05
MBN	2007	0.72 1,48 0.13	0.44 2,48 0.82	0.50 1,48 0.46	0.92 2,48 0.08	0.81 1,48 0.06	**0.04** 2,48 3.31	0.89 2,48 0.11
	2008	0.8 1,66.9 0.04	0.42 2,66.9 0.87	**<.00** 2,66.9 20.48	0.65 2,66.9 0.43	0.65 2,66.9 0.42	0.71 4,66.9 0.53	0.35 4,66.9 1.12
DOC	2007	0.36 1,44 0.85	0.73 2,44 0.32	0.22 1,44 1.54	0.93 2,44 0.07	0.80 1,44 0.06	0.51 2,44 0.67	0.61 2,44 0.49
	2008	0.41 1,71 0.67	0.33 2,77 1.12	0.64 2,71 0.45	0.42 2,71 0.88	0.42 2,71 0.87	0.41 4,71 1.00	0.68 4,71 0.57

Table 4-4. *(contd*

Sources	Year	Warming Pr> F *df* (num,den) F	Defoliation Pr> F *df* (num,den) F	Precipitation Pr> F *df* (num,den) F	W× D Pr> F *df* (num,den) F	W×Pr Pr> F *df* (num,den) F	D×Pr Pr> F *df* (num,den) F	W×D×Pr Pr> F *df* (num,den) F
DON	2007	0.43	0.85	0.41	0.80	0.53	0.86	0.86
		1,44	2,44	1,44	2,44	1,44	2,44	2,44
		0.63	0.16	0.69	0.22	0.39	0.15	0.15
	2008	0.74	0.9	0.32	0.42	0.64	0.54	0.68
		1,71	2,71	2,71	2,71	2,71	4,71	4,71
		0.13	0.07	1.16	0.93	0.48	0.80	0.61

[a] Microbial biomass carbon (g/kg), [b] Microbial biomass nitrogen (g/kg), [c] Dissolved organic carbon (g/kg),[d] Dissolved organic nitrogen (g/kg)

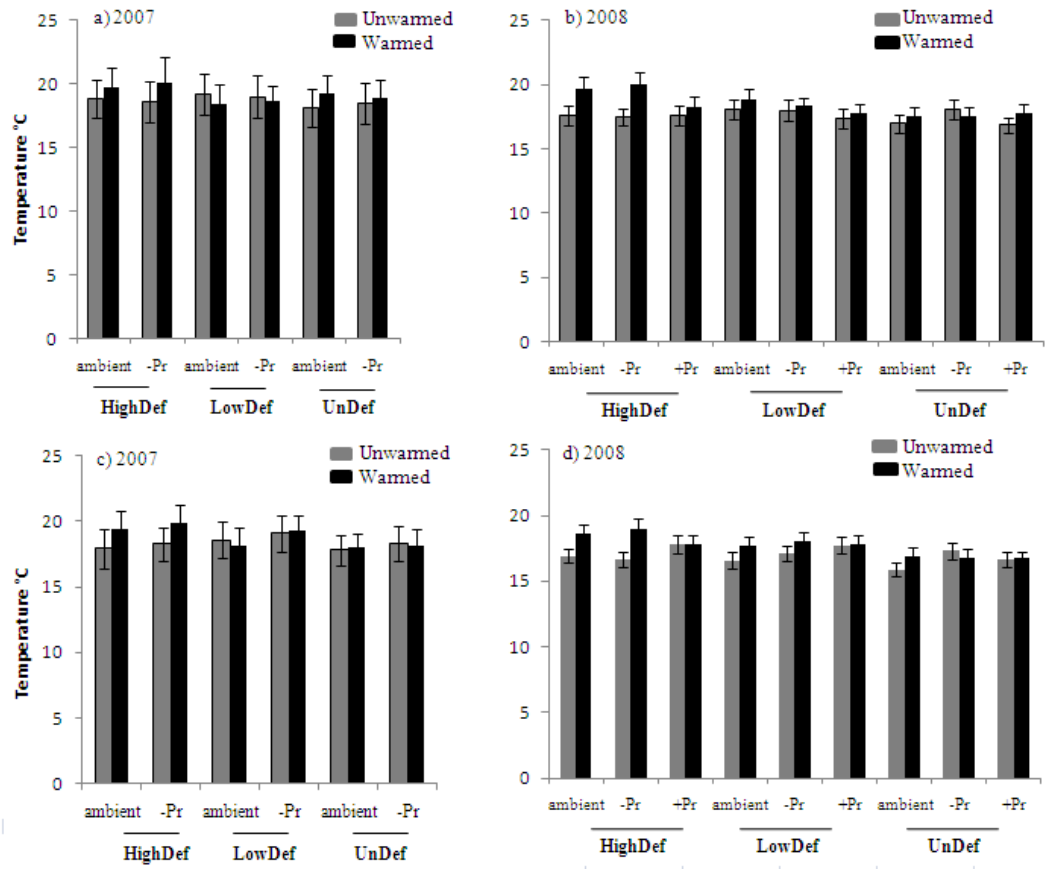


Figure 4- 1. Defoliation, precipitation and warming effects on average daily soil temperature in the 0-5 cm soil depth in 2007 (a) and 2008 (b), and in the 5-20 cm soil depth in 2007 (c), 2008 (d). Error bars represents SEs.

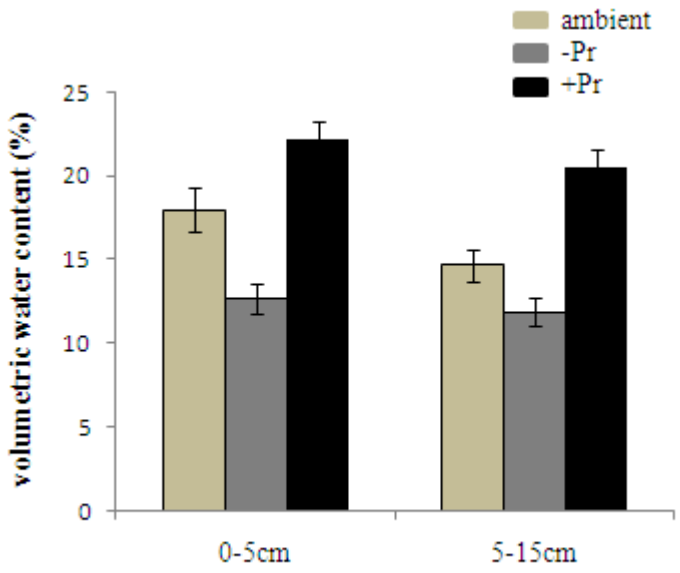


Figure 4- 2. Precipitation effects on average daily soil volumetric water content at 0-5 cm and 5-20 cm soil depths in 2008. Error bars represents SEs.

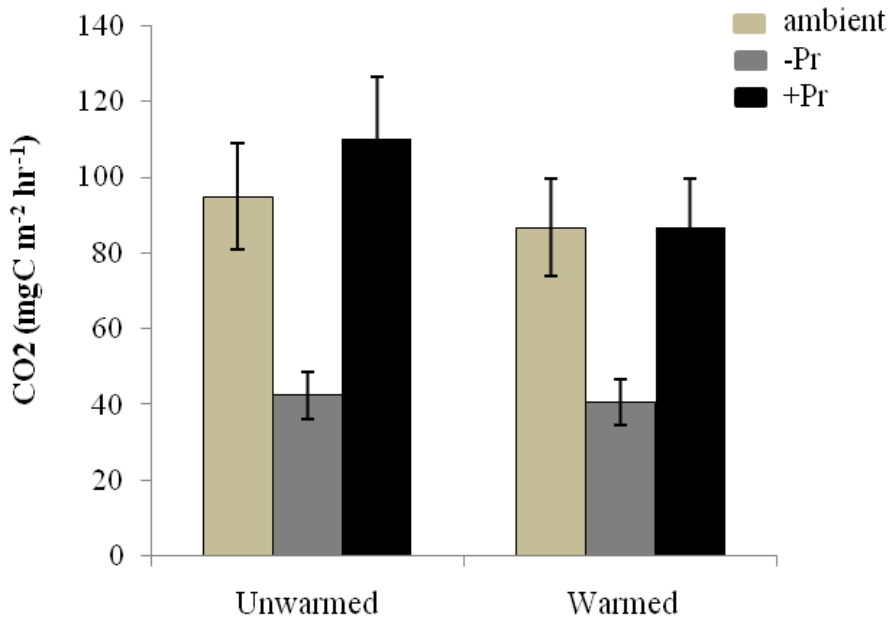


Figure 4- 3. Precipitation and warming effects on soil carbon dioxide efflux in 2007. Error bars represents SEs.

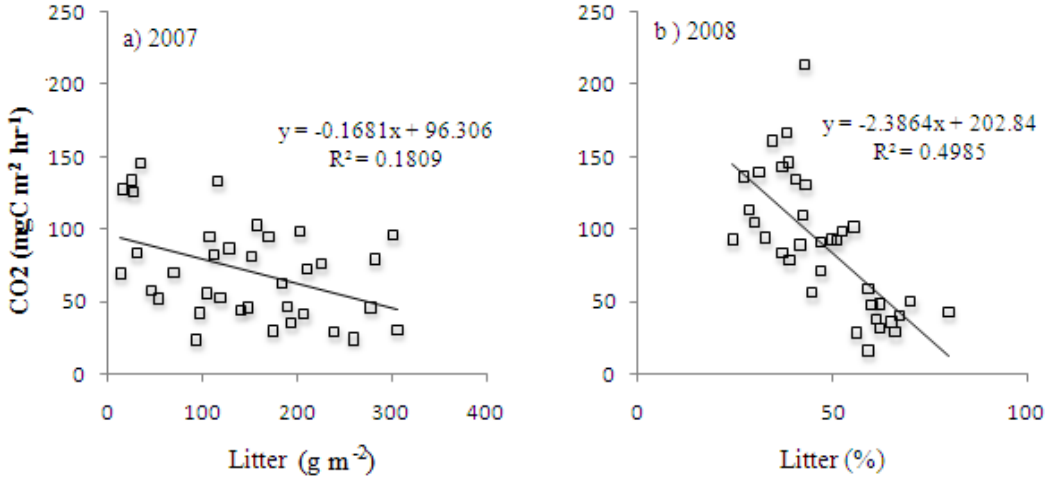
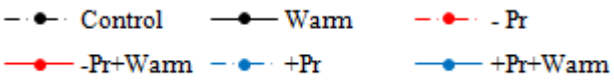


Figure 4- 4. Rate of soil carbon dioxide efflux as a function of litter quantity in a) 2007, b) 2008.



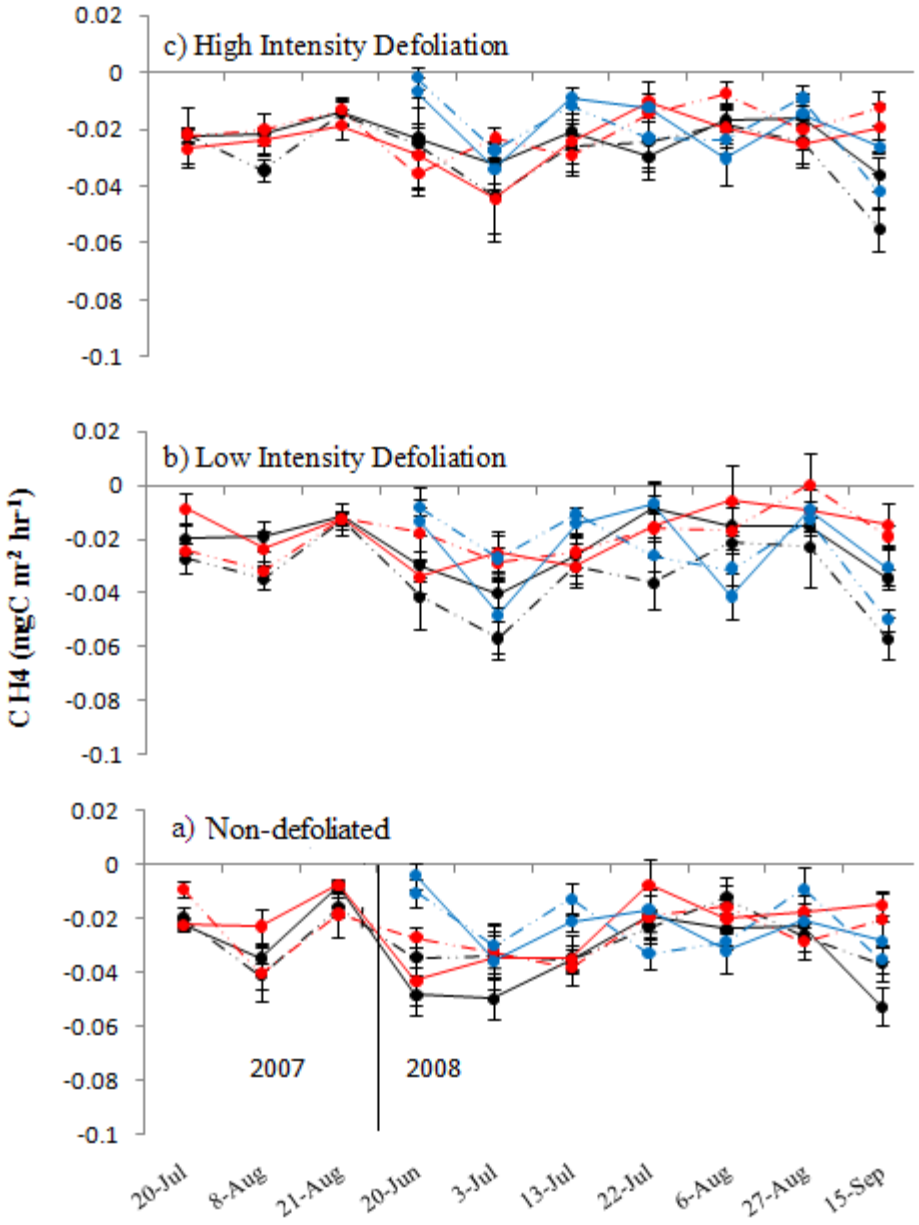


Figure 4- 5. Defoliation, precipitation and warming effects on soil methane efflux in the 2007 and 2008 growing seasons (a) Soil methane efflux at high intensity defoliation, (b) Soil methane efflux at low intensity defoliation, (c) Soil methane efflux at no defoliation. Error bars represents SEs.

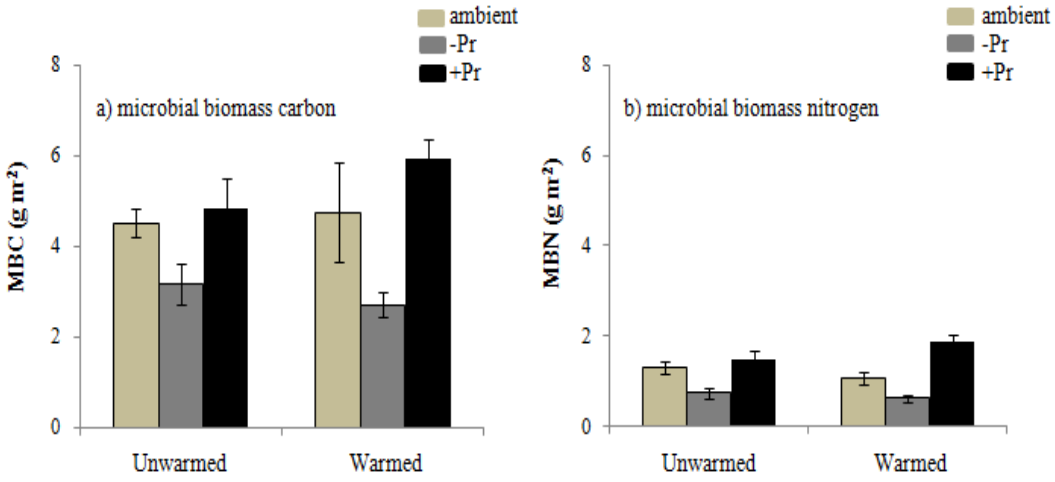


Figure 4- 6. Precipitation and defoliation effects on soil microbial biomass at 0-5 cm soil depth in 2008, a) soil microbial biomass carbon, b) soil microbial biomass nitrogen. Error bars represents SEs.

Chapter 5 Consequences of Precipitation, Warming, and Defoliation on Soil Microbial Community Structure and Function in a Native Grassland (Ecosite B)

Introduction

Northern temperate grassland ecosystems have been impacted by climate change over the past several decades (IPPC, 2001). The projection of climate change in grassland ecosystems across the Prairie Provinces of Canada predicts an increase of 2 °C in mean annual temperature and changes of ±10% in precipitation by 2020s (Sauchyn *et al.,* 2009). Climate change may have wide-ranging effects on ecological processes and ecosystem functions (Rustad *et al.,* 2001). Of particular concern is the impact of climate change on both the sustainability of biotic processes (Jarvis *et al.,* 2005) and their associated C sequestration (Rustad *et al.,* 2001; de Graaff *et al.,* 2006) within terrestrial ecosystems, which in turn, can be further influenced by grazing in grassland ecosystems (Reeder & Schuman, 2002). Several studies have been carried out to evaluate the effects of climate change (e.g., Coughenour & Chen, 1997; Rustad *et al.,* 2001) and grazing practices (e.g., Reeder & Schuman, 2002; Zhou *et al.,* 2002) on soil biotic processes. While these investigations have reported that changes in climate and grazing patterns were important in regulating soil nutrient dynamics, the impact of climate change and grazing on soil microbial community composition and functional diversity has been far less studied and remains poorly understood.

Bacteria and fungi comprise 90% of soil microorganisms (Rinnan & Baath, 2009) and play key roles in soil biotic processes such as nutrient cycling (Ingham *et al.,* 1985), and hence ecosystem C budget (de Graaff *et al.,* 2006; Six *et al.,* 2006). For many years, these two microbial groups have often been lumped into one decomposer category (Uphoff *et al.,* 2006), despite their different roles in the decomposition processes (Rinnan & Baath, 2009). Soil bacteria and fungi differ in metabolism (Six *et al.,* 2006) and C substrate utilization (Carroll & Wicklow, 1992). For example, Wardle (2002) reported that fungi utilized C associated with soil organic matter while bacteria rely on labile carbon that mostly exists in soil water. Another important difference is the higher C: N ratio in fungal than in bacterial cells (Sylvia *et al.,* 2005), suggesting that fungi growth may make a greater contribution to C sequestration. Therefore, shifts in soil microbial community composition may have important implications on overall soil C budgets (Six *et al.,* 2006).

Soil microbial community composition can be influenced by abiotic and biotic factors (Myers *et al.,* 2001). For example, studies in temperate heathland during drought suggest that soil fungi could dominate over bacteria and accelerate decomposition rates of more complex substrates (Jensen *et al.,* 2003) or even influence soil function (Whitford, 1989). Fungi and gram-staining groups of bacteria were found to be responsive to higher temperature, with declining relative abundance of fungi and gram-negative bacteria in an incubation study of grassland soil (Feng & Simpson, 2009).

Moreover, given the influential role of management practices on plant characteristics and soil microclimate, grazing management could cause shifts in soil microbial community structure (Ingram *et al.,* 2008) or composition (Zhang *et al.,* 2005). The extent to which climate and grazing strategies alter soil microbial communities depends on their interactive relationship, which could lead to different net responses. Such complexities possibly explain the contradictory observations in earlier studies where warming led to a decrease in soil bacteria (Rinnan *et al.,* 2007) and fungi (Frey *et al.,* 2008; Feng & Simpson, 2009) or an increase in soil bacteria (Bàrcenas-Moreno *et al.,* 2009) and fungi (Zhang *et al.,* 2005). Further research is needed to improve our understanding of the effects of climatic factors and grazing practices on soil microbial composition and function for sustainable management, as well as the prediction of potential feedback in grassland ecosystems under future climate change.

As a part of a larger project evaluating the impact of climate change and grazing intensity on rangeland ecosystems in the Canadian Prairie Provinces (Alberta, Manitoba, and Saskatchewan), the objective of this study was to investigate the influences of defoliation intensity, warming and altered precipitation on soil microbial community structure and function in a northern temperate grassland.

Material and Methods

2.1. Site Description

The study was conducted in a temperate grassland at the University of Alberta Research Station near Kinsella (53°05 N, 111°33 W), Alberta, Canada. The site is located within the grassland-forest ecozone and is dominated by *Festuca hallii*, *Artemisia frigida, Elymus trachycaulus, Pulsatilla patens, Koeleria macrantha, Oxytropis campestris,* and *Stipa curtiseta*. The main reason for choosing this site was its susceptibility to future climate change. In this ecozone, fescue prairie will give way to mixed prairies (Vandall *et al.*, 2006) under predicted future warmer climates. The areas soils are Orthic Dark Brown Chernozems (Howitt, 1988), with a sandy loam texture at both 0-5 cm (6% clay, 31% silt, 63% sand) and 5-15 cm (11% clay, 27% silt, 62% sand) soil depths. The long-term (1971-2000) average annual and growing season precipitation and temperature were 431.3 and 312.2 mm, and 2.4 and 13.9 °C, respectively. The weather conditions at sampling time (July) were cooler (by 1.8 ºC) and wetter (47.1 mm) in 2008 (temperature: 18.5 °C, precipitation: 55.3 mm) than in 2007 (temperature: 20.3 °C, precipitation: 8.2 mm).

2.2. Experimental Design

In early May 2007, ninety 2 × 2 m plots were established in a completely randomized block (CRB) design with a total of five blocks. The experiment used three treatments, including warming (ambient and warming), altered precipitation (ambient and ±50% precipitation), and defoliation (non-defoliation, low intensity defoliation and high intensity defoliation).

Circular open-top chambers (OTCs) with 2 m diameter and 0.4 m height and 60 degree side angle have been found to be an effective tool for temperature

enhancement in northern ecosystems (Marion *et al.,* 1997), and were used to simulate warming. In addition to an non-defoliated treatment, defoliation was achieved with manual aboveground defoliation (i.e. clipping) to stubble heights of 7 cm (low intensity) or 3 cm (high intensity), using a trimmer in June 2007 and 2008. To change the amount of precipitation, transparent rainout shelters were used as described in Chapter 4.

2.3. Soil Sampling

Composite soil samples were collected from 4 soil cores (2 cm diameter × 5 cm deep) at 0-5 cm soil depth on July 20 of 2007 and 2008. Soil samples were then immediately placed in a cooler and transported to the lab at the University of Alberta. Fresh soil samples were promptly sieved through a 4 mm mesh and all visible plant material removed. Two subsamples were obtained for soil microbial community structure (phospholipids fatty acid or PLFA) and physiological function (Biolog™) analysis. The sample for microbial community structure analysis was stored in a deep freezer at -20 °C and the other one kept at 4 °C for soil microbial functional diversity analysis.

2.3. Soil Microbial Community Structure

Shifts in soil microbial community structure were determined by PLFA analysis using 10 g of freeze-dried soil in 2008, following the method of Bossio *et al.* (1998). Identification of PLFAs followed the standard protocol of MIDI (1995) on a Hewlett Packard 5890 A Gas Chromatograph (Hewlett-Packard Co.,

Avondale, PA, USA). The nomenclature of fatty acids in this study followed Bossio *et al.* (1998). The PLFA signatures i14:0, 14:0, i15:1c, i15:0, a15:0, 15:0, i16:1c, i16:0, a16:0, 16:0, 2OH-16:1, 17:0, i17:0, a17:0, 17:0 cy, 18:1ω7c, 18:0, 19:0 cy are considered to be predominantly bacterial (Federle *et al.*, 1986; Tunlid *et al.*, 1989; Frostegård *et al.*, 1993; Frostegård & Bååth, 1996; Schmitt *et al.*, 2008; Amir *et al.*, 2008; Baniulyte *et al.*, 2009). The PLFA signatures 16:1ω5c, 16:1ω7c +15i 2OH, 18:1ω9c, 18:2ω6.9c are considered indicative of fungal fatty acids (Federle *et al.*, 1986; Frostegård & Bååth, 1996; Olsson *et al.*, 1998; Mikola & Setälä , 1999; Kourtev *et al.*, 2003). PLFAs were also categorized into possible groups of gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0) (O'Leary & Wilkinson, 1988), gram-negative bacteria (2OH-16:1, 17:0 cy, 18:1ω7c, 19:0 cy) (Amir *et al.*, 2008; Schmitt *et al.*, 2008; Hamer & Makeschin, 2009), actinomycetes (10 Me16, 10 Me17, 10 Me18, 10 Me19) (McKinley *et al.*, 2005; Joergensen & Wichern, 2008), and arbuscular mycorrhizae fungi (AM) (16:1ω5c) (Rahman, 2008).

PLFAs were expressed as relative abundance (mol% of the total PLFAs) in a given sample. The ratio of relative abundance of fungi- to-bacteria (F: B) was computed to assess changes in soil microbial community composition (Zhang *et al.*, 2005; Frey *et al.*, 2008). The Shannon diversity index (*H*) was calculated according to the following equation:

$$H = -\sum p_i \ln p_i \qquad (1)$$

where p_i is the proportion represented by each PLFA relative to their total.

2.4. Soil Microbial Physiological Function

Community-level physiological profile (CLPP) was studied in 2007 and 2008 to characterize the physiological function based on carbon substrate utilization patterns. Two distinct Biolog™ microplates (Biolog Inc., Hayward CA., USA), ECO and SF-N2, were used to study the physiological function of bacteria and fungi, respectively. Bacteria were extracted from 1 g of fresh soil in 100 mL 0.85% sterile NaCl solution. Biolog™ ECO plates were inoculated with 120 µL diluted (10^{-3}) suspension per well. Microplates were then incubated in the dark at 25 °C for 7 days and optical density measured at 0, 24, 48, 72, 96, 120, 144 h on a Biolog MicroStation™ reader (Biolog Inc., Hayward CA., USA).

To extract soil fungi, the protocol for bacteria extraction was modified following guidelines from Biolog Inc. for the SF-N2 plates (Biolog Inc., Hayward CA., USA). To prevent bacterial growth, we used streptomycin sulfate and chlortetracycline antibiotics (Zak & Parkinson, 1984; Dobranic & Zak, 1999). Ultraviolet (UV) light and an autoclave were used to sterilize all equipment, and preparation of microplates was done under a laminar-flow hood to prevent contamination.

Color development of individual C substrates over time was computed to calculate C substrate utilization (Garland & Mills, 1991), representing microbial physiological function on organic compounds. The metabolic potential in Biolog microplates was expressed as the average well color development (AWCD)

(Garland & Mills, 1991). In addition, to test the effects of warming, precipitation, and defoliation on the potential utilization of organic compounds, the AWCD area was calculated for the major organic compounds (Guckert *et al.*, 1996) in ECO plates (carbohydrate, polymer, carboxylic acid, phosphorylated chemical, amino acid, amine, ester; Jena *et al.*, 2006) and SF-N2 plates (carbohydrate, polymer, carboxylic acid, phosphorylated chemical, amino acid, amine, ester, alcohol, nucleoside; Petrucci *et al.*, 2002). The Shannon diversity index was calculated using equation (1), where p_i was the proportion represented by each C substrate utilization relative to the total use.

2.5. Statistical Analysis

All multivariate analyses for PLFAs and CLPP data sets were performed using PC-ORD version 5.10 (McCune & Mefford, 2006). Non-metric multidimensional scaling (NMS) (Kruskal, 1964; Mather, 1976) with the autopilot option and Sørensen distance measure was used to examine changes in soil microbial community structure and functional diversity caused by the treatments. The main matrix consisted of relative abundance of individual PLFAs in soil microbial community structure and the area under the curve of individual C substrates in soil microbial functional diversity analysis. The second matrix consisted of explanatory variables, including volumetric water content, soil temperature, soil nitrogen supply rate, microbial biomass carbon and nitrogen (Chapter 4), dissolved organic carbon and nitrogen (Chapter 4), soil carbon and nitrogen content (Nyanumba *et al.*, unpublished), plant diversity and biomass

(White *et al.*, unpublished), soil microbial taxonomic groups (from the PLFA data set), and groups of organic compounds (from the Biolog data set). The relationship between plots and associated explanatory variables in ordination space were then examined by superimposing the variables as joint plots, which are determined by Pearson and Kendall correlations of explanatory variables with ordination axes. Multi-Response Permutation Procedures (MRPP) with Euclidian distance tested the differences in soil microbial community structure and functional diversity with respect to the treatments.

All univariate analyses were performed using SAS v 9.1, using the GLIMMIX macro, transforming the data with gamma distribution, if necessary, to overcome the over-dispersion problem in the dataset (Littel *et al.,* 1996) (i.e., presence of an excess number of zeros). Shannon diversity index, AWCD, metabolic activity on organic compounds, the relative abundance of microbial taxonomic groups, and the fungi-to-bacteria ratio were analyzed with normal distribution and identity link-function (Shannon diversity index, ratio of fungi-to-bacteria) or gamma distribution and log link-function (AWCD, metabolic activity on organic compounds, microbial taxonomic groups). Shannon diversity index data were then subject to multiple regression analysis with the above mentioned explanatory variables, using the *proc stepwise* procedure with an alpha value adjustment of slentry = 0.1. The CLPP dataset was analyzed by year. An alpha value of 0.1 was used for significance due to low replication necessitated by logistical constraints (Scheiner & Gurevitch, 2001).

Results

3.1. Soil Microbial Physiological Function

3.1.1 Soil Bacterial Physiological Function

NMS ordination represented 90% (final stress =15.7, final instability = 0.00001) and 96% (final stress = 11.1, final instability = 0.00001) of total variance in bacterial substrate utilization, respectively, in 2007 and 2008. No clear separation was apparent in bacterial metabolic activity due to warming (MRPP, 2007: $p = 0.85$, A= -0.004; 2008: $p = 0.70$, A= -0.002), defoliation (MRPP, 2007: $p = 0.63$, A= -0.003; 2008: $p = 0.33$, A= 0.001) and precipitation (MRPP, 2007: $p = 0.54$, A= -0.001; 2008: $p = 0.11$, A= 0.007). Pearson correlation analysis showed that there were strong positive correlations between carbohydrate substrates and axis 1 in 2007 (r= 0.85) and 2008 (r=0.77).

The physiological function of soil bacteria varied with treatments. Precipitation influenced the utilization by bacteria of amino acids (p = 0.097), amines (p = 0.031) in 2007, as well as carboxylic acids (p = 0.062), polymers (p = 0.008) and phosphorylated chemicals (p = 0.092) in 2008. In general, precipitation exclusion suppressed the utilization of amino acids and amines by 9% and 21%, respectively, in 2007, and of carboxylic acids and polymers by 17% and 16%, respectively, in 2008. Changes in phosphorylated chemicals were more complex due to a three-way interaction of warming x precipitation x defoliation. A decline in polymer utilization was evident due to precipitation exclusion in check (i.e. non-defoliated and unwarmed) plots, as well as those plots exposed only to warming, by 36% and 22%, respectively. Defoliation changed amino acids

in 2007 ($p \leq 0.065$). Bacteria utilized 27% less amino acids in high intensity defoliation compared to non-defoliated plots.

Warming, precipitation and defoliation did not affect bacterial functional diversity (Shannon diversity index based on CLPP data) (Table 5-1).

3.1.2. Soil Fungal Physiological Function

NMS ordination explained 96% (final stress = 8.5, final instability = 0.00000) and 95% (final stress = 11.6, final instability = 0.00000) of total variance in fungal substrate utilization, respectively, in 2007 and 2008, based on the two- and one- dimensional solutions. However, no separation in fungal substrate utilization was observed with respect to warming (MRPP, 2007: $p = 0.15$, A= 0.005; 2008: $p = 0.26$, A= -0.002), defoliation (MRPP, 2007: $p = 0.27$, A= 0.003; 2008 $p = 0.41$, A= -0.000), or precipitation (MRPP, 2007: $p = 0.15$, A= 0.005; 2008: $p = 0.17$, A= 0.005). There was also little correlation between explanatory variables and C utilization in 2007. Nevertheless, in 2008, carbohydrates (r = -0.24), polymers (r = -0.36) and amino acids (r = -0.38) were negatively correlated with axis 1.

The metabolic potential (AWCD) of fungi responded to warming ($p = 0.04$) in 2007 and precipitation ($p = 0.08$) in 2008. Fungi AWCD decreased by 11% due to warming in 2007, and increased by 16% in the precipitation addition treatment relative to the ambient and precipitation exclusion treatments in 2008. Fungal physiological activity on polymers, carboxylic acid, phospholipid chemicals, amines, amino acids, carbohydrates, esters, and alcohol varied with precipitation and warming treatments, as well as their interaction. The transient effect of

warming caused soil fungi to utilize 16% less carboxylic acids, polymers, phosphorylated chemicals, amino acids, amines, alcohol, and nucleoside in 2007. Precipitation exclusion suppressed the fungal utilization of polymers and alcohol substrates by 10-11% in 2007 ($p \leq 0.036$). In contrast, precipitation addition caused soil fungi to utilize more (by 11- 31%) phospholipids, amino acids and alcohol in 2008 ($p \leq 0.098$). Finally, the utilization of carbohydrates, phospholipids, amino acids, and alcohols by fungi was affected by a defoliation x precipitation interaction in 2007 ($p \leq 0.099$). Defoliation effects were only evident in reduced precipitation treatments, with fungi utilizing 5-13% less of these substrates at low intensity defoliation, and 16-25% more at high intensity defoliation compared to non-defoliated plots.

Fungal functional diversity was relatively similar between treatments, with the exception of responses to warming ($p = 0.05$) and defoliation ($p = 0.07$) in the first year of study (Table 5-1). In addition, little of the variability in bacterial and fungal functional diversity was explained by the explanatory variables based on multiple regressions ($R^2 = 0.05$, $p \leq 0.1$).

3.2. Soil Microbial Community Structure

Non-metric multidimensional scaling (NMS) ordination was able to explain 92% of total variance in relative abundance of microbial PLFA in a two-dimensional solution (final stress =29.83, final instability =0.07). No visual separation in PLFAs was observed with respect to the treatments. The MRPP analysis supported the visual assessment of the ordination for defoliation (MRPP:

p = 0.51, A= -0.003) and precipitation (MRPP: p = 0.1, A= 0.01). Despite the lack of visual separation in ordination due to warming (Fig. 5-1), the significant effect of warming on soil microbial community structure was apparent (MRPP: p = 0.004, A= 0.03). As indicated by Pearson correlation analysis, a strong negative correlation was observed between the PLFAs of actinomycete and axis 1 (Table 5-2).

Although soil microbial community composition represented by the fungi: bacteria ratio did not respond to warming, defoliation or precipitation treatments ($p > 0.1$), the relative abundance of soil bacteria ($p = 0.01$), actinomycete ($p = 0.02$), and fungi ($p = 0.04$) varied as a result of the warming x defoliation interaction (Fig. 5-2). Relative abundance of bacteria was opposite that of fungi and actinomycete, increasing due to warming within high intensity defoliation plots, and decreasing in low intensity and non-defoliated plots (Fig. 5-2). However, the response in actinomycete was also more complex, influenced by warming x precipitation ($p = 0.05$) and defoliation x precipitation interactions ($p = 0.001$). Surprisingly the maximum relative abundance of actinomycete was observed with reduced precipitation, regardless of defoliation presence (Fig. 5-3). Finally, warming sharply increased the relative abundance of gram-positive bacteria by 42%.

Warming, precipitation, and interactions of warming with precipitation and defoliation affected microbial community diversity based on Shannon diversity indices derived from PLFA data (Table 5-1). The highest microbial diversity was found within precipitation exclusion plots with warming (Fig. 5-4). Warming

resulted in marked increases in microbial diversity across precipitation exclusion (from 1.16 to 2.09), precipitation addition (from 0.93 to 1.62), low intensity defoliation (from 0.8 to 1.5), and non-defoliated plots (from 0.98 to 1.8). However, no correlation was found between microbial diversity and explanatory variables.

Discussion

Two years of warming, defoliation and precipitation treatments caused minimal effect on metabolic potential in this grassland soil, although soil microbial community structure and utilization of a few organic substrates changed in response to the treatments. Likewise, we found no response in the fungi: bacteria ratio and a transient response in metabolic potential (AWCD) to the treatments over two years. In fact, treatment effects on the utilization of organic compounds such as amino acids, amines and polymers did not change the overall metabolic potential and C substrate utilization in the system. Our findings are in contrast with previous studies in grassland ecosystems (e.g., Zhang *et al.*, 2005), and instead suggest the stability of ecosystem function in this grassland soil is high under short-term perturbations as represented by the warming, precipitation and defoliation treatments, even though caused significant changes in soil microclimate (Chapter 4)

4.1. Soil Microbial Physiological Function

In previous studies, soil metabolic potential was found to be sensitive to herbivory and climatic parameters (Zhang *et al*., 2005; Bell *et al.,* 2008; Rinnan *et al.,* 2009). We found limited changes in bacterial and fungal functional diversity as well as their metabolic potential (AWCD). However, we did observe that metabolic activities of both bacteria and fungi on an array of C substrates varied with the treatment. Shifts in utilization of C substrates might be caused by sensitivity of root exudates to defoliation (Murray *et al.,* 2004), warming (Lemaire & Millard, 1999), and precipitation (Pinton *et al.,* 2001), which could influence soil microbial community in the rhizosphere (Bardgett *et al.,* 1999). A direct evidence of changes in the root exudates can be significant responses in plant richness to the warming by defoliation interaction in 2007 and 2008 as well as precipitation main effect in 2008 growing season in the study site (Bork *et al.,* 2009).

However, the overall weak response in overall metabolic potential of soil microbes to the treatments might be the result of relatively high plant diversity in this grassland ecosystem (Coupe, 2003). There is experimental evidence to support the positive linkage between above- and belowground functional diversity (Rodriguez-Loinaz *et al.,* 2008). In this case, the relatively high plant diversity across this study site might have resulted in high belowground functional diversity in soils of this ecosystem (Coupe, 2003), in turn contributing to the stability observed in the microbial functional group.

Although differences were seen in the diversity of bacterial and fungal communities, which may partially occur following by changes in plant diversity

due to warming x defoliation (Bork *et al.,* 2009), these changes did not occur within functional groups, as evidenced by a relatively weak response in the functional diversity of soil bacteria and fungi based on Biolog microplate data. This result suggests a high degree of functional redundancy may exist in microbial communities within the soil matrix (Mukerji *et al.,* 2006). As a result, even if climatic and physical disturbance changed soil microbial diversity at the community scale, the microbial functional diversity that is critical for energy and nutrient flux in the soil system may not be influenced, thereby contributing to the observed stability in soil function within this temperate grassland.

Direct supporting evidence for stability in this soil matrix was evident in that the imposed physiological stress of precipitation exclusion led to an increase rather than a decrease, in soil microbial community diversity, as indicated by the Shannon diversity index. Given that drought stress can depress soil microbial community diversity in favor of those faster adapting microbes (Atlas, 1984; Bottner, 1985), soil microbial communities in this system may have a high potential to adapt quickly to drought stress. Such rapid adaptation to the new microenvironment may result in improved stability in soil functioning over the longer term, and therefore result in a more sustainable ecosystem.

4.2. Soil Microbial Community Structure

Consistent with previous studies (Zogg *et al.,* 1997; Zhang *et al.,* 2005; Feng & Simpson, 2009), about 1 °C of warming shifted soil microbial community structure at this site, together with bacterial and fungal diversity (Bardgett *et al.,*

1999), even when combined with other treatments. The role of temperature in the biological world has long been established (Van't Hoff 1898). One main explanation for changes in soil bacterial and fungal diversity is that warming might favor microbial population growth (Bardgett *et al.,* 1999) in correspondence to their optimal temperature (Violante, 2002). The average higher temperature in July 2008 (18.5 °C) comparison to long-term (1971-2000) July temperature of 16.9 °C, may also provide favorable micro-climate for microbial growth, particularly in warmed plots. Alternatively, warming-driven changes in the aboveground community (i.e., in plant biomass and richness; Zak *et al.,* 2003), and hence substrate availability, may mediate effects on the belowground community (Bardgett *et al.,* 1999). However, the latter explanation may not apply here because the effect of warming on the aboveground plant productivity was relatively weak (White, unpublished data).

However, effects of warming on the relative abundance of bacteria and fungi were related to defoliation intensity, similar to responses in plant richness in the study site (White, unpublished data). When the effect was significant, warming increased the relative abundance of bacteria at high intensity defoliation, but increased the fungi within low intensity and non-defoliation treatments. Such changes may occur because of the effects of management practices on substrate quality (i.e., the ratio of C:N) and associated bacterial and fungal activity (Bossuyt *et al.,* 2001). Changes in litter quality due to altered plant richness under warming x defoliation (White, unpublished data), warming reduced plant richness in high intensity defoliation and increased in the absence of defoliation, could also

have contributed to our results. Additionally, the availability of soil inorganic N can negatively influence the fungi-to-bacteria ratio (Bardgett & McAlister, 1999). Increased soil inorganic-N under high intensity defoliation (data not shown) likely explains the increase in soil bacterial abundance in that treatment. Furthermore, the significant impact of warming x defoliation was also dependent on soil temperature (Table 4-1) with the increase of 2.1, 0.07, and 0.69 °C due to warming treatment, respectively, at high, low, and non-defoliated plots. Therefore, higher soil temperature favored the soil bacteria at high intensity defoliation, in contrast with previous finding (Zhang *et al.*, 2005). These results demonstrate that the food-web and decomposition process in highly disturbed and grazed areas were driven primarily by decomposer bacteria, while decomposer fungi are of greater importance in less disturbed systems (Bardgett *et al.*, 2001).

The increase in bacteria under high intensity defoliation and in fungi decomposers at low intensity defoliation has several important ecological implications. For example, soil fungi are vital for the degradation of dead plant, animal and insect biomass, particularly complex polymers associated with those substrates (Maier *et al.*, 2009). As a result, the higher abundance of soil fungi may lead to the decomposition of more complex organic compounds, which are believed to sequester C for longer time periods. Another example is the role of soil bacteria and fungi in their ability to form biofilm, a complex aggregate of microorganisms associated with the soil surface or plant root in the rhizosphere (Davey & O'Toole, 2000), and known to facilitate nutrient exchange. We did not investigate biofilm structure and formation in this study, but speculate that

grazing intensity can influence nutrient exchange in this soil by modifying biofilm properties.

In addition, shifts in actinomycete population could result in changes to C decomposition. The increase in actinomycete population in the precipitation exclusion treatments across all defoliation plots suggests that actinomycete adapt relatively quickly to drought stress in this system (Davet, 2004). Because actinomycete are involved in decomposing complex organic compounds such as cellulose and lignin, the greater abundance of actinomycete may result in greater potential for the breakdown of complex C compounds under drier conditions.

However, despite shifts in soil microbial community structure, no significant changes were observed in the ratio of fungi: bacteria. This is in contrast with our expectation and earlier findings in grassland ecosystems (Zhang *et al.*, 2005). One likely explanation may be the opposing and offsetting response of biomarkers for bacteria and fungi with respect to our treatments. For example, while warming increased the abundance of bacteria under high intensity defoliation, the abundance of fungi decreased under the same condition, thereby stabilizing the ratio of fungi: bacteria. Although this might suggest that the ratio of fungal- to bacterial-based C decomposition could be maintained in a changing environment, the overall degree of organic matter decomposition may differ due to shifts in soil microbial community structure. These findings implicates the controlling role of temperature, precipitation, and aboveground plant biomass removal over the microbial community structure and hence organic carbon decomposition.

Conclusions

Traits of the soil microbial community (i.e., structure and substrate utilization) can be influenced by changing climatic conditions and defoliation intensities. However, we observed that the metabolic potential (AWCD) of soil bacteria and fungi were stabilized in the short-term. When combined with the fairly constant diversity in microbial functional groups under treatments, this provided evidence for enhanced functional stability in this temperate grassland. Furthermore, stability in microbial functional diversity coincided with enhancement of microbial community structural diversity under certain treatments imposed in this study. Increased stability and diversity suggests that soil microorganisms in this temperate grassland contribute to the resilience and adaptation of soil functioning towards the imposed stresses (such as drought). As a result, this grassland ecosystem maintained overall ecosystem function under both climatic and management (i.e. defoliation) disturbances, at least in the short term. Long term studies are necessary to investigate whether future climate change could lead to a more marked shift in C utilization and microbial community composition in this grassland soil.

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Table 5- 1. Analysis of variance for the microbial structural and functional Shannon diversity index in the PLFA and CLPP datasets.

Sources	Yr	PLFA			ECO			SF-N2		
		[1]*df* nN/nD	F	p	*df* nN/nD	F	p	*df* nN/nD	F	p
Warming	2007	-	-	-	1/44	1.5	0.22	1/48	7.5	**0.05**
(W)	2008	1/71	37.7	**0.00**	1/68	0.8	0.35	1/67	0.07	0.8
Defoliation	2007	-	-	-	2/44	1.35	0.26	2/48	5.4	**0.07**
(D)	2008	2/71	2.75	0.17	2/68	1.01	0.37	2/67	1.38	0.35
Precipitation (P)	2007	-	-	-	1/44	2	0.16	1/48	1.03	0.36
	2008	2/71	16.1	**0.01**	2/68	1.26	0.29	2/67	0.37	0.71
W×D	2007	-	-	-	2/44	0.76	0.47	2/48	1.79	0.27
	2008	2/71	8.85	**0.03**	2/68	0.96	0.38	2/67	0.58	0.60
W×P	2007	-	-	-	1/44	0.00	0.98	1/48	2.03	0.22
	2008	2/71	12.5	**0.01**	2/68	0.48	0.62	2/67	0.92	0.46
D×P	2007	-	-	-	2/44	2.17	0.12	2/48	1.2	0.39
	2008	4/71	3.45	0.12	4/68	1.82	0.13	4/67	0.85	0.55
W×D×P	2007	-	-	-	2/44	0.18	0.83	2/48	2.52	0.19
	2008	4/71	4	0.1	4/68	0.19	0.94	4/67	1.32	0.39

[1]Degree of freedom, *df* Nn/Dn, are numerator/denominator, respectively.

Table 5- 2. Pearson correlation (r) of explanatory variables with ordination axes.

Sources	Axis 1	Axis 2	Sources	Axis 1	Axis 2
Actinomycete	-0.86	-0.50	Microbial biomass C	0.16	0.04
Bacteria	0.68	0.03	Microbial biomass N	0.15	0.05
Fungi	-0.62	-0.11	Dissolved organic C	0.11	0.06
G + bacteria	-0.82	-0.42	Dissolved organic N	0.14	0.05
G – bacteria	-0.70	-0.61	Soil C content	0.07	0.14
Mycorrhizae	-0.71	-0.34	Soil N content	0.07	0.14
F: B	-0.28	0.38	Plant Diversity	0.12	0.11
MSC[a]	-0.51	0.01	NO_3-N supply rate	-0.09	-0.02
Moist[b]	0.25	0.09	NH_4-N supply rate	0.06	0.04
VWC[c]	0.04	0.01	Aboveground Biomass	0.13	0.08
Temperature	-0.09	-0.06	Graminea Biomass	0.16	0.06

[a] Some of misc. fatty acid associated with any particular taxonomic groups, [b] Soil gravimetric moisture content, [c] Soil volumetric water content

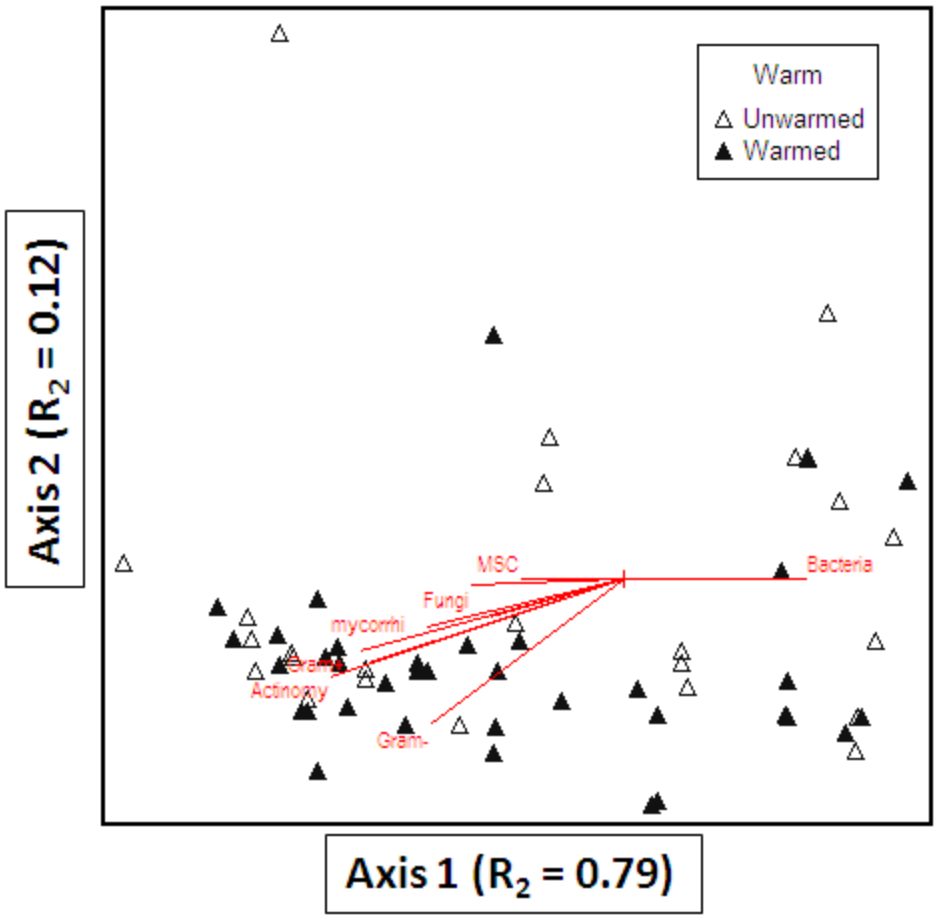

Figure 5- 1. NMS ordination of PLFA relative abundance by warming treatment in 2008. Vectors are based on summed abundance of specific taxonomic PLFAs and explanatory variables.

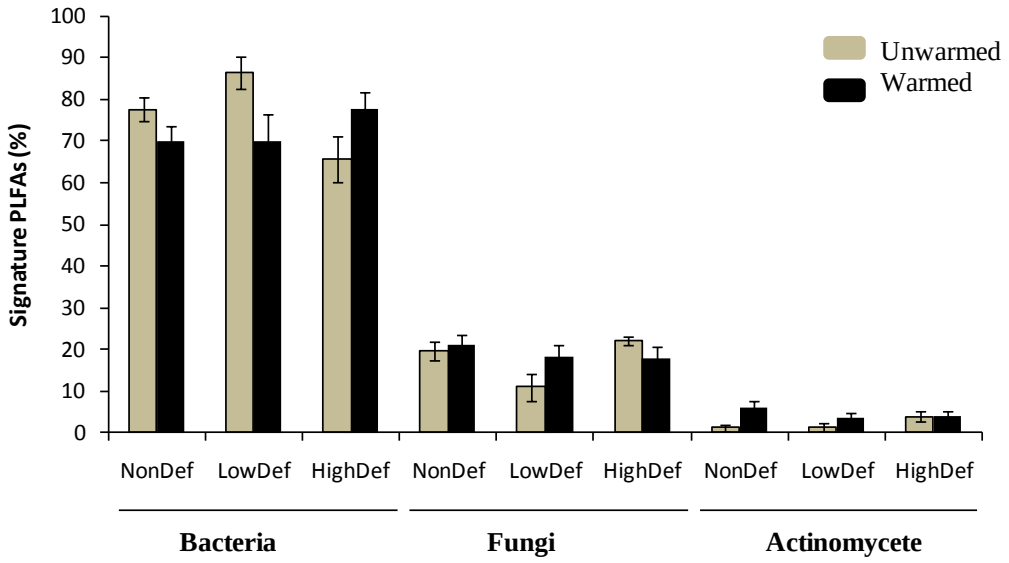

Figure 5- 2. Percentage of relative abundance of PLFA signature of a) bacteria, b) fungi, c) actinomycete as influenced by the warming and defoliation interaction in 2008. Error bars represents SEs.

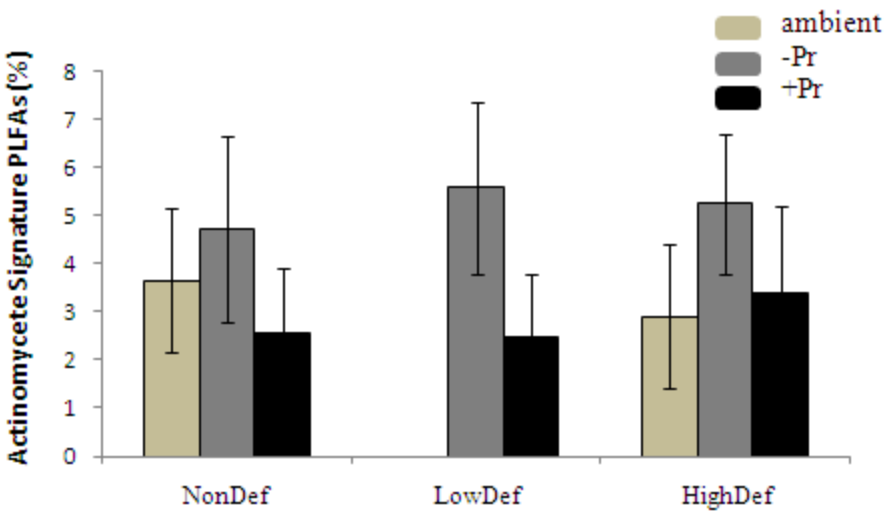

Figure 5- 3. Percentage of relative abundance of PLFA signature of actinomycete as influenced by the precipitation and defoliation interaction in 2008. Error bars represents SEs.

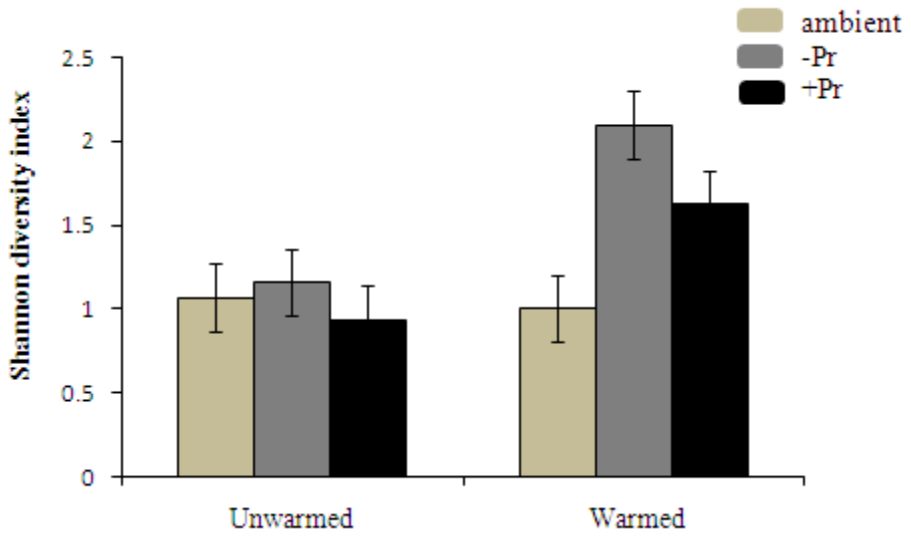

Figure 5- 4. Precipitation and warming interactive effects on microbial structural diversity in 2008. Error bars represents SEs.

Chapter 6 Synthesis and Conclusions

Global changes such as climatic warming and altered precipitation, together with management practices such as grazing intensity, have the potential to influence ecosystem functions, including biogenic greenhouse gas (GHG) emissions and microbial activity in grassland ecosystems. As it is difficult to extrapolate experimental observations from single-factor experiments to natural systems where multiple factors are at work simultaneously, in this dissertation I explored changes in two important aspects of rangeland ecosystems, including 'biogeochemical fluxes' and 'microbial community' responses to multifactor experiments including climate and grazing intensities.

More specifically, I examined soil carbon (C) and nitrogen (N) cycling along with soil microbial structure, composition, and physiological function, in a native temperate grassland soil. Understanding carbon and nitrogen cycles and the factors affecting 'microbial community' may help to elucidate C balance and the potential positive feedbacks to global climate change in grassland ecosystems. Additionally, the result of this research could have implications for policy makers and rangeland managers.

1. Soil Microbial and Biogeochemical Responses to Climate Change and Grazing Management

I found that soil C, N turnover, and microbial community responses to climatic parameters and grazing management varied in time and space (i.e. study

site). Our observations on soil C and N cycling, together with microbial properties over the growing seasons and years, suggest that the consequence of climate change and grazing management could be influenced by intra- and inter-annual variability in northern native temperate grassland ecosystems (Chapters 2, 3, 4, and 5).

Additionally, observations during the same growing season (2007) in two relatively nearby study areas (ecosite A and B) in a native northern temperate grassland (Chapters 2, 3, 4, and 5) suggest the responses of soil C and N turnover and microbial properties to grazing management and climatic warming are site-specific. In other words, both climatic variability and natural heterogeneity in soil and vegetation may be affecting soil C and N dynamics (Chapters 2 and 4) as well as microbial changes (Chapters 3 and 5) in response to climatic and management parameters in this native temperate grassland ecosystem. For instance, the release of soil C in the form of CH_4, a potent greenhouse gas, showed varying responses to defoliation and climatic parameters in this study, with no changes in ecosite A (Chapter 2) but significant changes in response to warming and defoliation at ecosite B (Chapter 4).

Other direct evidence was observed in the observed responses in soil microbial community structure. Soil microbial structure (indicated by the PLFA signatures) responded to warming and defoliation at ecosite B (Chapter 5) but not ecosite A (Chapter 3). Baseline responses (i.e. deviation from control treatments) to defoliation and climatic warming appeared to depend on plant diversity (i.e. evenness and richness), organic matter content, soil types and soil water

availability. Previous studies (Natural Regions Committee, 2006; Lamb *et al.*, 2007; White, unpublished) have shown that ecosite B, with a Dark Brown Chernozomic soil, had much less biomass production, soil organic matter, water content, and plant diversity than ecosite A, a Black Chernozomic soil. These differences may explain why overall treatment effects on soil C and N cycling and microbial properties were more limited and transient at ecosite A (Chapters 2 and 3) compared to ecosite B (Chapters 4 and 5).

To further outline these differences, the role of soil organic matter and plant diversity may differ in their impact on biogeochemical and microbial activities. Soil organic matter is the keystone of belowground function as it supplies C and N to soil biota and contributes to ammonification (Fig. 1-1). On the other hand, differences in plant diversity can alter soil microbial diversity (Porazinska *et al.*, 2003), for example via root residue contributions to the soil (Fig. 1-1), which in turn, may affect soil organic matter content (Fig. 1-1). Thus, differences in plant diversity and soil organic matter may contribute to the differing responses in biological communities belowground within the two ecosites studied. However, still other factors (i.e., soil water content) can also directly affect subsoil function (Fig. 1-1). Therefore, site characteristics can have considerable knock-on effects on the soil belowground community, including responses to climatic and management influences in temperate grassland ecosystems.

Yet another potential explanation for the stronger observed responses at ecosite B could be the role of precipitation (Chapters 4 and 5), which could have exacerbated the impacts of the other two treatments (defoliation and warming) on

soil C and N dynamics at ecosite B. Regardless of such different and site-specific responses in some components of C and N pools and microbial properties, some other components were influenced by our treatments in similar manner at both study areas.

Soil microbial community composition, as measured by fungi: bacteria ratios, appeared to be stable under climatic and defoliation disturbances in both study areas. I also observed similar carbon substrate utilization by soil bacteria and fungi in both study sites in response to climatic parameters and defoliation, despite the fact that overall C utilization by soil fungi (but not bacteria) was vulnerable to treatments. Similarly, I found that soil microbial diversity showed significant responses to treatments whereas microbial functional diversity appeared stable across the treatments in both study areas.

Attempts to generalize conclusions on the important components of soil C budget, including microbial community composition (Chapters 3 and 5), microbial functional diversity (Chapters 3 and 5), overall C utilization (Chapters 3 and 5), microbial biomass (Chapters 2 and 4), soil N mineralization (Chapter 2), dissolved organic C and N (Chapter 4), and the potential for GHG emissions in both study areas, are difficult. Nevertheless, overall results suggest that these northern temperate grassland ecosystems are relatively resistant to the impacts of climate change, particularly warming, in the short-term, even when faced with simultaneous disturbances such as livestock grazing, which was also observed in aboveground community. One theory to explain the relatively resistant in the belowground function in this temperate grassland ecosystem, is that successional

status, showing a relative stability in the grassland community in later successional state (Grime *et al.,* 2000). The studied temperate grassland was historically moderately grazed site may suggest a mid-successional states, explaining the weak responses in belowground community to defoliation and warming. Furthermore, this temperate grassland ecosystem historically experienced the cattle grazing and temperature fluctuation and even rapid temperature rise over the decades (Natural Regions Committee, 2006). This might explain high resistance of these belowground communities to defoliation and 1°C warming over a two-year study. However, variation in precipitation appears to be the key factor affecting these grassland ecosystems as we observed the dominant effect of moisture on soil C and N turnover as well as microbial activity (Chapters 4 and 5). The high sensitivity of the belowground C and N dynamic to precipitation may result from high summer moisture index (SMI) and relatively dry climatic condition in this subregion (Natural Regions Committee, 2006). Furthermore, the study years (2006-2008) were relatively dry year given the lower average precipitation amount compare to the long-term average (Chapter 1). Thus, changes in precipitation amount during the dry years could have pronounced impacts on belowground functioning in these ecosystems. Therefore, the sensitivity of these temperate grasslands to precipitation in terms of belowground community activity could affect ecosystem function as the climate of the regions changes, either towards wetter or drier conditions.

2. Ecosystem Feedback on Global Climate Change

One important aspect of our study was the exploration of changes in global warming potential (GWP) (i.e., greenhouse gas GHG emission) in relation to climatic and grazing parameters, in this native temperate grassland ecosystem. Soil respiration data in both study areas provide experimental evidence for the rapid acclimatization and/or adaptation of soil respiration to climatic warming. Moreover, rapid acclimatization and adaptation of soil respiration to warming (Chapters 2 and 4) was similar in both study areas. However, we have not investigated the main cause of this acclimatization, and this merits further study. For example, depletion of labile C could weaken the potential positive feedback to global climate change in northern temperate grassland ecosystems.

Although the evolution of other trace gases (e.g. N_2O and CH_4) might be a substantial contributor to global warming, our measurements showed that these northern temperate grassland may not be a major N_2O or CH_4 contributor to global warming (Chapters 2 and 4). Instead, the grasslands studied here were an overall CH_4 sink, and the magnitude of N_2O contribution to global warming was fairly small, considering their equivalency to CO_2. As a result, these northern native temperate grasslands appear to be expressing a high degree of resilience and resistance following defoliation and despite a warmer climate, creating little to no climate-microbe feedback . While net GHG balance may resist changes by grazing practices in a warmer world, strong changes may be observed in drier or wetter conditions as once again, the amount of precipitation positively influenced soil CO_2 respiration.

The consequence of climate change is not only limited to the impacts of global warming in native temperate grassland ecosystems because GHG potential and C loss changed under normal and widely fluctuating precipitation in this system. Although temperature, precipitation, and grazing impacts can not be viewed in isolation because of their potential interactions (Chapters 4 and 5), the impacts of precipitation were generally dominant over the other parameters (Chapters 4 and 5). Changing precipitation may therefore have both direct and indirect effects on soil microbial activity, in turn influencing potential GHG emissions and associated contributions to atmospheric warming and climate change. Direct effects include the impacts of precipitation on soil C consumption and respiration (i.e., greenhouse gas emission modulated by soil biota), whereas the indirect effects are mediated by the aboveground community (i.e. net primary production), which alters soil physiochemical conditions and hence microbial activity (Bardgett *et al.*, 2008).

In the present study, although I did not differentiate between the magnitude of direct and indirect precipitation effects on soil C balance and microbial activity, the overall results clearly showed that precipitation was the factor leading to microbe-climate feedback in the ecosystem. Increased precipitation could accelerate the net transfer of C to the atmosphere in the form of CO_2 flux and discharge of C in the form of dissolved organic C through hydrologic leaching (Chapter 4). Thus, the potential of C loss via release from the soil to the atmosphere in the form of CO_2 might eventually stimulate these native temperate

grassland ecosystems, particularly if climate changes to wetter conditions and moisture becomes less limiting for growth.

3. Management Implications and Future Research

So far, numerous publications describe the proper grazing management strategies for grassland ecosystems during drought years. However, this study showed that changes in subsoil community function during wet conditions could be more dramatic in the context of global climate change, accelerating soil C loss and increasing climate-microbe feedback. Precipitation changes therefore appear to be the dominant factor capable of affecting change to these northern temperate grassland ecosystems under climate change. Thus, a key message is that proper mitigation strategies may be needed to offset the consequences of changes in precipitation on belowground functioning and C sequestration potential. In fact, the development of adaptation strategies directed at coping with altered precipitation impacts on belowground functioning in temperate grasslands should be one of the priorities for rangeland managers and policy makers.

Economic viability is central to the establishment of mitigation strategies that help cope with the ecological impacts of climate change in rangelands. As interactions between changes in precipitation and defoliation were evident in this study (Chapters 2, 3, 4, and 5), I propose that under future conditions affected by altered precipitation regimes, grazing management should be considered a useful tool in grassland ecosystems to mitigate the risk of precipitation change on potential GHG release.

For example, grazing has been shown to affect litter quantity (accumulation and depletion) (Christie, 1979; Naeth *et al.*, 1991). Additionally, litter accumulation negatively influenced soil C respiration in this study site (Chapter 4). Acting together, the effect of grazing on litter accumulation and the negative effect of litter on soil CO_2 efflux, may indicate that a conservative grazing management strategy that increases litter accumulation in this system may decrease soil C loss to the atmosphere in these northern temperate grasslands. In the process, litter accumulation may mitigate the climate-microbe feedback associated with wetter conditions. This example illustrates but one of the options available for grazing managers to offset potential negative effects of precipitation on soil C storage in these grasslands.

Beneficial grazing practices must also incorporate reliable information on soil C balance under a changing climate. Reductions in recommended livestock stocking rate, avoiding early season grazing or continuous grazing regimes, even in wetter climates or under longer growing seasons, are some of the strategies available to enhance soil organic C. Nevertheless, ecologists must strive to provide a proper management and adaptation framework for temperate grassland ecosystems when faced with increased precipitation. As the adaptation of grasslands and impacts of grazing are likely to be site-specific, as well as vary over time, one critical step in global climate change sciences is linking small scale studies to the larger climatic patterns evident at the regional scale (Christensen *et al.*, 2001; Zwiers & Zhang, 2003; Drinbock *et al.*, 2003). Therefore, from an ecological perspective, a move must be made toward both the individualistic

assessments and their extrapolations in order to improve rangeland management and policy-making decisions.

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