Grass emerges like the tip of an iceberg
in a land too dry for forest,
too wet for desert, shows
only its hair. On the surface,
foliage, flowers, blades
and seeds while beneath,
so much unseen,
so much unknown.

– Diane Buchanan “The Warp and Woof” in *Writing the Land*

“The middle ground is a mess. It is fascinating to study, and rich in wonderful biology. But by studying it, do not expect universal rules, even simple contingent and general rules, to emerge. If and when they do, treasure them.”

– John H. Lawton, 1999, *Oikos* 84(2)

University of Alberta

Consequences of altered precipitation, warming, and clipping for plant productivity, biodiversity, and grazing resources at three northern temperate grassland sites

by

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Abstract

There is limited understanding about how altered precipitation and warming associated with climate change affect grassland systems. Also, although grasslands commonly support herbivores, it is unclear how grazing influences responses to climate change. To address these knowledge gaps, I carried out a fully controlled and factorial three-year, multi-site experiment simulating climate change and grazing (via clipping). This experiment was conducted at three sites, chosen to broadly represent northern temperate grassland in the region, and each of Canada's prairie provinces. I increased air temperature by 2-4°C, reduced precipitation by 60%, and clipped plants at low and high intensity. At one site, I also applied added (+60%) precipitation. I monitored an array of responses, including plant biomass and biodiversity, and grazing resources.

Shoot biomass decreased strongly with reduced precipitation and clipping, and tended to decrease with warming. However, shifts in root: shoot ratio and associated root biomass responses enabled stability of total biomass. With respect to grazing resources, herbage availability and quality decreased with reduced precipitation and warming; decline in herbage availability was less pronounced with warming than reduced precipitation.

To assess biodiversity responses, I evaluated indirect and direct treatment effects on species richness and evenness. Across sites, richness declined with environmental changes associated with all three treatments. However, evenness responses varied by site, and were overall more resistant. I also assessed changes in similarity between the seed bank and aboveground vegetation at one location.

Precipitation and clipping affected similarity between the seed bank and vegetation, while warming did not.

Across sites, responses were generally consistent, except for the driest site, which remained largely resistant to reduced precipitation. Generally, the grasslands were highly responsive to warming, altered precipitation, and clipping, with negative implications for ecosystem function and biodiversity. However, productivity and biodiversity responses were asynchronous; productivity was more responsive to precipitation and clipping, while richness was more sensitive to increased air temperature. As well, results suggest that management will not substantially influence responses to climate change. Overall, maintenance of total biomass suggests that ecosystem function is relatively resistant to climate change, but climate change has negative ramifications for biodiversity and grazing resources.

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1. INTRODUCTION

1.1 Climate change and ecology

Over the last thirty years, climate change has become a major focus of ecological research. A "Web of Science" search for ecology-related articles on the topic of "climate change" reveals an average of over 1,375 annual citations in the four-year period from 2007-2011, compared to only 30 annual citations from 1980-1985. Ecologists are interested in how factors related to climate change affect an array of variables in biological systems. Arguably, this is simply lending new context to long-standing questions ecologists have commonly asked about the drivers of biological systems. The difference is that instead of a theoretical exercise, ecologists are now increasingly asked to provide predictions that are used for management decisions and public policy surrounding climate change (Pettorelli, 2012). Indeed, a culture of controversy has developed around climate change predictions, furthering the need for accurate science in this area (Gleick *et al.*, 2010).

So how will climate change affect biological systems? Globally, we find mean annual temperature and precipitation, both elements of climate change, largely control a suite of important biological variables including species richness and annual net primary productivity (Rosenzweig, 1968, Hawkins *et al.*, 2003). But how will these patterns of variation respond to the gradual increase in temperature, altered precipitation patterns, and extreme weather events predicted to occur with climate change? How will the effects of climate change compare to other global change factors that control variables like biodiversity (Kreft *et al.*, 2007) and ecosystem function (Davidson *et al.*, 2007), such as nitrogen deposition and human land use/land cover (Vitousek, 1994)? Just as ecological laws are difficult to identify due to ecological patterns being largely contingent on the environment and organisms considered (Lawton, 1999), we can expect significant variability among responses to climate change.

1.2 Approaches to climate change research

Ecologists use an array of approaches in understanding the responses of biological systems to climate change. These approaches can be grouped into three main categories: observational, modeling, and experimental (Rustad, 2008). Observational approaches, although restrained by the limitations of any observational science (Underwood *et al.*, 2000), provide important corroborative and novel evidence of responses to climate change occurring in the present (Sagarin *et al.*, 2010). Long-term datasets, often compiled at multiple sites, have shown responses of phenology, range, evolution, and productivity to climate change (Parmesan *et al.*, 2003). Another type of observational approach is that of “space-for-time”, where responses are assessed along natural gradients corresponding to the factors of interest.

Experimental approaches generally manipulate single or multiple factors in whole ecosystems or components of ecosystems. This allows responses to individual factors to be isolated, and for increased mechanistic understanding of responses alone and in combination (Rustad, 2008). However, many climate change experiments are short-term and thus may not be analogous to real-world responses (Rustad, 2001). They are also logistically limited in terms of scale (Norby *et al.*, 2004), and the infrastructure involved in producing the experimental treatments may produce undesired or unknown effects (Shaver *et al.*, 2000).

Finally, modelling approaches explicitly test concepts and processes, and integrate existing knowledge in doing so. Overall they provide a more general and comprehensive understanding of responses. There have been repeated calls for researchers to design studies that better integrate these three approaches (Rustad, 2008, Dunne *et al.*, 2004, Brown *et al.*, 2011), to both avoid the shortfalls associated with each approach and take advantage of their strengths.

1.3 Climate predictions

A variety of climate models are used to understand variation in past climate and develop future projections. Results from these models are the basis for studies on the effects of climate change on biological systems. The most complex of these models are Atmosphere-Ocean General Circulation Models (AOGCMs)**.** The Intergovernmental Panel on Climate Change (IPCC) is a United Nations body created to compile and assess current climate change science, and is broadly considered the authority on the topic (Anonymous, 2001). To form their most recent climate predictions, the IPCC primarily used a collection of 23 AOGCMs from 14 different institutions involved in climate research (IPCC, 2007). Estimates from different AOGCMs differ based on uncertainty in the data, incomplete knowledge of ecosystems, and inherent imperfections in the models (Knutti, 2008), but together are considered to provide valuable information concerning past and future climatic trends (Reichler *et al.*, 2008).

The 2007 IPCC report found that average global surface temperature has increased 0.74±0.18 °C over the last century (1906-2005), with an accelerated rate of warming over the last fifty years (IPCC, 2007). It is very likely that this warming is caused mainly by increases in anthropogenic greenhouse gases (IPCC, 2007). Projected climatic conditions are inherently variable, largely because future climate will be a function of future emissions. However, if emissions rates remain constant, global average surface temperature is expected to rise another 1.8 °C in the next eighty years, compared to 1980-1999 temperatures. Climate change is not restricted to temperature; there have also been changes to the amount of precipitation in many large regions, and increases in both the occurrence of drought and heavy precipitation events. However, due to the inherent natural variability in precipitation, changes in precipitation have been more difficult to assess than those of temperature (IPCC, 2007).

1.4 Climate change and grasslands

Climate change has not been uniform globally, with terrestrial areas, and northern latitudes in particular, experiencing the most pronounced warming (IPCC, 2007). Northern temperate grasslands are within the area experiencing greater-than-average changes in climate, and are dynamic systems that are likely sensitive to climate change (Gonzalez *et al.*, 2010). These grasslands are also heavily influenced by disturbance, including fire and drought, and grazing by livestock in particular is a common land use (Gibson, 2009). However, as of yet there is little comprehensive understanding of how grasslands respond to climate change, especially in interaction with grazing (White *et al.*, 2011). As well, biodiversity and ecosystem function in native temperate grasslands have been identified as highly threatened because of widespread land conversion and limited conservation efforts in this biome (Hoekstra *et al.*, 2005). The many ecosystem services and functions provided by grasslands, including the provision of critical wildlife habitat, carbon sequestration and bio-fuel production (Gibson, 2009), further the need to understand how these systems will respond to climate change.

1.5 Research approach and rationale

Temperature and precipitation, as components of climate change, and grazing or clipping, as types of disturbance, are three of many ecological drivers that can influence aspects of the plant community (Figure 1-1). I used a primarily experimental approach, with some integration of gradient and modelling approaches, to understand these influences. These drivers can have independent or interactive effects on the plant community, and I built this into my experimental and analytical framework. I was interested in responses in two specific aspects of the plant community: biodiversity and ecosystem function, as well as implications for an ecosystem service: grazing resources (Figure 1-1).

I conducted my research in three northern temperate grassland sites across the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba. The sites chosen were native grassland, rather than tame, because of its conservation and biodiversity value (Peltzer, 2000). These sites are along the northern extent of the Great Plains grassland, which extends south to northern Mexico. Despite the economic importance of this region for agriculture, specifically beef cattle production (Vaisey, 1999), there is little known about how this region will respond to climate change. I determined the main and interactive effects of climate change and grazing on a suite of variables by experimentally manipulating temperature and precipitation, as well as simulating grazing, in a factorial, replicated multi-site experiment. I was part of a multi-disciplinary team involved with this experiment; litter decomposition, soil invertebrate community, and microbial respiration were all examined within the project. My research focussed on the responses of ecosystem function and biodiversity as related to the plant community; a summary of my research questions follows.

1.6 Specific research questions/objectives

Chapter 2

1. Is *total plant biomass* responsive to reduced precipitation, warming, and clipping?
2. How do *shoot* and *root biomass* respond to reduced precipitation, warming, and clipping?
3. How do *changes in allocation (root: shoot ratios)* explain responses of total plant biomass to reduced precipitation, warming, and clipping?

Chapter 3

1. What is the influence of growing conditions (warming and altered precipitation) on *accumulated herbage* production, including contributions from graminoids and forbs, and does this vary with clipping intensity?
2. What is the influence of growing conditions (warming and altered precipitation) on *regrowth biomass* of herbage, including contributions from graminoids and forbs, following early season clipping, and does this vary with intensity of clipping?
3. What is the influence of growing conditions (warming and altered precipitation) on *herbage quality* in graminoid and forbs, and does this vary with clipping intensity?

Chapter 4

1. What are the main and interactive effects of reduced precipitation, warming, and clipping on *similarity between aboveground vegetation and seed bank composition* at a northern temperate grassland site?
2. What are the relative impacts of reduced precipitation, warming, and clipping on *similarity between aboveground vegetation and seed bank composition* at a northern temperate grassland site?

Chapter 5

1. Is *biodiversity resistant or reduced* by the environmental drivers associated with reduced precipitation, warming, and clipping?

2. Are changes in *biodiversity* with reduced precipitation, warming and clipping driven by *indirect responses*?
3. Are *responses of biodiversity* to environmental drivers associated with reduced precipitation, warming, and clipping predominantly *site-specific?*

Chapter 6

1. What are *current approaches* to experimental climate change research in temperate grasslands?
2. What are *future directions* of experimental climate change research in temperate grasslands?

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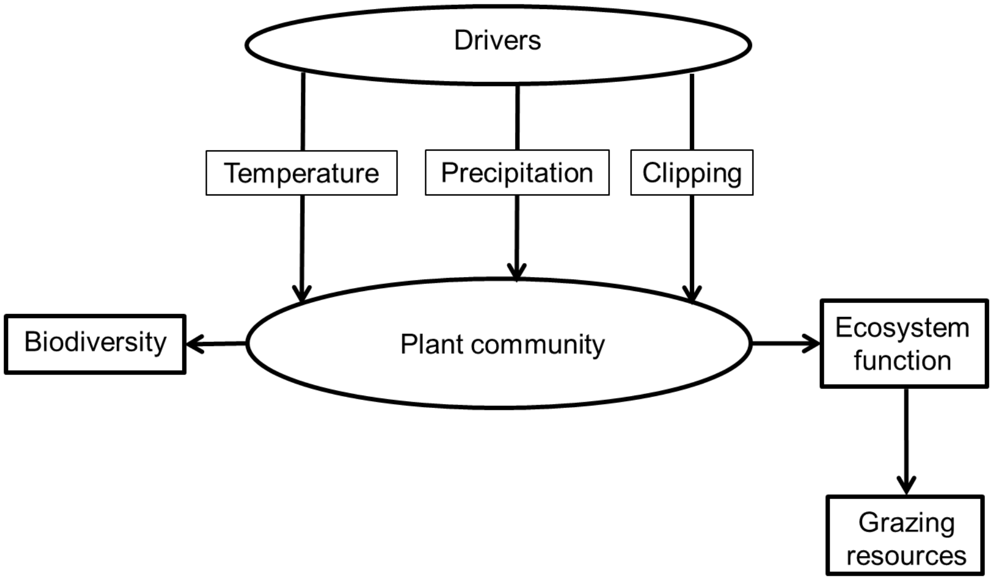

Figure 1-1. Schematic depicting conceptual approach to research, depicting temperature, precipitation, and clipping as drivers that may influence aspects of the plant community, including biodiversity, ecosystem function, and grazing resources.

2. NORTHERN GRASSLAND BIOMASS RESPONSES TO REDUCED PRECIPITAITON, WARMING AND CLIPPING ACROSS THREE SITES

2.1 Introduction

Grasslands are the largest terrestrial biome on Earth, and provide valuable ecosystem services, including carbon sequestration, wildlife habitat and forage for livestock (Gibson, 2009). In the last century, average global air temperatures have increased by 0.74 °C, and precipitation patterns have been altered, with increases in some regions and decreases in others (Solomon, 2007). Even in areas where precipitation increases, increased variability in precipitation coupled with increased evaporation due to warming may cause increase drought frequency (Solomon, 2007). Climate is highly linked to plant biomass production, and early observational studies suggest grassland productivity is responding to climate change (Nemani *et al.*, 2003, Zhou *et al.*, 2001).

Subsequent studies involving experimental manipulation of precipitation and temperature in grasslands generally demonstrate decreases in shoot biomass with reduced precipitation (Wu *et al.*, 2011), and while the effects of warming on shoot biomass vary in both direction and magnitude, meta-analyses reveal the dominant trend is for shoot growth to increase (Wu *et al.*, 2011, Rustad *et al.*, 2001). Most studies have not assessed how root biomass responds to climatic factors (Wu *et al.*, 2011), nor how climatic factors might interact with grazing (White *et al.*, 2011), the dominant land use in grasslands (Gibson, 2009). We address this lack of information by assessing responses of both shoot and root biomass to simulated climate change and simulated grazing in an experiment replicated at three sites across western Canada.

There are many mechanisms, both direct and indirect, by which warming can affect plant biomass. Direct effects of warming include heat stress or altered photosynthetic rates, and indirect effects include changes to nitrogen mineralization or soil moisture availability (Shaver *et al.*, 2000). These varied mechanisms may contribute to the observed variability in grassland biomass responses to warming (Lin *et al.*, 2010). Responses to warming are also expected to vary according to initial environmental conditions, as well as properties of the dominant plant species and their interspecific relationships (Shaver *et al.*, 2000). The effects of reduced precipitation on grassland biomass are more consistent, with generally decrease plant biomass (Wu *et al.*, 2011). However, grassland plant biomass can be unresponsive to decreased precipitation (Frank, 2007), or even increase if drought-tolerant species gain a competitive advantage (Gilgen *et al.*, 2009).

Some of the variability in grassland response to climatic factors may also be due to inconsistent methodology among studies. Using consistent methodology in replicating our experiment at three sites enables us to identify general responses among sites with broadly similar climates. Moreover, given the potential for interactions between precipitation and temperature manipulation (Norby *et al.*, 2004, Carlyle *et al.*, 2011) to cause effects that are not evident when examining individual factors in isolation (Folt *et al.*, 1999), we utilize a fully factorial design to evaluate interactions among treatments.

The importance of belowground processes for understanding ecosystem responses to climate change is increasingly recognized (Pendall *et al.*, 2008, Norby *et al.*, 2000). However, most knowledge of grassland biomass responses to reduced precipitation and warming is derived from studies that exclude root biomass: in a recent meta-analysis of the effects of temperature and precipitation change on terrestrial ecosystems, only six of 38 studies included root biomass (Wu *et al.*, 2011). In contrast, roots comprise the vast majority — up to 90% — of plant production in grassland (Steinaker *et al.*, 2005, Hui *et al.*, 2006). Further, roots can play a key role in a variety of processes, including plant competition (Casper *et al.*, 1997) and carbon sequestration (Jones *et al.*, 2004). Interpretation of shoot responses without consideration of root responses may give an incomplete or misleading picture of total plant responses. For example, although drought may decrease aboveground biomass, the simultaneous maintenance of, or increase in, belowground biomass, may result in a quick recovery of aboveground biomass (Shinoda *et al.*, 2010). Belowground plant responses to environmental change may be more important than those aboveground in understanding long-term responses to climatic conditions, especially in systems like grasslands where most production is belowground.

Another aspect largely missing from studies of climate change in grasslands is grazing. All grasslands are grazed to some extent by large vertebrate herbivores (Gibson, 2009), and herbage production is strongly linked to climate (Craine *et al.*, 2010). Grazing alone directly affects both above- (Milchunas *et al.*, 1993) and belowground (Frank, 2007, Clark, 2012) biomass. Further, through indirect effects on micro-environmental conditions, grazing has the potential to mitigate or exacerbate effects of climate change (Klein *et al.*, 2004). In contrast to climate, grazing can be managed. Despite this, we know of no published study that has assessed the responses of plant biomass to the simultaneous manipulation of temperature and precipitation, along with plant biomass removal.

Research questions

1. Is *total plant biomass* responsive to reduced precipitation, warming, and clipping?
2. How do *shoot biomass* and *root biomass* respond to reduced precipitation, warming, and clipping?
3. How do *changes in allocation (root: shoot ratios)* explain responses of plant biomass to reduced precipitation, warming, and clipping?

2.2 Methods

Site descriptions

Research was conducted at three sites, one in each of Canada's prairie provinces: Alberta (AB), Saskatchewan (SK) and Manitoba (MB). Sites were chosen to be broadly representative of northern temperate grassland in the region and represent each of Canada's prairie provinces. Other requirements were that researchers be able to control management at the site, and have easy access to sites.. Details on the biophysical details of the three grassland sites are summarized in Table 2-1. The AB site (53.016539°N, 111.539898°W) was located at the University of Alberta Kinsella Research Station, approximately 140 km southeast of Edmonton, within the Aspen Parkland, a savannah-like mosaic of rough fescue grassland and aspen stands. The AB experimental area was positioned on the east slope of a small hill characteristic of the hummocky moraine known as “knob-and-kettle” terrain. The SK site (49.30039°N, 104.633961°W) was at the Gap Community Pasture, 130 km south of Regina, in a mixed grassland with isolated shrubs and trees in lower areas with sufficient moisture. This experimental area was a flat hilltop in a landscape of gently rolling grassland hills, also considered “knob-and kettle” terrain. The MB site (50.781249°N, 100.593395°W) was in Riding Mountain National Park, about 200 km west of Winnipeg, within a landscape of boreal forest and aspen stands, with patches of rough fescue grassland. The MB experimental area was flat, within a forest landscape. All grasslands were dominated by native, perennial species, and thought to be free of previous tillage.

All sites were historically grazed by bison and other ungulates until bison were extirpated during European settlement in the late 1800`s. Until inception of the experiment, the AB site was moderately grazed by cattle, deer and moose, and the SK site was moderately grazed by cattle, deer, and pronghorn antelope. Cattle grazing at the MB site ended in 1970, although elk, moose, and deer remain. At all sites we used fencing to prevent grazing by large mammals during the study.

Experimental design

In summer 2007, we initiated a three-year manipulative experiment at each site to determine the interactive effects of reduced precipitation, warming, and clipping on a suite of response variables. Clipping was used to simulate grazing and ensure uniform removal of vegetation. Grazing by cattle would have been non-uniform within plots and damaged experimental infrastructure. At SK and MB, a fully randomized design was employed, while a randomized blocked design was used in AB to account for the slight slope and an additional watering treatment (discussed in Chapters 3 and 4). A random number-generating spreadsheet was used to randomly assign treatments to plots. Sampling plots consisted of circles 2 m in diameter, separated from adjacent plots by at least 0.5 m. At each site, there were five replicate plots of each treatment combination.

Treatments

Precipitation trends under climate change are more difficult to assess (Christensen, 2007). Although precipitation is predicted to increase at high latitudes (Dore, 2005), observed increases have been lowest in the prairies than in other parts of Canada (Zhang *et al.*, 2010), and increased variability may lead to drier periods (Sushama *et al.*, 2010).

Precipitation was reduced in half the plots to simulate the drier conditions predicted for the region (Sushama *et al.*, 2010). The other half of plots received ambient (control) precipitation. Precipitation was manipulated using rain-out shelters comprised of wood frames 60 cm above ground on the low end, and 120 cm above ground on the high end, with 2.5 m by 2.5 m plastic tops (Dura-Film Super 4™ 6-mil polyethylene film; AT Plastics, Edmonton, Alberta, Canada; Appendix A). To maintain consistency of other environmental conditions between precipitation treatments, shelters were installed over all plots regardless of treatment. In the reduced precipitation treatment, small perforations in the plastic prevented approximately 60% of rainfall from reaching the ground, while the ambient precipitation treatment had larger holes to allow complete entry of rainfall but controlled for any effects of the structure itself.

Half the plots received a passive warming treatment using fibreglass open-top chambers (OTCs; Sunlite-HP, Solar Components Corporation/Kalwall Corporation, Manchester, NH, USA), which increase air temperatures by 2-4 °C (Marion *et al.*, 1997). This increase is within the 4.5 °C increase predicted for the region within the next 50 years (Nyirfa and Harron, 2002). OTCs were 2 m in diameter and 40 cm high, with sides positioned at a 60° angle to the ground (Marion *et al.*, 1997). Both OTCs and rain-out shelters were installed in spring (May) of each year and removed in mid-September well after the first frost. The area within the OTC, or equivalent area in ambient control plots, was considered the main plot area, while clipping and precipitation manipulation encompassed a 2.5 m by 2.5 m area fully enveloping the OTC.

Clipping treatments were applied annually in late June, with plots either not clipped (i.e. controls), or clipped to a height of 7 cm (low intensity) or 3 cm (high intensity) above the ground. These clipping levels are consistent with local grazing practices in Aspen Parkland (Burkinshaw *et al.*, 2009). Clipping treatments were applied throughout the full 6.25 m^2 area under the rain-out shelter. In AB and SK, vegetation was removed with a mower set at the appropriate height, while in MB a string trimmer was used because of uneven ground and shrubs. In all plots, a permanently marked central 50 cm x 50 cm subplot was clipped by hand to quantify biomass removal and reduce disturbance. Clipped biomass from this subplot was dried and weighed.

Sampling

We collected standing live shoot and root biomass annually in July to correspond with peak biomass (Steinaker *et al.*, 2008). Shoot biomass was clipped to ground level from a 10 cm by 100 cm quadrat, with a different quadrat sampled each year. Previous years' litter was separated from standing live shoot biomass, and the latter dried to constant biomass and weighed. In determining shoot biomass, we included biomass removed during the initial clipping treatment for those plots. Thus, measures of shoot biomass consist of aggregate biomass from the June clipping treatment added to standing live shoot biomass sampled in July.

Root biomass was measured in two cores, 5-cm-diameter and 20 cm deep, taken adjacent to the shoot harvest area. Root cores were washed with a 2 mm sieve, dried and weighed; root biomass was averaged between the two cores. We added shoot biomass to root biomass to determine total plant biomass, and further divided root biomass by shoot biomass to calculate root: shoot ratio. We note that shoot biomass measures represent a single year's growth, and root biomass measures represent multiple years' growth. This difference is due to root life-spans that typically exceed one year in our region (Macdougall *et al.*, 2011), and to more effective differentiation of living and dead material aboveground than belowground. As a result, we expect this difference should introduce a lag effect, causing any treatment-induced changes in root biomass to appear slower than changes in shoot biomass, even if the effect size of a treatment on current year's growth were the same above and belowground.

Environmental measurements

To confirm efficacy of the treatments, environmental variables were measured May-September in two replicates of each treatment combination at each site. Soil moisture, assessed as % volumetric water content (%VWC), and soil temperature were recorded at 0-5 cm depth every 30 minutes using Decagon ECH2O EC-TM soil moisture and temperature probes (Decagon Devices Inc., Pullman, WA, USA) placed at the top of each permanent sampling plot. Air temperature 25 cm aboveground was recorded every 30 minutes using Onset HOBO Pendant Temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) shielded with PVC pipes. Two Davis Rain Collector II buckets at each site (Davis Instruments, Hayward, CA, USA) measured ambient precipitation, along with two temperature probes 25 cm aboveground measuring ambient temperature.

Statistical analysis

Plant response variables included total plant biomass, shoot biomass, root biomass, and root: shoot ratio, all from the third year of data collection (2009). Due to the lag effects described above and elsewhere (Rustad, 2001), we report only the final year, as these data represent the greatest cumulative impact of treatments (Appendix A). Plant response variables were log-transformed to successfully meet assumptions of normality and equality of variances. To identify patterns among sites, we performed an analysis of variance (ANOVA) using general linear mixed models for each of the response variables, with site as a random effect, and reduced precipitation, warming, and clipping as fixed factors. To further understand how responses differed among sites, we also analysed each site individually. As the AB site was blocked, we used a general linear mixed model with block as a random factor. SK and MB were not blocked, and for these sites we used general linear models with the three treatments as fixed factors. We focus statistically significant ($p \leq 0.05$) results. All analyses were performed using the PASW STATISTICS 19 for Windows.

To evaluate efficacy of the treatments in altering environmental conditions, we analyzed 2009 average daily soil moisture, soil temperature and air temperature. Environmental data were average daytime measurements (8 AM to 6 PM) from mid-May to early September. These data satisfied statistical assumptions without any transformations. We analyzed these data in a mixed model with site as random effects, and reduced precipitation, warming, and clipping as fixed factors.

2.3 Results

Total plant biomass

For all three sites considered together, experimental treatments had no effect on total biomass (Figure 2-1a, Table 2-2). For sites considered separately, experimental treatments also had no effect on total biomass (Figure 2-1b,c, Table 2-2), with one exception: in SK, total plant biomass in low intensity clipped plots was significantly higher than non-clipped or high intensity clipped plots (Figure 2-1c, Table 2-2).

Shoot biomass

Shoot biomass generally decreased with the treatments, although the decrease in shoot biomass with warming was only marginally statistically significant (p=0.076, Figure 2-2a). For all three sites considered together, shoot biomass significantly decreased in response to reduced precipitation and both clipping levels (Figure 2-2a, Table 2-2). Shoot biomass decreased 22% in response to reduced precipitation, 22% under low intensity clipping, and 43% under high clipping, averaged across sites. Shoot biomass responses to low intensity clipping were therefore of the same magnitude as those from changes in precipitation, while high intensity clipping further reduced shoot biomass regardless of precipitation regime. Decreases in shoot biomass (9%) with warming were less pronounced than with other treatments.

Analyzed separately, the three sites responded similarly to warming and clipping: none responded significantly to warming, while clipping consistently significantly decreased shoot biomass at all three sites (Figure 2-2b, Table 2-2). Precipitation responses varied among sites. In AB, reduced precipitation significantly decreased shoot biomass (Figure 2-2b, Table 2-2). In MB, there was no significant (p=0.070) decrease in shoot biomass with reduced precipitation, although visual inspections suggest that shoot biomass weakly decreased (Figure 2-2c, Table 2-2). While shoot biomass in SK did not show a strong main response to precipitation or warming, it was affected by a three-way interaction (Table 2-2), with warming decreasing shoot biomass, but only under ambient precipitation and low or high clipping (Figure 2-2d).

Root biomass

Across all sites, root biomass was affected by a significant three-way interaction among treatments (Figure 2-3a, Table 2-2). This reflected root biomass increasing 36% with warming when accompanied by ambient precipitation and low or high intensity clipping, but decreasing with warming by 19% under ambient precipitation with no clipping. This significant three-way interaction was present at SK as well (Figure 2-3d). Although not statistically significant, similar patterns were observed at AB and MB (Figure 2-3b,c). There was also a significant two-way interaction between precipitation and clipping at MB, reflecting a decrease in root biomass with clipping and reduced precipitation (Figure 2-3c, Table 2-2)

Biomass allocation

Root: shoot ratios increased significantly with all treatments across all sites (Figure 2-4a, Table 2-2). Root: shoot ratios increased 39% under reduced precipitation, 19% with warming, and 41% and 72% with low and high clipping severity, respectively. There was also a significant three-way interaction among treatments (Table 2-2), as root: shoot ratios increased with warming under ambient precipitation, and clipping (either low or high intensity). At individual sites, reduced precipitation significantly increased root: shoot ratios in AB and MB, and clipping significantly increased root: shoot ratio at all sites (Figure 2-4b,c,d, Table 2-2). SK was also affected by a significant three-way interaction following the same pattern as that across sites (Figure 2-4d, Table 2-2). Although AB and MB did not display statistically significant three-way responses, visual inspection of the data confirms that root: shoot ratios followed this same trend at all sites (Figure 2-4b,c). Root: shoot ratio in MB also varied significantly with a precipitation by clipping interaction, as reduced precipitation increased root: shoot ratio, except under high clipping intensity (Figure 2-4c, Table 2-2).

Treatment efficacy

Approximately 29±2.2% (low intensity clipping; mean ±1 SE) and 54 ±3.5% (high intensity) of shoot biomass was removed per plot in AB; 34±22% and 63±12% in SK; and 48±31% and 69±16% in MB. Rain-out shelters, OTCs, and the clipping treatments influenced the environmental conditions within plots. The reduced precipitation treatment significantly decreased soil moisture by an average of 6.6 %VWC across sites (Appendix A, Table 2-3). Soil moisture was decreased significantly by 4.7 %VWC in low intensity clipping plots and 3.5 %VWC in high intensity clipping plots.

Soil daytime temperature was affected by a three-way interaction between the treatments (Appendix A, Table 2-3). Soil temperature increased 2.2 °C in warmed relative to unwarmed plots, under ambient precipitation and clipping (low or high intensity). Average daily daytime temperature was significantly increased by both OTCs and rain-out shelters. Average daily daytime air temperature increased 0.5 °C in reduced precipitation plots and 2.0 °C in warmed plots across sites (Appendix A, Table 2-3).

2.4 Discussion

Despite some site-specific variation, patterns of biomass response across the three sites were remarkably similar. Large changes in both shoot and root biomass were observed, yet total biomass remained largely unaffected by the treatments. Changes to root: shoot ratios were larger than would be expected based on shoot or root responses alone. Any changes in shoot biomass and root biomass were in opposite directions, contributing to marked shifts in root: shoot ratio. The lack of net change in total biomass is thus partly reflective of shifts in root: shoot ratio. As well, shoots were more responsive to the main effects of the treatments than were roots. In these grassland ecosystems, where the majority of biomass is belowground, larger changes in root biomass may be necessary to impact total plant biomass.

The responses of shoot biomass to reduced precipitation and clipping were consistent with other published responses, and support the general finding that reduced precipitation reduces shoot biomass in grasslands (Wu *et al.*, 2011). Along with suppressing plant physiological processes such as photosynthesis (Chaves *et al.*, 2002), reduced precipitation can decrease nutrient availability or directly cause mortality (Wu *et al.*, 2011). At the SK site, decreases in shoot biomass with reduced precipitation occurred only in plots that were unwarmed and clipped. As SK is the site with the lowest mean annual precipitation, and normally experiences moisture deficits in mid to late summer, plants at this location may tolerate low soil moisture, provided clipping is not present as an added stress. Heisler-White (2009) also found that site-specific responses to altered precipitation were contingent on average soil water availability. Shoot biomass in MB, the site with the highest annual mean precipitation, was less responsive than in AB, which in turn displayed the largest proportional decrease in shoot biomass under reduced precipitation. Similar to the present study, Heisler-White (2009) found that sites with intermediate rainfall were more sensitive to changes in precipitation. With high ambient precipitation, such as in MB, moisture perhaps is sufficient to maintain shoot biomass even after a reduction in precipitation.

The observed decrease in shoot biomass with increasing severity of clipping is consistent with the majority of grazing experiments in grasslands (Milchunas *et al.*, 1993). Along with directly removing biomass, grazing affects plants through changes to biomass allocation and photosynthetic rates, as well as indirectly via the modification of light, water, and nutrient availability. Indeed, we observed decreased soil moisture due to clipping. Although in some situations grazing of grasslands has been shown to cause overcompensation for lost biomass (McNaughton, 1979, Klein *et al.*, 2007), we did not observe this phenomenon.

In comparison to precipitation and clipping, experimental warming has had highly variable effects on grassland shoot biomass in previous studies (Wu *et al.*, 2011, Grime *et al.*, 2000, Lin *et al.*, 2010). We found warming to be the treatment eliciting the weakest shoot biomass response. Although increased grassland biomass in response to warming has been found elsewhere and attributed to increased nutrient availability or photosynthesis (Lin *et al.*, 2010, Rustad *et al.*, 2001), we did not observe this response. We also did not find a decrease in soil moisture with warming, which is known to dampen the positive effects of warming (Kardol *et al.*, 2010). Although not assessed in this study, decreases in shoot biomass with warming can arise from physiological changes owing to high leaf temperatures (Kardol *et al.*, 2010), changes in community composition (Shaver *et al.*, 2000), or even plant species interactions (Suttle *et al.*, 2007). In our study it is unclear if shoot biomass was simply relatively unresponsive to warming, or if positive and negative effects of warming were in counterbalance to leave shoot biomass unchanged.

Relative to shoot biomass, the influences of temperature, water, and grazing on grassland root biomass are not well understood. In contrast to shoot biomass, we found no main effect of precipitation on root biomass, which is consistent with the idea that root biomass, although controlled by precipitation, is generally less sensitive to precipitation than its above-ground counterpart (Sharp, 1989).

In a review, warming was found to have no effect on grassland root biomass (Wu *et al.*, 2011). However, we found root biomass increased with warming, specifically increased soil temperature, particularly at the site with the highest average summer temperatures (SK). Both soil temperature and root biomass only increased in combination with ambient precipitation and low or high intensity clipping. This result is similar to Xu *et al.* (2012) who found that favourable effects of increased soil temperature on root biomass were contingent on the availability of sufficient precipitation to facilitate root growth, and more pronounced with clipping. This increase in root biomass with warming provides evidence that we captured responses of production rather than changes in decomposition, as warming would be expected to accelerate root decomposition (Wildung *et al.*, 1975), leading to decreased root biomass.

The lack of main response of root biomass to clipping is consistent with the finding of Milchunas *et al.* (1993) that grazing has no consistent effect on root biomass in grasslands. However, our observed lack of response may also indicate that the clipping regimes we tested were within the tolerances for these particular communities, as other studies in the region indicate grazing can alter root biomass (Hild *et al.*, 2001, Dormaar *et al.*, 1994, Johnston *et al.*, 1971). Finally, our assessment of root biomass did not extend below 20 cm, and thus will not reflect changes in deeper roots, which can respond to biomass removal despite resistance of shallow roots (Coupe *et al.*, 2009).

Shoot and root biomass responses to the treatments were generally in opposite directions, contributing to the lack of response in total plant biomass, and the substantial shifts in root: shoot ratio. As well, shoot biomass was more responsive to the main treatment effects than root biomass, and thus shifts in root: shoot ratio likely reflect mostly decreases in shoot biomass. Neither shoot nor root biomass had significant responses to the main effect of warming, but because any effects were in opposite directions, root: shoot ratios exhibited an increase with warming. Shinoda *et al.* (2010) found that despite a large reduction in shoot biomass with drought, shoot biomass rapidly recovered after treatments ceased. They attributed this resilience to the large root biomass that had been maintained despite the drought.

Root: shoot ratios generally increase with reduced resources, as plants shift allocation of carbohydrates toward root development to increase resource uptake (Bloom *et al.*, 1985, Frank, 2007). However, extensive water stress can have a detrimental effect on fine root mortality, reducing total root biomass (Green *et al.*, 2005, Meier *et al.*, 2008). Similarly, Xu *et al.* (2012) found that changes in root: shoot ratios with warming and clipping depended on inter-annual precipitation. This may explain why we found increased root: shoot ratios with warming under ambient precipitation and clipping, but not under reduced precipitation. When applied simultaneously, the combined stress of the three treatments may have prevented a shift to root biomass.

The treatments we examined produced expected effects on response variables. Reduced precipitation decreased soil moisture, as did clipping to a lesser extent, which is an expected response to reduced vegetation cover (Sala *et al.*, 1992). Warming had the desired effect of increasing air temperature. The observed increase of 2.0°C from our OTCs was on the lower end of that observed in others studies (2°- 4°C), but efficacy of OTCs differs by weather conditions, latitude and time of day (Marion *et al.*, 1997). The rain-out shelters also increased air temperatures, although less so than the OTCs. This increase in temperature could be due to changes in vegetation structure associated with the reduced precipitation treatment, or to increased coverage of plastic in the reduced precipitation compared to the ambient precipitation shelters.

Our findings of relatively stable total plant biomass under experimental climate change and clipping at three sites across the Canadian prairies have implications for understanding grassland ecology and management. The interpretation of responses largely depends on whether shoot, root or total biomass is considered. The decreases in shoot biomass in response to reduced precipitation or clipping could have ramifications for ecosystem function including the provision of livestock forage and wildlife habitat. Nonetheless, the maintenance of total plant biomass, despite changes in root biomass in response to certain treatment combinations, has positive implications for ecosystem sustainability in the short term.

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Table 2-1. Characteristics of the three study sites[1].

	AB	SK	MB
Latitude	53.016539°N	49.30039°N	50.781249°N
Longitude	111.539898°W	104.633961°W	100.593395°W
Dimensions of experimental site	40 m x 100 m	25 m x 80 m	33 m x 55 m
MAP (mm)	431.3	386.3	506.5
MAT (°C)	2.8	3.6	1.6
2007, 2008, 2009 growing season precipitation (mm)	241.8, 232.9, 168.9	273.2, 348.4, 241.2	369.1, 405.1, 277.3
2007, 2008, 2009 growing season average temperature	13.8, 13.5, 13.8	16.5, 15.2, 15.2	14.7, 13.8, 14.3
Long-term growing season precipitation (mm)	314.7	262.4	349.3
Long-term growing season average temperature (°C)	14.0	15.2	14.4
ANPP (g/m^2)	190.8±28.5	131.8±20.3	281.9±56.2
Natural subregion	Aspen parkland	Mixed grassland	Aspen parkland
Plant growth forms (approx. % composition of total biomass)	Graminoid – 70 Forb – 30 Shrub – <1	Graminoid – 80 Forb – 20 Shrub – 0	Graminoid – 40 Forb – 50 Shrub – 10
Dominant species	*Festuca hallii*, *Hesperostipa curtiseta*, *Elymus trachycaulus*	*Hesperostipa curtiseta*, *Pascopyrum smithii*, *Carex* spp.	*Poa secunda*, *Carex* spp., *Monarda fistulosa*
Richness (species/0.25m^2 ±1 SE)	8.4±0.4	6.2±0.2	9.6±0.8
Soils	Orthic Black Chernozem	Orthic Dark Brown Chernozem	Orthic Dark Grey Chernozem

[1] Aboveground net primary productivity (ANPP) and species richness from control plots averaged over three years of study (2007-2009). Growing season refers to May-September. All climate information is from nearest weather station (less than 80 km from site) with available records (Environment Canada, 2012). MAP (Mean Annual Precipitation), MAT (Mean Annual Temperature), and long-term growing season averages are calculated from at least 15 years of data from 1971-2000. Plant species nomenclature follows United States Department of Agriculture (USDA) PLANTS Database (2012). Soil taxonomy follows that of Soil Classification Working Group (1998).

Table 2-2. Multi-site and individual site ANOVA results for the effects of reduced precipitation (P), warming (W), and clipping (C) on total plant biomass, shoot biomass, root biomass, and root: shoot ratio. Significant p-values (≤.05) are bold.

Response variable	Source of Variation	All sites		AB		SK		MB	
		F_{df}	P	F_{df}	P	F_{df}	p	F_{df}	p
Total plant biomass	P	$1.09_{1,165}$	.299	$3.83_{1,44}$	.057	$0.07_{1,47}$	.792	$0.02_{1,48}$	.900
	W	$0.04_{1,165}$	.850	$0.31,_{44}$	.581	$0.64_{1,47}$	.429	$0.00_{1,48}$	.980
	C	$2.40_{2,165}$	.094	$0.20,_{44}$	.817	**$3.28_{2,47}$**	**.046**	$1.49_{2,48}$	.235
	P × W	$0.01_{1,165}$	.911	$1.84,_{44}$	.182	$0.46_{1,47}$	.501	$0.16_{1,48}$	.696
	P × C	$1.87_{2,165}$	.157	$0.92,_{44}$	.408	$0.83_{2,47}$	.441	$2.73_{2,48}$	.076
	W × C	$1.66_{2,165}$	.193	$0.38,_{44}$	.688	$0.78_{2,47}$	.464	$1.48_{2,48}$	.239
	P × W × C	$2.10_{2,165}$	.126	$0.48,_{44}$	.623	$1.68_{2,47}$	.197	$0.35_{2,48}$	.706
Shoot biomass	P	**$20.36_{1,165}$**	**<.001**	**$33.88_{1,44}$**	**<.001**	$0.21_{1,47}$	.652	$3.43_{1,48}$	.070
	W	$3.20_{1,165}$	.076	$1.61_{1,44}$	.211	$1.71_{1,47}$	.198	$0.92_{1,48}$	.342
	C	**$21.76_{2,165}$**	**<.001**	**$7.18_{2,44}$**	**.002**	**$18.16_{2,47}$**	**<.001**	**$5.32_{2,48}$**	**.008**
	P × W	$1.28_{1,165}$	.241	$1.39_{1,44}$	.244	$1.57_{1,47}$	.216	$0.00_{1,48}$	.985
	P × C	$1.51_{2,165}$	.223	$1.10_{2,44}$	.344	$0.05_{2,47}$	.954	$1.06_{2,48}$	.355
	W ×C	$0.22_{2,165}$	.804	$0.69_{2,44}$	.509	$0.14_{2,47}$	.955	$0.15_{2,48}$	.861
	P × W × C	$1.27_{2,165}$	.283	$0.34_{2,24}$	.716	**$4.41_{2,47}$**	**.018**	$0.18_{2,48}$	.839
Root biomass	P	$0.34_{1,165}$	.559	$0.00_{1,44}$	.982	$0.01_{1,47}$	.942	$1.15_{1,48}$	.289
	W	$0.61_{1,165}$	.437	$0.04_{1,44}$	.841	$1.31_{1,47}$	.259	$0.16_{1,48}$	.691
	C	$0.21_{2,165}$	.809	$0.39_{2,44}$	.682	$1.67_{2,47}$	.200	$0.56_{2,48}$	.576
	P × W	$0.014_{1,165}$	.905	$1.22_{1,44}$	.276	$0.67_{1,47}$	.417	$0.16_{1,48}$	.688
	P × C	$2.81_{2,165}$	.063	$1.09_{2,44}$	.344	$1.11_{2,47}$	.337	**$5.34_{2,48}$**	**.008**
	W × C	$1.40_{2,165}$	.250	$0.40_{2,44}$	.673	$1.17_{2,47}$	.319	$1.35_{2,48}$	.270
	P × W × C	**$3.60_{2,165}$**	**.029**	$0.64_{2,44}$	.534	**$3.43_{2,47}$**	**.041**	$0.63_{2,48}$	.536
Root: shoot ratio	P	**$17.94_{1,165}$**	**<.001**	**$28.19_{1,44}$**	**<.001**	$0.13_{1,47}$	.725	**$5.44_{1,48}$**	**.024**
	W	**$4.12_{1,165}$**	**.044**	$1.08_{1,44}$	.304	$2.98_{1,47}$	.091	$1.28_{1,48}$	.264
	C	**$17.02_{2,165}$**	**<.001**	**$7.90_{2,44}$**	**.001**	**$7.43_{2,47}$**	**.002**	**$4.79_{2,48}$**	**.013**
	P × W	$1.11_{1,165}$	.295	$0.17_{1,44}$	.679	$2.09_{1,47}$	.155	$0.04_{1,48}$	.852
	P × C	$2.44_{2,165}$	.090	$0.90_{2,44}$	.413	$0.81_{2,47}$	.451	**$3.86_{2,48}$**	**.028**
	W ×C	$0.24_{2,165}$	.790	$0.89_{2,44}$	.420	$0.97_{2,47}$	.387	$0.07_{2,48}$	.931
	P × W × C	**$4.08_{2,165}$**	**.019**	$0.68_{2,44}$	.511	**$7.00_{2,47}$**	**.002**	$0.65_{2,48}$	.525

Table 2-3. Multi-site ANOVA results for the effects of reduced precipitation (P), warming (W), and clipping (C), as well as their interactions, on 2009 soil moisture, soil temperature, and air temperature. Significant p-values (≤.05) are bold.

Response variable	Source of variation	F_{df}	p
Soil moisture	P	**$17.45_{1,57}$**	**<.001**
	W	$1.23_{1,57}$	.272
	C	**$3.15_{2,57}$**	**.0.51**
	P × W	$1.71_{1,57}$	.197
	P × C	$1.06_{2,57}$	.354
	W × C	$1.44_{2,57}$	.246
	P × W × C	$1.00_{2,57}$	.374
Soil temperature	P	$0.008_{1,57}$	.931
	W	$1.139_{1,57}$	.290
	C	$2.647_{2,57}$	.080
	P × W	$0.980_{2,57}$	.326
	P × C	$0.093_{2,57}$	.911
	W × C	$0.206_{2,57}$	.815
	P × W × C	**$4.435_{2,57}$**	**.016**
Air temperature	P	**$7.90_{1,57}$**	**.007**
	W	**$4.00_{1,57}$**	**.05**
	C	**$8.20_{2,57}$**	**.001**
	P × W	$2.72_{1,57}$	.104
	P × C	$0.34_{2,57}$	.715
	W × C	$3.02_{2,57}$	.057
	P × W × C	$2.09_{2,57}$	.133

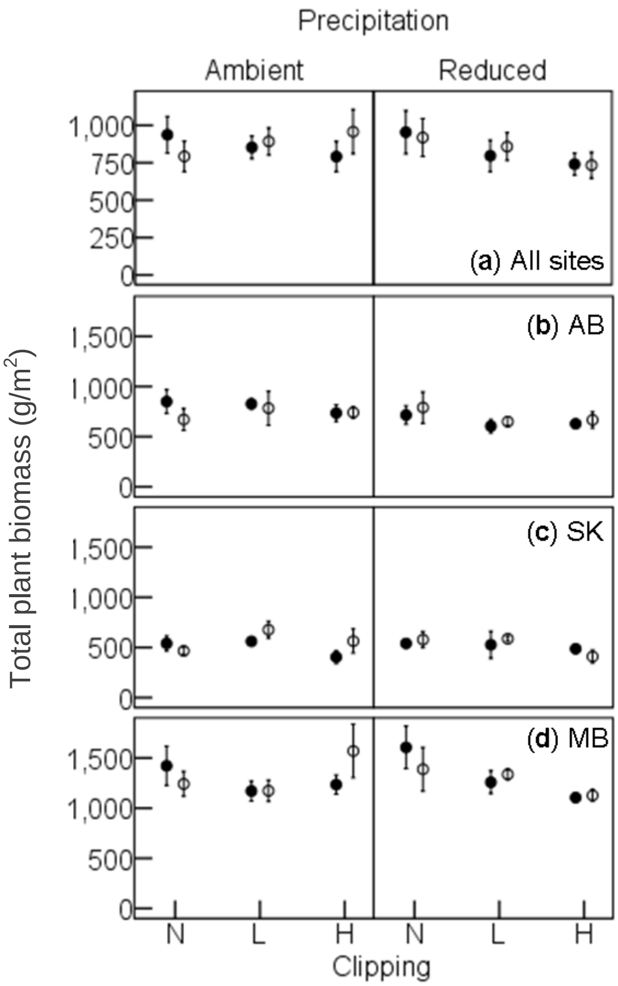

Figure 2-1. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, "N"; low intensity, "L"; high intensity, "H") on total plant biomass for all sites (a), AB (b), SK (c), and MB (d). Error bars represent ±1 SE; n=5.

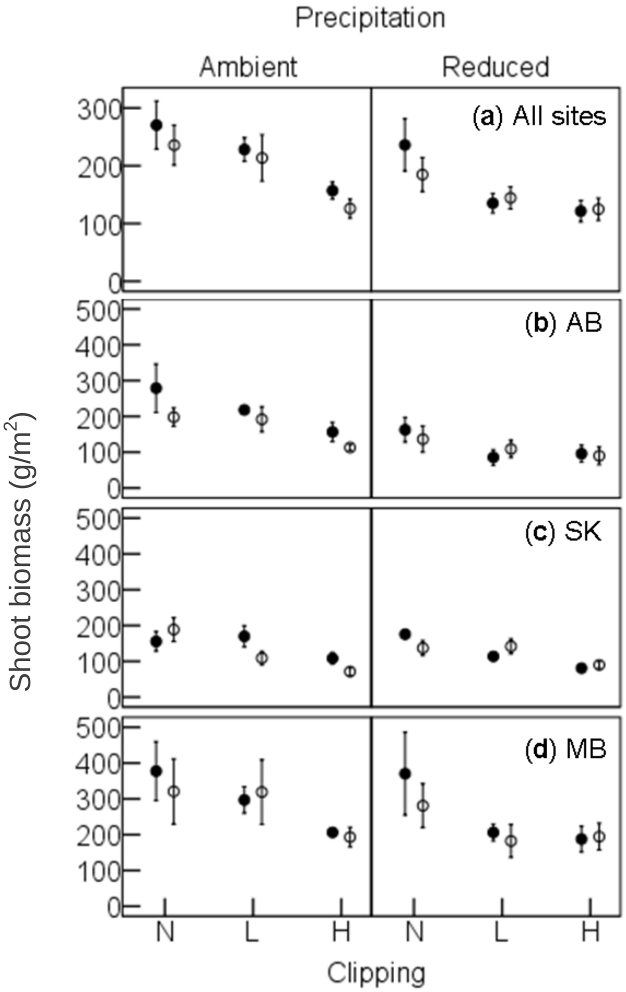

Figure 2-2. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, “N”; low intensity, “L”; high intensity, “H”) on shoot biomass for all sites (a), AB (b), SK (c), and MB (d). Error bars represent ±1 SE; n=5.

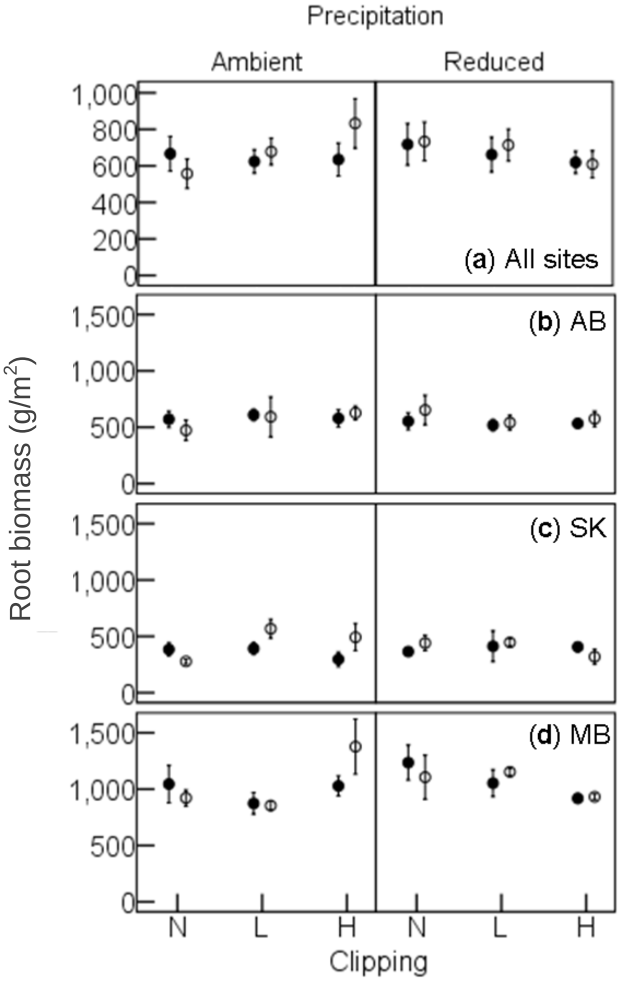

Figure 2-3. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, “N”; low intensity, “L”; high intensity, “H”) on root biomass for all sites (a), AB (b), SK (c), and MB (d). Error bars represent ±1 SE; n=5.

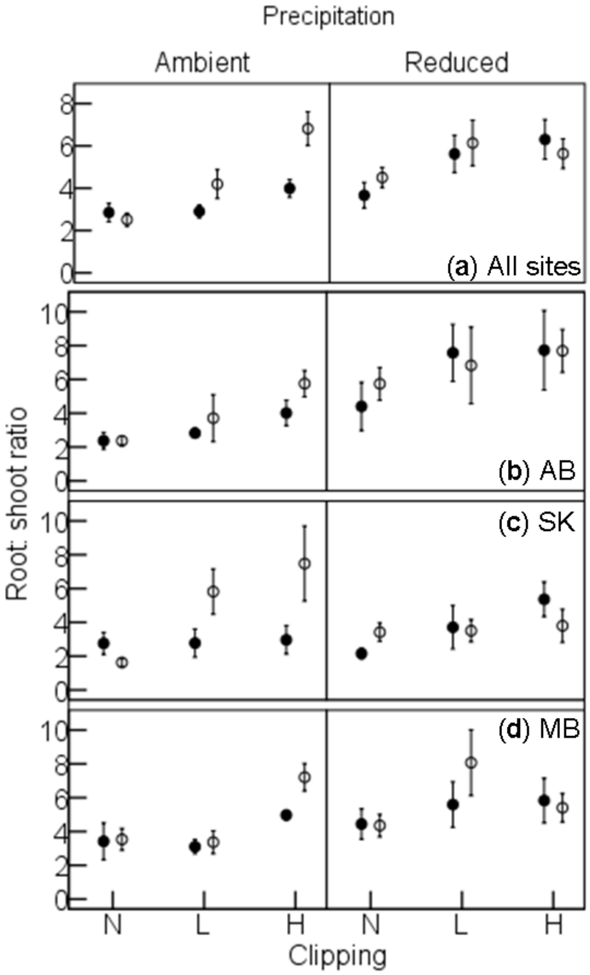

Figure 2-4. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, "N"; low intensity, "L"; high intensity, "H") on root: shoot ratio for all sites (a), AB (b), SK (c), and MB (d). Error bars represent ±1 SE; n=5.

3. IMPLICATIONS OF ALTERED PRECIPITATION, WARMING, AND CLIPPING FOR GRAZING RESOURCES IN THE CANADIAN PRAIRIES

3.1 Introduction

The magnitude of climate change has been variable depending on location, with accelerated warming in high latitudes (Trenberth, 2007). Over the last century average temperatures have increased 1.5°C across the Canadian prairies, which represents the largest temperature change across Canada (Zhang *et al.*, 2010). Further increases up to 4.5°C are forecast for the region in the next 50 years (Nyirfa and Harron, 2002).

Precipitation trends under climate change are more difficult to assess (Christensen, 2007). Although precipitation is predicted to increase at high latitudes (Dore, 2005), observed increases have been lowest in the prairies than in other parts of Canada (Zhang *et al.*, 2010), and increased variability may lead to drier periods (Sushama *et al.*, 2010). Warming and altered precipitation have the potential to impact rangeland productivity (Izaurralde *et al.*, 2011), with socio-economic repercussions (Finger *et al.*, 2010). Indeed, there is evidence that productivity at northern latitudes is increasing due to climate change, though this trend is inconsistent across North America (Zhou *et al.*, 2001).

Several studies have evaluated climate impacts on plant production, but results have varied (Chapter 2) and few studies have addressed impacts on grazing resources. Further, climate studies rarely include grazing intensity as an additional treatment, although grazing can affect vegetation, and producers could use this information to adjust grazing if necessary (Izaurralde *et al.*, 2011). In Canada's prairie provinces, cattle grazing is a major economic activity on an estimated 13 million ha (Vaisey, 1999). Despite this, little is known about how altered climate might affect grazing resources, or how these changes may be mitigated by changes in grazing intensity.

Graminoid and forb, collectively termed "herbage", quantity can respond to climate and grazing via changes in accumulated herbage biomass during the growing season. There can also be shifts between growth forms (i.e. graminoid and forb biomass). Regrowth following early season grazing may also be affected, altering subsequent grazing opportunities later in the growing season. Climate change may also alter herbage quality (Craine *et al.*, 2010), including crude protein (CP) concentration, a measure derived from plant nitrogen content. Ruminants have minimum CP requirements and optimal performance requires a balance between protein and energy; thus, even small decreases in CP can have implications for livestock (Poppi and McLennan, 1995).

Managing range resources under altered climate requires accurate information on herbage responses; Morgan (2008) advises that production in the Great Plains will increase with warming. Indeed, meta-analyses of warming have concluded plant growth will increase in grasslands (Rustad *et al.*, 2001; Lin *et al.*, 2010; Wu *et al.*, 2011). This is further supported by well-known global relationships involving annual temperature and net primary productivity (Rosenzweig, 1968). However, warming may not have positive effects on biomass if plants are optimally adapted to their current (i.e. lower) temperature (King *et al.*, 1995; Bertrand *et al.*, 2008).

Temperature affects plant biomass via direct and indirect mechanisms, contributes to varied effects of warming on productivity (Shaver *et al.*, 2000). For example, warming may decrease soil moisture (Kardol *et al.*, 2010), in turn limiting plant biomass. Despite the trend for plant productivity to increase with warming (Wu *et al.*, 2011), experimental warming can also decrease grassland productivity, either alone (Klein *et al.*, 2007) or in interaction with changes in precipitation (Hoeppner and Dukes, 2012). Moreover, little research has examined specific effects of temperature on regrowth, although King et al. (1998) showed that regrowth of three *Festuca* species decreased with warming.

On a continental scale, precipitation is considered the most important driver of grassland distribution and productivity (Milchunas, 1994; Knapp and Smith, 2001; Huston and Wolverton, 2009). Although the relationship between peak biomass and precipitation is generally positive (Sims, 1978; Wu *et al.*, 2011) some studies have found no relationship (Frank, 2007) or even a negative relationship (Gilgen and Buchmann, 2009) between biomass and precipitation. Moreover, most of our understanding of altered precipitation effects on production rely on observational studies, which may show different results than manipulative studies (Nippert *et al.*, 2006). Recent investigations manipulating timing and distribution of precipitation are revealing complex grassland productivity responses to precipitation (Fay *et al.*, 2011) that further vary by local landform and topography (Heisler-White *et al.*, 2009). There is also significant variation in precipitation effects on regrowth following defoliation, despite the known importance of moisture in altering regrowth (Hobbs, 1996). Improved regrowth has been observed under both restricted soil moisture (van Staalduinen and Anten, 2005) and surplus moisture conditions (Fanselow *et al.*, 2011).

Both short- (Biondini and Manske, 1996) and long-term (Milchunas and Lauenroth, 1993) grazing are known to influence rangeland production. Under certain conditions, such as a long history of grazing, low grazing intensity and high soil moisture, grazing can cause overcompensation leading to increased biomass (McNaughton, 1979; Frank and McNaughton, 1993); however, a review of global studies (Milchunas and Lauenroth, 1993) and a study from the Canadian prairies (Clarke, 1942) indicate growing season grazing decreases production. Overcompensation not-withstanding, regrowth following biomass removal depends on many factors, including temperature and precipitation, as well as grazing intensity (Fanselow *et al.*, 2011).

Biomass responses to precipitation (Knapp *et al.*, 2002), warming (Lin *et al.*, 2010), and grazing (Bork *et al.*, 2012) also vary by growth form. Shrub growth can increase with warming (Klein *et al.*, 2007; Lin *et al.*, 2010), but shifts between graminoids and forbs may be of greater concern in rangelands without a woody component, as graminoids are favoured for cattle forage (Holcheck, 1984). Shifts in biomass among herbage components in response to climate may be more important than changes in total biomass. In addition to direct effects of climate and grazing, herbage responses may be further complicated by species interactions. For example, Dunnett and Grime (1999) found that while grasses grown in monoculture increased with warming, they declined with warming when grown in combination with forbs.

Both climate and grazing can impact herbage quality, including CP concentrations. Recent grazing generally enhances CP through the initiation of young photosynthetic regrowth, but a long history of grazing can decrease CP (Milchunas *et al.*, 1995). Generally, conditions that accelerate plant maturity like increased temperature and decreased precipitation, have a negative effect on CP (Buxton, 1996). However, variation from this trend has been observed for precipitation (Hayes, 1985; Craine *et al.*, 2010). Levels of CP also vary with growth form (Holcheck, 1984; Bork *et al.*, 2012); thus, shifts in growth form arising from climate would further affect CP availability (Klein *et al.*, 2007).

Precipitation, temperature and grazing can also interact to alter herbage quantity (Klein *et al.*, 2007) and quality (Walter *et al.*, 2012). For example, decreases in herbage quantity and quality from warming can be mitigated by simulated grazing (Klein *et al.*, 2007). Where the effects of temperature and precipitation depend on land uses such as grazing, producers may alter grazing practices to maintain long-term productivity. Despite calls for more studies that include grazing in conjunction with climate (Izaurralde *et al.*, 2011), we know of no published study that has simultaneously manipulated precipitation, temperature and grazing (or equivalent) on herbage characteristics.

We examined how experimentally altered precipitation, warming and clipping (i.e. simulated grazing) affect herbage quantity and quality in three northern temperate grasslands distributed across western Canada, at the northern boundary of the Great Plains. Our replicated, multi-factorial study should provide a more robust assessment of the impact of climate on herbage production and quality in the Canadian prairies, and contribute to our understanding of how grazing opportunities may change under various climate change scenarios. Specific research questions included:

1. What is the influence of growing conditions (warming and altered precipitation) on *accumulated herbage* production, including contributions from graminoids and forbs, and does this vary with clipping intensity?
2. What is the influence of growing conditions (warming and altered precipitation) on *regrowth biomass* of herbage, including contributions from graminoids and forbs, following early season clipping, and does this vary with intensity of clipping?
3. What is the influence of growing conditions (warming and altered precipitation) on *herbage quality* in graminoid and forbs, and does this vary with clipping intensity?

3.2 Methods

Study sites

Research was conducted at three sites, one in each of Canada's prairie provinces: Alberta (AB), Saskatchewan (SK) and Manitoba (MB). See Chapter 2 and Table 2-1 for detailed site description. The AB site was on the University of Alberta Kinsella Research Ranch 140 km southeast of Edmonton, in a fescue grassland on a thin Orthic Black Chernozem soil within the Aspen Parkland natural subregion. Dominant species include *Festuca hallii* [Vasey] Piper, *Hesperostipa curtiseta* (Hitchc.) Barkworth, and *Elymus trachycaulus* (Link) Gould ex Shinners. The SK site was a mixed grassland on an Orthic Dark Brown Chernozemic soil at "the Gap" Prairie Farm Rehabilitation Administration (PFRA) community pasture 130 km south of Regina, within the Dry Mixed Grassland natural subregion. Dominant vegetation included *Hesperostipa curtiseta* (Hitchc.) Barkworth, *Pascopyrum smithii* (Rydb.) Á. Löve, and dryland *Carex* spp. The third site is a plains rough fescue (*Festuca halli*) grassland community on an Orthic Dark Gray Chernozem soil at Riding Mountain National Park in MB, about 200 km west of Winnipeg, again within the Aspen Parkland natural subregion. Plant cover at this site is dominated by *Poa secunda* J. Presl, dryland *Carex* spp., and *Monarda fistulosa* L. Plant species nomenclature follows the United States Department of Agriculture (USDA) PLANTS Database (2012), and soil taxonomy follows the Soil Classification Working Group (1998).

The AB and SK sites have a history of moderate cattle grazing, which ended just prior to this experiment. The MB site had not been grazed by cattle since 1970, but elk, moose and deer continue to forage in the area. At the start of the study we fenced all sites to deter entry by large mammals. Vegetation at all sites is predominately cool season, perennial and native. Based on control plots, biomass at both AB and SK are dominated (approximately 75%) by graminoids, with the remainder comprised of forbs, and minimal (<1%) shrubs at AB. In contrast, the MB site is mostly (50%) forbs, with 40% graminoid and 10% shrub biomass.

Experimental design

In April-May 2007 we implemented a three year manipulative experiment at each site to determine the interactive effects of temperature (warmed or ambient control), precipitation (reduced and ambient precipitation in AB, SK and MB, with an additional added precipitation treatment in AB beginning in 2008), and growing season clipping (none, low intensity in June, or high intensity in June) on a suite of response variable. At each site, a randomized factorial design with five replicates of each treatment combination was established (90 plots in AB; 60 plots in each of SK and MB)**.** In AB the 90 plots were composed of five fully replicated blocks to account for topographical variation and the additional precipitation treatment. Details on treatment efficacy are in Chapter 2 and 4.

Warming was achieved with open-top chambers (OTCs), as per standard International Tundra Experiment (ITEX) design (Marion, 1997). OTCs were made of Sunlite-HP (Solar Components Corporation, Manchester, NH, USA), a flexible fibreglass greenhouse material allowing transmission of visible light, but inhibiting infrared radiation and advective cooling, resulting in a temperature increase within the chamber of 2-4°. Air temperature 25 cm aboveground was recorded every thirty minutes using Onset HOBO Pendant Temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) shielded with PVC pipes. The OTCs were 2 m in diameter at the base and 40 cm high, with the sides positioned at a 60° angle to the ground, resulting in a top opening of 1.6 m. The area within the OTC, or equivalent area in ambient control plots, was considered the main plot area, while clipping and precipitation manipulation encompassed a 2.5 m by 2.5 m area fully enveloping the OTC.

Rain-out shelters were used to produce treatments of ambient or reduced (-60% growing season) precipitation. In addition, AB had an added (+60%) precipitation treatment during 2008 and 2009. Rain-out shelters were 2.5 m by 2.5 m wood frames 60 cm above ground on the low end, and 120 cm above ground on the high end. Shelter tops were overlain with plastic (Dura-Film Super 4 6-mil polyethylene film; AT Plastics, Edmonton, AB, Canada) cut using either small slits to permit entry of 40% of rainfall, or for the ambient and added precipitation treatments, larger slits to permit entry of all rainfall but control for any effects of the structure itself (i.e. changes in wind or light, etc.). At AB, the 60% of water removed from the reduced precipitation treatment was collected, quantified, and redistributed onto the added precipitation treatments by hand watering within 48 hrs after each precipitation event. Except in the first year, when set up of rain-out shelters was delayed until June, OTCs and rain-out shelter tops were installed each year after snowmelt in early May, and removed in mid-October after the first killing frost and before snowfall.

In mid-summer (June 15-30), plots were clipped either to a stubble height of 7 cm (low intensity) or 3 cm (high intensity), or left non-clipped. These intensities were representative of conservative and aggressive grazing for these grasslands. A mower was used for clipping in AB and SK, while a string trimmer was used in MB due to more robust vegetation. At all sites a central 50 cm by 50 cm permanent sample plot was hand-clipped. Clipped vegetation from this permanent sample plot was sorted to graminoid, forb and shrub components, dried to constant mass, and weighed.

Vegetation Sampling

Annually at peak biomass in late July, we quantified vegetation biomass by clipping a previously unsampled 10 x 100 cm quadrat to ground level, and sorted biomass to graminoid, forb, shrub, and litter (including standing dead and detached material). Non-litter biomass in previously clipped plots constituted "regrowth biomass", while biomass in non-clipped plots was peak current annual above-ground growth. Within previously clipped plots, "accumulated biomass" was calculated by adding regrowth to initial biomass removed during June clipping. Samples were dried at 65°C for 72 hours, and weighed. Accumulated biomass samples were ground to 1 mm using a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and nitrogen (N) concentration (%) was determined by dry combustion (CE440 Elemental Analyzer) at the University of Alberta Biogeochemical Analytical Service Laboratory, according to standard analytical methods (AOAC, 1995). Total % N was then converted to % CP by multiplying by a conversion factor of 6.25 Although exact ratios of total N to CP differ among organic material, (Sriperm *et al.*, 2011), 6.25 is commonly used in the literature to estimate plant crude protein (Craine *et al, 2010.;* Klein *et al.* 2007).

Data analysis

Treatment, site and year effects, as well as their interactions, were tested using linear mixed model analyses of variance (ANOVAs) for repeated measures, specifying a compound symmetry covariance structure for the repeated measure (year). We analysed only the second and third year of data (2008 and 2009), as the added precipitation treatment and some control infrastructure were not established in 2007 (See Appendix A for 2007 accumulated herbage data). When necessary, natural log- or square root-transformations were used (Appendix B) to successfully meet ANOVA assumptions of normality and equal variance, although data in tables and Fs are derived from original data to maintain interpretability. Site, year, precipitation, clipping and warming were included as fixed factors, with replicate plots random. For all response variables, we conducted a supplemental analysis of the AB data that included the added precipitation treatment. In these AB-only analyses, we included block as a random factor, with variance components as the covariance structure.

Accumulated herbage, forb and graminoid biomass responses were analyzed across all clipping levels in a full factorial model. Additionally, we assessed regrowth herbage, forb and graminoid biomass following low or high intensity clipping. By definition, there was no regrowth in non-clipped plots. Analysis of CP was done separately for non-clipped vegetation and clipped vegetation, as CP levels of these treatments were expected to vary due to obvious differences in plant phenological stage at sampling.

All ANOVA analyses were performed using general linear mixed models in SAS (Version 9.1.3, SAS Institute Inc.). Pair-wise mean comparisons were conducted on all significant effects using Tukey tests ($p<0.05$). In follow up to the ANOVA analyses, we conducted bivariate correlations between accumulated grass and forb biomass at AB to assess changes in the contribution of each growth form. We calculated Pearson's correlation coefficients and used one-tailed significance tests to compare these variables within warmed and unwarmed plots separately, using PSAW Statistics 18 Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com).

3.3 Results

OTC efficacy

OTCs were effective in raising average daytime (9 AM to 6 PM) air temperature. On average, daytime air temperatures in OTCs from May to September 2008 were elevated 2.4±0.1 °C in AB, 1.1±0.06 °C in SK, and 1.2±0.08 °C in MB, and 3.3±0.1 °C AB, 1.5±0.1 °C in SK, and 1.5±0.1 °C MB in 2009. Nighttime temperatures (6 PM to 9 AM) were also elevated 3.3±0.1 °C in AB, 1.4±0.1°C in SK, and 1.5±0.1 °C in MB.

Spatial and temporal variation in responses

Accumulated herbage biomass was 17.5% greater in 2009 than 2008 (p = 0.003); this response was driven by graminoids ($p < 0.001$) rather than forbs (p = 0.40, Table 3-1). Accumulated herbage also differed by site ($p < 0.001$), with MB 44.6% greater than AB, and AB 17.8% greater than SK. This pattern also occurred for accumulated graminoid and forb biomass ($p < 0.001$), though the difference between AB and SK was not significant for graminoids (Table 3-1). Regrowth biomass of total herbage and graminoids ($p < 0.01$) but not forbs ($p > 0.06$) differed among sites via the same pattern as accumulated biomass.

Regrowth within each herbage component differed ($p < 0.01$) between years (greater in 2009) but only in low rather than high intensity clipped plots.

Within non-clipped plots, graminoid CP concentration differed by site ($p < 0.001$), but forb CP did not ($p = 0.10$, Table 3-1). In clipped plots, both graminoid and forb CP differed ($p < 0.001$) by site. CP did not differ by year ($p > 0.2$) in either non-clipped or clipped plots.

Accumulated biomass

Warming reduced ($p = 0.049$) accumulated herbage by 7.9% across all three sites (Table 3-2). This reduction was attributed primarily to a reduction in graminoid biomass ($p = 0.088$) rather than forb biomass ($p = 0.70$). Warming also interacted with precipitation and site to alter forb biomass ($p = 0.03$); warming impacted forb biomass under varied precipitation in AB, and when this site was examined including moisture addition, resulted in a precipitation x warming interaction ($p = 0.03$). Warming increased forb biomass under low precipitation ($p = 0.057$), but decreased forb biomass under higher precipitation, particularly ambient precipitation (Figure 3-1). Correlation of grass and forb biomass for AB in ambient and reduced precipitation plots indicated relationships between these components were linked to warming. A positive relationship existed between grass and forb biomass ($r = -0.30$, $p = 0.01$) without warming, which was instead negative ($r = 0.23$, $p = 0.04$) with warming.

Changes in clipping intensity impacted accumulated herbage ($p < 0.001$), graminoid and forb biomass (Table 3-2). Clipping also interacted with site (forb biomass, $p = 0.045$) and year (accumulated herbage, $p = 0.01$; forb biomass, $p =$

0.02). Overall, low and high intensity clipping reduced accumulated herbage ($p < 0.05$) by 12.7% and 31.5%, respectively, relative to plots receiving no prior clipping (Table 3-2). Furthermore, while forb biomass declined under light and high intensity clipping, graminoids declined only with high intensity clipping (Table 3-2).

Observed reductions in forb were limited to AB (maximum decline of 50.6%) and MB (maximum decline of 51.5%) only ($p < 0.05$), with SK having stable forb biomass ($p > 0.05$) among clipping treatments (data not shown). Clipping x year effects reflected increasing separation among clip treatments from 2008 to 2009 with respect to accumulated herbage and forb biomass. In 2008 only non-clipped (156.2±9.3 g/m^2) and high intensity (124.7±9.3 g/m^2)) clipped plots differed ($p < 0.05$) in accumulated herbage, while in 2009 accumulated herbage biomass in low intensity (171.3±9.4 g/m^2) clipped plots was depressed ($p <0.05$) compared to no clipping (205.3±9.3 g/m^2), but elevated ($p <0.05$) compared to high intensity (123.1±9.3 g/m^2) clipping. A similar effect was evident for forbs; accumulated forb biomass did not differ between low and high intensity clipping in 2008, but differed between all levels in 2009 (data not shown).

Decreases in precipitation generally reduced accumulated herbage (-24.5%), including that of graminoids (-21.5%) and forbs (-32.3%, Table 3-2). However, precipitation also interacted with site (all biomass components, $p \leq 0.02$), and site x year (graminoid, $p = 0.003$), indicating precipitation responses were variable among sites and study years. Reductions in accumulated herbage for example ($p < 0.05$), were limited to AB (-42.6%) and MB (-19.9%), but not

SK ($p = 0.48$), and were attributed largely to changes in graminoids, as forbs declined in MB (-41.6%) but not AB or SK ($p \geq 0.2$). The three-way interaction within graminoid biomass reflected inconsistent precipitation responses among sites and years. In AB, reduced precipitation decreased accumulated graminoid biomass in both years up to 50.9%, while at MB reduced precipitation decreased graminoids by 27.4% in 2008, with no decline in 2009. In SK reduced precipitation did not affect graminoid biomass ($p > 0.05$) in either year.

Precipitation impacts also tended to vary with clipping ($p = 0.058$) to affect graminoid biomass. Under ambient precipitation, graminoid biomass was greater ($p < 0.05$) under no clipping (131.1±7.5 g/m^2) and low intensity (147.5±7.6 g/m^2) clipping than under high intensity clipping (101.8±0.5 g/m^2). Under reduced precipitation however, graminoid biomass under low intensity clipping (99.4±7.5 g/m^2) did not differ ($p > 0.05$) from high intensity clipping (86.8±7.5 g/m^2).

Finally, inclusion of added precipitation in the analysis of data from the AB site indicated this treatment increased accumulated herbage biomass ($p < 0.001$) by 49.6% relative to the ambient treatment. This response was paralleled by an increase of 59.3% in graminoid biomass ($p < 0.001$) at this location. Precipitation also interacted with year to affect accumulated herbage ($p = 0.04$) and graminoid ($p < 0.001$) biomass, whereby herbage and graminoids under added precipitation increased by 53.9% and 48.2%, respectively, during 2009 compared to the previous year.

Regrowth biomass

Warming had little effect on most regrowth biomass ($p \geq 0.05$), with the exception of graminoid regrowth, which declined by 20.4% in high intensity clipped plots ($p = 0.02$, Table 3-3). In both clipping treatments, reduced precipitation led to decreased ($p < 0.01$) herbage regrowth by as much as 35.4%. Reductions in graminoid biomass ($p < 0.05$, both clipping treatments, Table 3-3), rather than forb biomass, were mainly responsible for the decline in regrowth herbage with reduced precipitation. Reduced precipitation decreased graminoid regrowth by 30.7% and 18.0% under low and high intensity clipping, respectively (Table 3-3). Effects of reduced precipitation on herbage and graminoid regrowth were further mediated by precipitation x site interactions in both clipping treatments ($p < 0.05$). Reduced precipitation decreased herbage regrowth in AB (-34.4%) and MB (-32.0%), but not SK ($p = 0.46$). Reductions in graminoid regrowth were restricted to AB (-51.9%).

Although there was no effect of precipitation on forb regrowth ($p > 0.05$), forb biomass tended to be lower (-47.7%) in low intensity clip plots ($p = 0.073$). There was also a precipitation x site interaction ($p = 0.04$) within high intensity clipped plots, whereby regrowth forb biomass decreased at the MB site only (by 60.5%) with reduced precipitation.

Analysis of AB data with the added precipitation treatment indicated this treatment increased ($p < 0.001$) herbage regrowth (+32.4% under low clipping; +89.7% under high clipping), again largely due to positive responses in graminoid regrowth (+32.8% low clipping; +96.6% high clipping). Forb regrowth responded ($p = 0.04$) to added precipitation in low intensity clipped plots only, increasing 72.2% compared to the ambient treatment.

Herbage quality

Warming had no significant effect on final graminoid ($p = 0.09$) or forb ($p = 0.73$) CP in non-clipped plots (Table 3-4). Also in the absence of early season clipping, reduced precipitation decreased ($p < 0.05$) overall graminoid and forb CP by absolute values of 0.6% and 0.9%, respectively (Table 3-4). However, a three-way interaction between precipitation, site, and year ($p = 0.03$) revealed that decreases in graminoid CP occurred primarily under reduced precipitation in AB during 2009 (CP fell from 11.9% to 8.8%). An additional four-way interaction involving warming ($p = 0.04$) at this location indicated that graminoid CP decreased from 10.5±0.6% CP to 8.5±0.6% CP with reduced precipitation, but only under warmed conditions. Forb CP content was affected ($p = 0.007$) by a precipation x warming x site interaction. In AB, forb CP in warmed plots decreased from 11.8±0.6% CP to 9.1 CP ±0.6% CP with reduced precipitation (p = 0.003). Additionally, reduced precipitation in SK decreased CP from 11.1±0.6% CP to 8.8±0.7% CP in non-warmed plots only ($p = 0.01$), but increased CP from 8.8±0.7% CP to 10.9±0.7% CP in warmed plots (p = 0.02). In MB, there were no precipation x warming effects ($p > 0.3$).

In plots exposed to clipping, reduced precipitation decreased ($p \leq 0.001$) graminoid and forb CP by 1% CP or more (Table 3-4). Forb CP was also affected by a precipation x clipping interaction ($p = 0.005$); decreases in forb CP with reduced precipitation were limited to low intensity clipping, where CP values declined from 12.5±0.3% CP to 10.8±0.3% CP.

Within plots exposed to clipping, warming decreased ($p = 0.04$) graminoid CP (Table 3-4), a trend that was also apparent ($p = 0.09$) in the absence of earlier clipping (Table 3-4). A similar reduction in forb CP ($p = 0.09$) was evident (from 12.4±0.2 % CP to 12.0±0.2% CP). Warming also interacted with year ($p < 0.05$) to alter graminoid CP; declines in CP (from 10.8±0.2% CP to 10.0±0.2 % CP) under warming occurred only in 2009. Forb CP was further affected by an interaction of warming x precipitation x clipping ($p = 0.05$); decreases in CP with warming from 11.5±0.4% CP to 10.2±0.4% CP occurred only under reduced precipitation and low intensity clipping. Increases in clipping intensity increased ($p = 0.001$) CP of both graminoids and forbs by 1% or more (Table 3-4). Clipping also interacted with year ($p < 0.05$) to alter graminoid CP, with greater CP in 2009 (11.2±0.2% CP) than 2008 (10.6±0.2% CP) under high intensity clipping; there was no year effect under low intensity clipping.

Incremental effects of added precipitation in AB on herbage quality were limited. While water addition increased graminoid and forb CP ($p < 0.05$) in comparison to reduced precipitation in both clipped (graminoid difference of 2.1% CP; forb 0.9% CP) and non-clipped (graminoid 2.0% CP; forb 2.7% CP) plots, no difference was observed relative to plots receiving ambient precipitation. A further precipation x year interaction in non-clipped plots indicated the difference in graminoid CP between reduced and added precipitation was significant only in 2009 (11.4±0.5% CP to 8.5±0.5% CP).

3.4 Discussion

Both climatic factors (i.e. warming and reduced precipitation) and clipping decreased season-long accumulated herbage, with similar magnitudes of response to precipitation and clipping, and smaller responses to warming. Effects of clipping and warming on accumulated biomass were largely consistent across sites, while responses to precipitation varied. Although negative effects of warmer air temperatures on plant growth has been attributed to soil drying (Smoliak, 1986), as the OTCs did not decrease soil moisture, and as biomass in SK responded to warming but not lower precipitation, we hypothesize some other mechanism is responsible for this finding. Notably, the decline in accumulated herbage with warming was largely due to graminoids rather than forbs. King *et al.* (1995) also showed that decreases in growth of three *Festuca* species, one of which is found at our sites, were due to increased temperature and not changes in soil moisture. The latter study suggested this was an adaptation to limit growth to periods of lower temperatures, thus restricting growth to early in the growing season when soil moisture is high**.** There are also numerous physiological responses of plants that could lead to decreased biomass under warming, such as responses to heat stress or deviation from optimum temperatures for photosynthesis (Walther, 2004). Unlike Klein et al. (2007), we found no evidence of interactive effects of warming and clipping on accumulated herbage.

Accumulated herbage declined with reduced precipitation in both AB and MB, but not SK. At SK, the driest site, plants may be adapted to arid conditions via high water use efficiency and other drought tolerance mechanisms (Fay *et al.*, 2002). However, this tolerance to reduced precipitation may be temporary, as grassland can exhibit lag responses to precipitation (Fabricante *et al.*, 2009), and annual precipitation is known to strongly influence grassland ANPP regardless of mean annual precipitation (Sala *et al.*, 1988). In AB, graminoids were mainly responsible for the decline in accumulated herbage with reduced precipitation, while in MB, forbs were mainly responsible. Grasses are generally more responsive to precipitation than forbs, as grasses are reliant on access to shallow water supplies through fibrous roots, whereas forbs can access deep water supplies (Patton *et al.*, 2007). However, responses of forbs and graminoids to precipitation can be species-specific, as may be occurring in MB, and are not always consistent with this explanation (Fay *et al.*, 2002).

One of the ways in which climate change is expected to impact plants is through altered species interactions (Adler *et al.*, 2012). This idea was reinforced in the current study by the underlying negative relationship between grass and forb biomass, but only with warming. As both warming and reduced precipitation decreased accumulated graminoid biomass, forbs may have been able to take advantage of decreased competition and respond positively despite adverse environmental conditions. Likewise, in the absence of warming, there was a positive relationship between the two, potentially driven by other favourable environmental conditions.

Accumulated herbage declined with increased clipping intensity. However, as has been observed in a number of studies (Harmens *et al.*, 2004; Li *et al.*, 2009), these decreases were most pronounced under high intensity clipping. In our study, this was likely because of the ability of graminoids to tolerate low intensity grazing. Grasslands across the northern prairies have a long grazing history, which likely allows mid to late seral grasses to be well adapted to withstand periodic herbivory and contribute to high diversity in these communities (Milchunas *et al.*, 1988).

Unlike Heitschmidt *et al.* (2005), there were no consistent interactive effects of biomass removal and precipitation treatments on accumulated biomass. However, consistent with the trend for reduced accumulated graminoid biomass under reduced precipitation and low intensity clipping, in a long-term study, Milchunas (1994) found that the effects of clipping were more severe with low precipitation. The response of graminoids to clipping can largely be explained by regrowth, as both favourable and unfavourable environmental conditions during regrowth influenced graminoids more than forbs. In contrast, forb responses to warming and precipitation were driven by early-season conditions rather than altered regrowth. Consistent with these findings, Hawkes and Sullivan (2001) concluded that monocots grew less after herbivory under low resources, but grew more after herbivory under high resources.

Overall, regrowth biomass was more sensitive to precipitation than to warming. The exception was at SK, where regrowth did not respond to reduced precipitation. With added precipitation in AB, regrowth was greater under high intensity than low intensity clipping, again driven by graminoids. There is some disagreement over whether low or high resources should lead to increased regrowth; our study is consistent with the compensatory continuum hypothesis, which states that herbivory tolerance is greater under high resources (Maschinski and Whitham, 1989). Despite this, we observed no net increase in accumulated herbage to suggest overcompensation in response to clipping in our grasslands. In contrast to precipitation, the only effect of warming on regrowth was a decrease in graminoids within high intensity clipped plots.

Generally, we found that both warming and reduced precipitation had negative effects on herbage quality. Especially in MB, where baseline graminoid protein concentrations were below 7%, the decrease of 1% in CP with reduced precipitation could bring CP near the critical maintenance level for livestock of 5% (Milchunas *et al.*, 2005). Furthermore, there is no evidence to suggest these changes in CP are transitory, as when treatments interacted with year, it was to magnify rather than minimize effects over time.

Decreases in CP with increased temperature have been observed in a number of studies (An *et al.*, 2005; Craine *et al.*, 2010), likely due to accelerated maturation and reduced leaf to stem ratios (Buxton, 1996). Minor reductions in precipitation are expected to increase CP by delaying maturation (Buxton, 1996). However, as we found, under more severe reductions in precipitation, CP may decrease, potentially due to nutrient translocation from shoots to roots (Hayes, 1985; Buxton, 1996) or forced senescence brought on by a lack of water. Supplemental rainfall is known to increase biomass and a subsequent dilution of N in plant tissues can decrease CP concentrations (Milchunas *et al.*, 1995), although increased N uptake can simultaneously compensate for this (Fanselow *et* *al.*, 2011). We observed no change in CP with added moisture relative to ambient precipitation, and thus increased precipitation is unlikely to augment or exacerbate CP losses. In general, interactions between warming and precipitation either were consistent with, or exacerbated, existing trends, except that CP values in SK increased with reduced precipitation (under warmed conditions).

Our results further support the idea that planning for altered herbage production under future climate conditions will be necessary (Morgan, 2008). However, this planning is difficult as there are many uncertainties in predicting plant responses to climate change. Aggregate herbage responses can also be complicated by species interactions (Suttle *et al.*, 2007) and interactions with other global change factors, such as rising CO_2 levels (Campbell, 2000). Although understanding agronomic responses to climatic factors at the scale of the individual ranch operation may not be possible, caution needs to be taken when applying knowledge from large spatial scales to small scales (Brown, 2008). For example, our consistent response of decreased herbage biomass with warming differs from that currently projected across large-regions of the northern Great Plains (Morgan, 2008), and from meta-analysis results (Rustad *et al.*, 2001; Lin *et al.*, 2010; Wu *et al.*, 2011). We therefore see the need for more studies across broadly similar grasslands to identify the underlying factors associated with observed variability in herbage quantity and quality responses.

Although increases in herbage quality from clipping may compensate for declines in herbage quantity (Milchunas *et al.*, 2005), responses of herbage quality and quantity under reduced precipitation and warming were both in the same direction. In other words, our results indicate that losses in herbage production under drought and warming will be exacerbated by decreased herbage quality. We saw evidence that graminoids, the more desirable plant group for herbage, will be particularly sensitive to warming, allowing forbs to prosper. However, graminoids also performed better than forbs with added precipitation; thus, the full implications of our research will ultimately depend on future climatic conditions.

Although the majority of our findings indicate decreased herbage production capabilities under future climates, the decline in both herbage quantity and quality with warming was less pronounced than that of reduced precipitation. Furthermore, it is unknown whether future precipitation regimes will be drier or wetter for the Canadian prairies, and thus, it is possible that increased precipitation may lead to increased herbage production and little to no changes in herbage quality. Due to uncertain predictions in future climate and vegetation responses, Izaurralde *et al.* (2011) suggests livestock producers use diversified crop-livestock production systems. We hope that our study can contribute by providing information likely to lead to more accurate region-specific predictions of grazing resources under future climatic and land use conditions.

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Table 3-1. Mean (SE) aboveground biomass and crude protein concentration of various vegetation components, as sampled across three northern temperate grasslands in western Canada.

Year	Site	Accumulated biomass (g/m^2)			Crude protein (%)	
		Herbage	Forb	Graminoid	Forb	Graminoid
2008	AB	128.8 (9.3)	34.6 (6.3)	94.1 (6.4)	10.9 (0.4)	9.8 (0.3)
	SK	109.9 (9.4)	16.1 (6.4)	93.8 (6.5)	10.3 (0.5)	8.7 (0.3)
	MB	186.6 (9.3)	61.9 (6.3)	124.7 (6.4)	9.9 (0.4)	6.9 (0.3)
2009	AB	152.1 (9.3)	42.0 (6.3)	110.2 (6.4)	10.4 (0.4)	9.8 (0.3)
	SK	109.9 (9.4)	19.3 (6.4)	109.2 (6.5)	9.9 (0.4)	8.4 (0.3)
	MB	219.1 (9.3)	72.1 (6.3)	146.9 (6.4)	9.6 (0.4)	6.7 (0.3)

Table 3-2. Accumulated mean (SE) herbage, forb and graminoid biomass in response to the main effects of warming, reduced precipitation and clipping treatments. Data are averaged across 2008 and 2009 and the three northern temperate grasslands in western Canada. Within a column and treatment, means followed by the same letter do not differ, p>0.05.

Treatment	Level	Accumulated biomass (g/m^2)		
		Herbage	Forb	Graminoid
Precipitation	Ambient	175.7a (5.6)	48.9a (3.5)	126.8a (4.3)
	Reduced	132.6b (5.5)	33.1b (3.5)	99.5b (4.3)
Warming	No	160.5a (5.5)	42.1a (3.5)	118.4a (4.3)
	Yes	147.8b (5.6)	39.9a (3.5)	107.9a (4.3)
Clipping	None	180.8a (6.8)	59.0a (4.3)	121.7a (5.3)
	Low intensity	157.8b (6.8)	34.4b (4.4)	123.5a (5.3)
	High intensity	123.9c (6.8)	29.6b (4.3)	94.3b (5.3)

Table 3-3. Mean (SE) regrowth biomass, including accumulated herbage, grass and forb components, in response to the main effects of warming and reduced preciptation,, following either low or high intensity clipping earlier in the growing season. Data are pooled across years and across three northern temperate grasslands in western Canada. Within a column and treatment, means followed by the same letter do not differ, p>0.05.

Treatment	Level	Regrowth biomass (g/m^2) – Low intensity clipping			Regrowth biomass (g/m^2) – High intensity clipping		
		Herbage	Forb	Graminoid	Herbage	Forb	Graminoid
Precipitation	Ambient	110.8a (7.3)	30.6a (5.8)	80.2a (4.6)	54.1a (2.6)	16.3a (2.2)	37.8a (2.3)
	Reduced	71.6b (7.1)	16.0a (5.7)	55.6b (4.5)	42.9b (2.6)	11.6a (2.2)	31.0b (2.4)
Warming	No	91.7a (7.1)	21.1a (5.7)	70.7a (4.5)	51.4a (2.6)	13.1a (2.2)	38.3a (2.3)
	Yes	90.6a (7.3)	25.5a (5.8)	65.1a (4.6)	45.5a (2.6)	14.8a (2.2)	30.5b (2.4)

Table 3-4. Mean (SE) crude protein concentrations (%) in the forb and graminoid biomass components in response to warming and reduced preciptation, both with and without clipping during the growing season. Data are pooled across years and across three northern temperate grasslands sites in western Canada. Within a column and treatment, means with the same letter do not differ, p>0.05.

Treatment	Level	Crude protein (%) - No Clipping		Crude protein (%) - With Clipping	
		Forb	Graminoid	Forb	Graminoid
Precipitation	Ambient	10.6a (0.24)	8.7a (0.2)	12.7a (0.2)	11.0a (0.1)
	Reduced	9.7 b(0.3)	8.1b (0.2)	11.7b (0.2)	9.8b (0.1)
Warming	No	10.2a (0.2)	8.6a (0.2)	12.4a (0.2)	10.6a (0.1)
	Yes	10.1a (0.3)	8.2a (0.2)	12.0a (0.20)	10.2b (0.1)
Clipping	Low intensity			11.7a (0.2)	9.9a (0.1)
	High Intensity			12.8b (0.2)	10.9b (0.1)

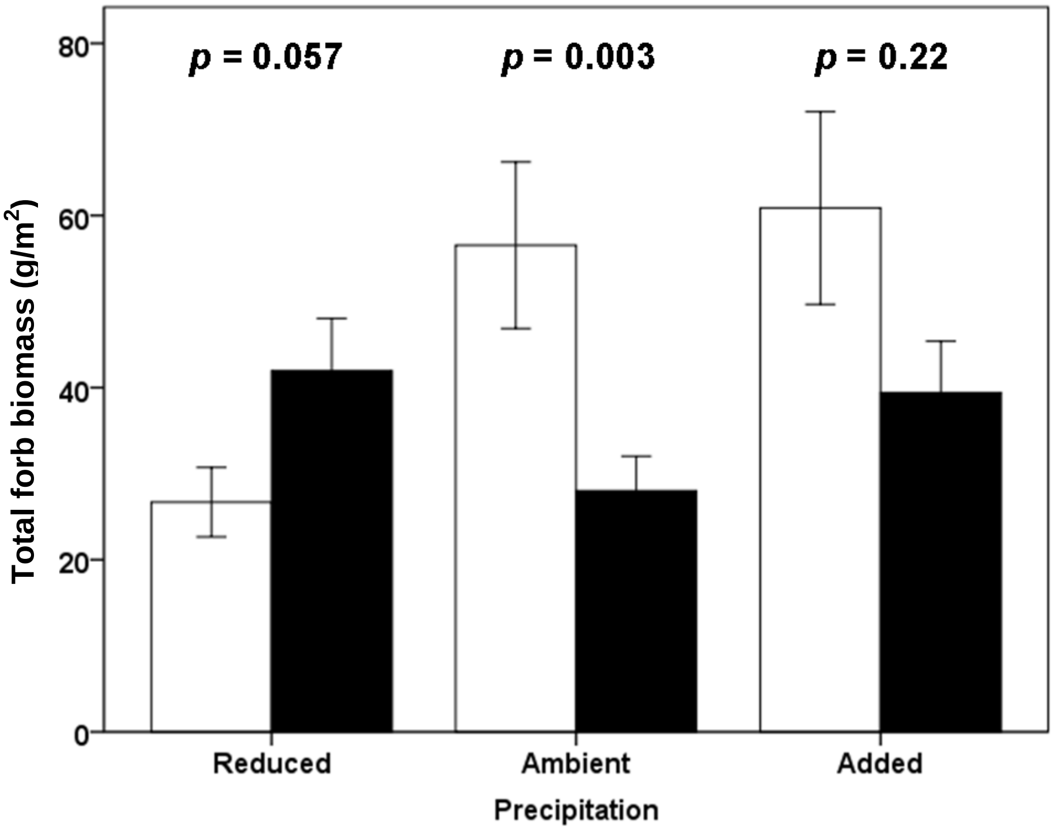

Figure 3-1. Mean accumulated (±SE) forb biomass at the AB grassland under control (white bars) and warmed (black bars) conditions and each of three precipitation treatments: reduced, ambient and added; n=5. *P* values indicate significance of pair-wise interactions within each precipitation value.

4. SIMILARITY BETWEEN GRASSLAND ABOVEGROUND VEGETATION AND SEED BANK SHIFTS WITH ALTERED PRECIPITATION AND CLIPPING, BUT NOT WARMING[1]

4.1 Introduction

The seed bank, defined as all viable seeds contained in the soil of a given area (Harper 1977), holds a record of previous vegetation, as well as potential permutations for future plant communities. The similarity between the seed bank and aboveground vegetation for a given community can be affected by factors such as disturbance, succession, and restoration, and is frequently studied in order to better understand the mechanisms controlling plant community composition (Hopfensperger 2007). Grassland plant community composition has been shown to respond to shifts in precipitation and increases in temperature associated with climate change (Kardol et al. 2010, Grime et al. 2000), as well as to the dominant land use in grasslands: grazing (Milchunas and Lauenroth 1993). However, we do not know how precipitation, warming, and grazing may interact to affect similarity between the seed bank and aboveground vegetation.

Identifying changes in similarity between the seed bank and aboveground vegetation (hereafter referred to as “similarity”) is important because the extent of similarity can indicate the potential for a seed bank to restore the aboveground community after disturbance, with increased potential for restoration from a seed bank that more closely mirrors the vegetation (Valko et al. 2011). On average,

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grasslands generally have higher similarity than wetlands or forests (Hopfensperger 2007), and thus can be expected to restore the aboveground community after disturbance. However, similarity varies among grasslands, with similarity generally higher in established grasslands or extreme growing conditions (Hopfensperger 2007), and communities dominated by annuals (Ungar and Woodell 1996, Peco et al. 1998).

No study has manipulated grazing and precipitation simultaneously to determine how they may interact to affect similarity, and despite its potential to affect plants both above (Walther 2004) and belowground (Walck et al. 2011), no study has manipulated temperature in regards to similarity. The effects of precipitation and grazing on similarity have been independently investigated in grasslands, but results have been highly variable. Precipitation has been shown to have variable effects on similarity over small time scales (Caballero et al. 2008, Gonzalez and Ghermandi 2008). Although similarity is generally thought to increase after a disturbance such as grazing (Ungar and Woodell 1996), as seeds in the soil can be the source for regeneration in disturbed areas, grazing has also been shown to decrease similarity (Peco et al. 1998, Osem et al. 2006). The highly variable results among studies have been attributed to differences in grazing regimes, environmental conditions and vegetation characteristics between sites (Osem et al. 2006).

To understand how these factors can affect similarity, we manipulated precipitation and temperature, and clipped vegetation (as a proxy for grazing), for three years in a northern temperate grassland and determined the response of similarity between the seed bank and aboveground vegetation. Grasslands are increasingly under the pressure of climate change and grazing from larger herds, and this information will aid us in understanding how the plant community may subsequently shift. As well, in contrast to studies manipulating only one factor, we investigated and compared the effects of multiple factors on similarity within a single grassland site.

4.2 Methods

Study site

This study was conducted at the University of Alberta Research Station near Kinsella, Alberta, Canada (53^{o}85’0”N; 111^{o}83’30”W). The area has a continental climate with average annual temperature of 2.8 °C and precipitation of 431.3 mm (Environment Canada, unpublished data). The study area is part of the Aspen Parkland natural sub-region and includes a mosaic of trembling aspen (*Populus tremuloides* Michx.) groves and rough fescue (*Festuca hallii* [Vasey] Piper) grassland (Sims 2000). The study site was a 40 m x 100 m field on an east-facing slope, consisting of diverse native grassland dominated by *Festuca hallii*, *Hesperostipa curtiseta* (A. S. Hitchc.) Barkworth and *Elymus trachycaulus* (Link) Gould ex Shinners, as well as many perennial forbs. The site is used for cattle grazing, though grazing was prevented for the duration of the experiment. Soils are thin Orthic Black Chernozems developed from glacial till (Howitt 1988., Soil Classification Working Group 1998).

Experimental design and treatments

In May 2007, a three year manipulative experiment was initiated to determine the effects of warming (control, warmed), precipitation (reduced, ambient, added), and clipping (none, low intensity, high intensity), on an array of grassland responses, including similarity between seed bank and aboveground composition. To account for differences in topography, we used a randomized complete block design, with each of the 18 treatment combinations occurring once in each of five replicate blocks, for a total of 90 plots, each approximately 4 m^2.

Plots received either ambient, reduced (approximately 60% less), or added (approximately 60% more) precipitation. Rain-out shelters, 60-120 cm tall wood frames with 2.5 m x 2.5 m plastic tops, were installed over all plots (Dura-Film Super 4™ 6-mil polyethylene film; AT Plastics, Edmonton, Alberta, Canada). Although the shelters themselves may have some effect on micro-environmental conditions, by installing sham shelters over the control plots we maintained consistency between treatments. The reduced precipitation treatment had small slits in the plastic that allowed approximately 40% of rainfall to reach the ground; water not falling through the plastic was collected in tanks using a gutter system, and quantified. The added and ambient precipitation treatments had larger slits and holes to allow complete rainfall entry but control for any unintended effects of the structure itself. To increase precipitation but not affect the timing of rainfall in the added plots, water collected from reduced precipitation plots was redistributed by hand to adjacent added precipitation plots within 48 hours after rainfall. Rainout shelters were installed in 2007, although the added precipitation treatment was first implemented in 2008.

Plots were either not clipped, or clipped in midsummer (June 15-30) to a stubble height of 7 cm (low intensity) or 3 cm (high intensity) above ground, corresponding to approximately 35% and 56% of standing annual biomass respectively. Clipping was conducted with a mower set at the corresponding height, except for the central 50 cm by 50 cm permanent sampling area, which was clipped by hand to limit disturbance. Clipped biomass was subsequently dried and weighed.

Half the plots were warmed by approximately 3°C using fibreglass open-top chambers (OTCs; Sunlite-HP, Solar Components Corporation/Kalwall Corporation, Manchester, NH, USA). The OTC design has been used previously to increase air temperatures by 2-4° C. OTCs were 2 m in diameter and 40 cm high, with sides positioned at a 60° angle to the ground (Marion et al. 1997). OTCs and rain-out shelters were installed in spring (May) of each year and removed in mid-October.

Two Davis Rain Collector II buckets (Davis Instruments, Hayward, CA, USA) measured ambient precipitation. Air temperature 25 cm above ground was recorded in two blocks (36 plots) every half hour using Onset HOBO Pendant Temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) shaded in PVC pipes. To calculate percent precipitation prevented entry by the rain-out shelter on a plot basis, we divided the amount of precipitation per m^2

collected in each tank, by the average of ambient precipitation per m^2 measured by the two rain gauges.

Aboveground vegetation

Vegetation sampling in each plot was concentrated within the central 50 cm by 50 cm sampling area. Foliar plant cover (%) by species was visually estimated mid-month in May, June, July, and August of 2009. Every July, aboveground shoot biomass was clipped from an independent 0.10 m^2 (10 cm by 100 cm) harvest strip. Elevated platforms were used by the researcher during estimation of plant cover, and each year a different harvest strip was clipped, to limit disturbance to the plots. Harvest biomass was determined by sorting out litter from live biomass, drying, and weighing. To determine total biomass, clipped and harvest biomass were determined per m^2, and added.

Seed bank

Seed bank composition in each plot was sampled in 2008 from soil with the litter layer removed. To sample the persistent seed bank, those species which retain their seed bank for more than 12 months (Thompson and Grime 1979), rather than the transient seed bank, it is recommended to sample when species germinating in the spring have begun to develop but no fresh seeds have been dispersed (Csontos 2007). We sampled in mid-July, near the ideal time (end of June) suggested for seed bank sampling in our climate (Milberg 1992, Milberg and Persson 1994).

Within each plot, one 7 cm diameter sample of 15 cm depth was taken using a soil auger, of which half was sub-sampled, and one 5 cm diameter sleeve core of 20 cm depth was taken using a root corer, both adjacent to the harvest strip. Thus, the total volume of soil sampled per plot was approximately 680 cm^3. Soil was successively washed through two sieves (6 mm and 0.212 mm mesh) to remove coarse (plant shoots, roots and soil invertebrates) and fine debris (TerHeerdt et al. 1996). The smaller sieve size has been used to capture grassland seeds and likely captures the majority of seeds here, but we recognise some smaller seeds may have passed through and affected the results. Remaining coarse debris was visually inspected for seeds. The soil and seeds in the smaller mesh size was spread in a thin layer over 3 cm of sterilized seedling starter mix in 25 cm x 25 cm greenhouse trays. Three control trays filled with only the seedling starter mix were used to identify any seed contamination within the greenhouse. Trays were exposed to supplemental heat (24 °C) and lighting (12 hours per 24 hour cycle), watered every two to three days, and rearranged randomly biweekly. Emerging seedlings were counted and removed as they became identifiable, with unidentifiable seedlings transplanted into pots and grown until identifiable. After five months, germination ceased and the top layer of the tray was raked; seedling assessment continued until no germination was evident, at seven months.

Similarity

We used Sørensen's quantitative measure C_N (Magurran 1988) to determine similarity between 2008 seed bank composition and monthly standing vegetation in 2009 for each plot, as vegetation composition is influenced by previous year persistent seed bank (Caballero et al. 2008). This is a quantitative similarity measure and thus incorporates both species presence/absence and species abundance. Relative abundances of seed bank and vegetation were used by converting to percentages prior to calculation of similarity. Similarity is calculated as $C_N = 2jN/(aN+ bN)$, where aN is the total vegetation cover of each plot, bN is the total number of seeds in each plot (both 100 in this case due to the conversion), and jN is the sum of the lower of the two abundances (vegetation cover or seed number) for species occurring both in the seed bank and aboveground in each plot.

Statistical analysis

Fixed effects of precipitation, clipping, and warming on similarity were then assessed using a mixed model, with block as a random factor, using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com).

Shifts in similarity could be due to changes in the composition of the seed bank or of the vegetative community. To parse out these roles, we measured the responses of the seed bank and aboveground community to the treatments. To describe these community responses to the treatments, we performed Unconstrained Non-metric Multidimensional Scaling (NMDS) ordinations using Sørensen's (Bray-Curtis) distance measure on the seed bank and aboveground community (McCune and Mefford 2006). Multi-Response Permutation Procedures (MRPP) were conducted for each treatment to determine treatment effects on community composition. Ordinations were performed using PC-ORD Version 5.10 (McCune 2002)). We used Indicator Species Analysis (ISA) to identify individual species underlying compositional differences in response to the main treatments (Dufrene and Legendre 1997).

4.3 Results

Treatment efficacy

Rainout shelters reduced precipitation in relation to ambient conditions by 64.7 ± 2.4% in 2008 and 59.9 ± 1.4% (mean ± 1SE) in 2009, with corresponding increases in precipitation within the added precipitation treatment. Clipping removed 57.4 ± 5.5 g/m² (low intensity clipping) and 76.7 ± 6.2 g/m² (high intensity clipping) in 2008, corresponding to 39% and 56% of total biomass. In 2009, clipping removed 74.8 ± 10.8 g/m² (low intensity) and 99.3 ± 16.3 g/m² (high intensity), 32% and 56% removal of total biomass, respectively. On average, growing season (May-August) air temperatures at mid-afternoon (1:30 PM) were 3.1 ± 0.15 °C greater in warmed plots than control plots in 2008, and 4.2 ± 0.16 °C greater in 2009.

Aboveground vegetation

The NMDS ordination of 2009 aboveground vegetation (Appendix C), along with MRPP analysis, indicated that precipitation ($p<0.001$, Appendix C) and clipping ($p<0.001$; Appendix C) affected aboveground community composition, but temperature did not. Pair-wise contrasts were significant for all levels of precipitation and clipping (Appendix C). The ISA analysis identified one indicator species: *Astragalus flexuosus* (added precipitation, $p=0.04$).

Seed bank

Both visual inspection of the ordination of the seed bank species and MRPP analysis indicated that only precipitation affected seed bank community composition ($p<0.05$; Appendix C). Pair-wise analysis revealed that this change in seed bank community composition was driven by differences between the added and reduced precipitation treatment ($p=0.02$; Appendix C). No species were associated with particular treatments in the ISA.

Similarity

Total species richness was one-third lower in the seed bank (22 species) than in aboveground vegetation (33 species). Six species present in the seed bank were not found in the aboveground vegetation, while 17 species present in the vegetation were not detected in the seed bank (Table 4-1). Similarity between the seed bank and aboveground vegetation as a response to treatments followed a similar pattern across all months, so we present July (peak biomass and richness) results only. Reduced precipitation increase similarity between the seed bank and aboveground vegetation ($p=0.04$; Table 4-2, Figure 4-1), and high intensity clipping decreased similarity ($p=0.05$; Figure 4-1). Warming had no effect on similarity ($p=0.73$; Figure 4-1). There were no significant interactions between any of the three treatments (Table 4-2).

4.4 Discussion

Clipping and precipitation have not been jointly investigated for effects on the similarity between seed bank and aboveground vegetation, and warming has not been tested independently. We found that the response of similarity was dependent on treatment, with both clipping and precipitation, but not warming, affecting similarity vegetation on a short timescale. The magnitude and direction of response varied with treatment, with clipping decreasing similarity and precipitation increasing similarity. There were no interactions between any of the factors, suggesting that the seed bank responds independently to the different factors.

Clipping may be expected to increased similarity, as germination success typically increases following release from dense plant cover (Kitajima and Tilman 1996, Peco et al. 1998). However, direct mechanical harm to seedlings and alteration of micro-environmental conditions that limit seed germination, such as may have occurred with our clipping treatment, are known to decrease in similarity (Bakker and Devries 1992). Alternatively, grazing-adapted adult plants that use vegetative propagation to maintain dominance in the community, rather than seedlings emerging from the seed bank, may have been able to fill any gaps caused by clipping (simulated grazing), subsequently decreasing similarity. Vegetation in North American grasslands east of the Rocky Mountains, such as at our site, evolved under and are adapted to bison grazing, and more recently cattle grazing (Milchunas and Lauenroth 1993).

The increase in similarity in response to reduced precipitation is consistent with the observed pattern of increased similarity in communities subject to extreme growing conditions, such as drought (Henderson et al. 1988, Peco et al. 1998, Gul and Weber 2001). The annual and biennial species that colonize under extreme conditions, such as reduced precipitation, rely on seed banks, unlike perennial species that rely primarily on vegetative reproduction (Henderson et al. 1988). In contrast to other studies in which seed bank expression in aboveground vegetation increased during wet years (Gutierrez and Meserve 2003, Caballero et al. 2008), which can provide the moisture necessary for seed bank germination (Clauss and Venable 2000), added precipitation had no effect on similarity. However, these studies have been limited to arid or semi-aid systems dominated by annuals and shrubs. In our grassland, a well-established continuous cover of perennial grasses and forbs may benefit from increased resources such as soil moisture, and thereby restrict seed bank germination or survival of seedlings via competition for soil nutrients or light (Buckland et al. 2001, Osem et al. 2006). Also, we had only applied our added precipitation treatment for three months at the time of sampling, possibly not sufficient to elicit vegetation changes.

Our system was insensitive to warming, with similarity and both the seed bank and aboveground community unresponsive to warming. A certain threshold treatment value can be necessary before similarity is affected (Wellstein et al. 2007) and it may be that more prolonged warming or a greater increase in temperature would affect similarity. Consistent with our finding, other studies have found that both the seed bank (Akinola et al, 1998) and the aboveground community (Grime et al. 2008) can be insensitive to warming.

The germinated seed bank density was unaffected by the treatments, and thus did not contribute to changes in similarity. The average density we found, 6,923 seeds per m^2 (data not shown), is similar to that previously identified within fescue prairie seed banks (Willms and Quinton 1995). Seed density is a function of soil volume, and the volume of soil recommended for seed bank studies varies. Our sample volume (681 cm^3) was above that suggested by Hayashi & Numata (1971) and Roberts (1981) but below others' recommendations (Oomes and Ham 1983, Thompson 1997). Rather than providing an exhaustive description of the seed bank, our intent was to document changes in similarity between the seed bank and above ground vegetation. Sampling intensity does not appear to be a constraint in the current study, given that we found rapid responses in similarity to the treatments over the study period. This is consistent with the finding of Caballero et al. (2008) where the relationship between seed bank and aboveground vegetation were closely linked at on a small time scale, from one to three years.

The seed bank showed a lack of response to both warming and clipping. Other studies have reported delayed responses of seed bank compared to responses of aboveground vegetation, with little effects from short-term manipulation of warming and precipitation (Akinola et al. 1998) or grazing and mowing (Peco et al. 1998) on seed banks. Composition of the seed bank community did, however, respond to short-term manipulations in precipitation. Changes in soil moisture can significantly affect seed longevity in the soil (Bekker et al. 1998). However, changes in similarity due to the precipitation treatment are still likely to be attributed to changes in aboveground vegetation, as only the reduced and added precipitation treatments differed in aboveground community, but similarity differed between the reduced and ambient treatments. *Pulsatilla patens* was indicative of reduced precipitation, and comprised 2% of seed bank abundance, and thus its increase with reduced precipitation may have contributed to the increase in similarity with reduced precipitation.

Although we conclude that changes in aboveground vegetation were generally responsible for the similarity response, we cannot discern from our study whether changes in similarity are due to changes in germination and establishment of new plants, or changes in the abundance of pre-existing plants. For example, it is possible that those species that have high presence in the seed bank are those that also increase in size or cover with reduced precipitation. Also, small changes in abundance of the most common species in the seed bank and vegetation (Table 4-1) would have the most profound effect on the similarity index, and may be largely responsible for observed changes in similarity.

We found that within our grassland site, similarity responded over the short-term to precipitation manipulation and clipping, with responses largely driven by changes to the aboveground community, rather than the seed bank. Site characteristics such as domination by perennials and grazing history may help explain our findings. We recommend more studies be conducted manipulating multiple factors at a single site in order to better understand variable responses of similarity across systems. As regeneration from seed is largely responsible for the introduction of plant species and associated changes in community composition (Grubb 1977), changes in the relationship between the seed bank and aboveground vegetation are important in understanding community dynamics.

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Table 4-1. Relative abundance by species (%) across all treatments in the seed bank for 2008 and aboveground vegetation for 2009.

Plant species	Seed bank	Vegetation
Achillia millefolium L.	-	0.22
Allium textile A. Nels. & J.F. Macbr.	-	0.12
Androsace septentrionalis L.	18	0.15
Artemisia campestrisI L.	-	0.053
Artemisia frigida Willd.	60	10
Antennaria parvifolia Nutt.	-	-
Astragalus flexuosus Dougl. Ex G. Don	1.6	2.6
Avenula hookeri (Scribn.) Holub	-	0.14
Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths	-	3.6
Campanula rotundifolia L.	0.15	-
Carex stenophylla Wohl. Ssp. Eleocharis (bailey) Hult.	2.4	21
Cerastium arvense L.	-	0.17
Chenopodium album L.	0.014	0.047
Cirsium arvense (L.) Scop.	0.023	-
Comandra umbellata (L.) Nutt.	-	2.4
Crepis tectorum L.	1.7	-
Elymus trachycaulus (link.) Gould ex Skinners ssp. Trachycalus	0.28	12
Epilobium glaberrimum Barbey	0.80	-
Erigeron caespitose Vent.	-	0.79
Erysimum inconspicuous (S. Wats.) MacM	0.26	0.11
Festuca hallii (Vasey) Piper	1.8	5.3
Gaillardia aristata Pursh	-	-
Geum triflorum Pursh	-	0.024
Gnaphalium palustre Nutt.	0.024	-
Hesperostipa curtiseta (A.S. Hitchc.) Barkworth	3.9	25
Heterotheca villosa (Pursh) Shinners var. hispida	-	0.19
Koeleria macrantha (Ledeb.) J.A.	1.4	3.8
Lygodesmia juncea (Pursh) D. Don ex Hook	-	0.014
Muhlenbergia cuspidata (Tarr. Ex Hook) Rydb.	-	-
Oxytropis campestris (L.) DC.	1.1	0.23
Phlox hoodii Richards.	-	0.050
Poa pratensis L.	0.40	0.24
Potentilla bipinnatifida (Pursh) D. Don ex Hook	2.3	0.31

Potentilla concinna Richards.	0.85	0.56
Pulsatilla patens (L.) P. Mill. ssp. multifida (Pritz.) Zamels	2.0	6.9
Rosa arkansana Porter	-	0.36
Selaginella densa Rydb.	-	-
Solidago missouriensis Nutt.	-	3.3
Sphaeralcea coccinea (Nutt.) Rydb.	-	0.22
Taraxacum officinale G.H. Weber ex Wiggers	0.13	0.024
Thermopsis rhombifolia (Nutt. ex Pursh) Nutt. ex Richards.	-	0.20
Tragopogon dubius Scop.	-	0.024
Urtica dioica L.	0.17	-

Table 4-2. Mixed model analysis of effects of precipitation (P), clipping (C), and warming (W) on similarity between 2008 seed bank and 2009 aboveground vegetation.

Source of variation	Similarity	
	$F_{,df}$	P
P	$5.342_{2,146}$	0.036
C	$9.411_{2,147}$	0.047
W	$0.031_{1,146}$	0.725
W * C	$0.424_{2,146}$	0.735
W * P	$2.459_{2,146}$	0.315
C * P	$0.692_{4,145}$	0.871
W * C * P	$0.302_{4,146}$	0.914

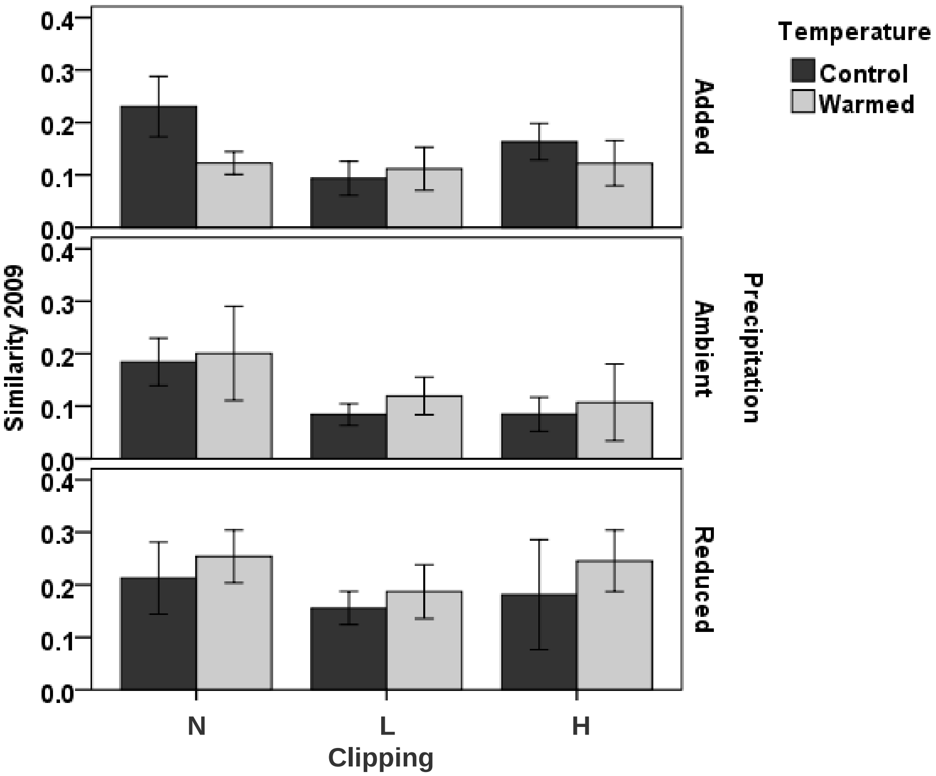

Figure 4-1. Effect of precipitation, clipping (N, none; L, low intensity; H, high intensity), and warming on similarity between 2008 seed bank and 2009 aboveground vegetation. Error bars are ±1 SE; n=5.

5. EVIDENCE OF BIODIVERSITY LOSS ASSOCIATED WITH EXPERIMENTAL WARMING ACROSS THREE NOTHERN TEMPERATE GRASSLAND SITES

5.1 Introduction

There is widespread concern over biodiversity loss in response to increasing temperatures and altered precipitation patterns associated with climate change (Tilman, 2012). Both factors directly and indirectly affect a multitude of biological processes, making biodiversity responses to climate change difficult to predict (Shaver *et al.*, 2000). However, identifying general biodiversity responses to climate change is important both because of the roles biodiversity may play in maintaining ecosystem function and reducing invasibility, as well as in feeding back to provide resistance to climate change itself (Hooper *et al.*, 2005).

Grasslands cover approximately 40.5 % of global land area, and are important repositories of biodiversity (Gibson, 2009). Prior studies suggest that plant biodiversity in grasslands generally increases with increased precipitation (Yang *et al.*, 2011, Zavaleta *et al.*, 2003, Hoeppner *et al.*, 2012), although timing of precipitation can further influence this relationship (Knapp *et al.*, 2002). The effects of warming on plant biodiversity are less clear; plant biodiversity has declined with warming in a number of studies (Klein *et al.*, 2004, Yang *et al.*, 2011). However, in others (Price *et al.*, 2000, Harmens *et al.*, 2004, Bloor *et al.*, 2010, Zavaleta *et al.*, 2003) biodiversity has been resistant, i.e. unchanged perturbation (*sensu* Pimm, 1984). Resistance of biodiversity in some systems versus reductions in others suggests potentially different mechanisms linking warming to community assembly in different communities. For example, biodiversity loss associated with warming experiments has been attributed to various processes such as decreased soil water availability (Yang *et al.*, 2011), heat stress (Klein *et al.*, 2004) or altered species interactions (Yang *et al.*, 2011). Understanding when these different processes are, or are not, important may help us understand why biodiversity is resistant to climate change in some systems, while responsive in others.

Alternatively, variability in responses may derive from the varied net results of effects acting in opposing directions (Grace *et al.*, 1999), rather than from diverse processes themselves. For example, Price *et al.* (2000) posit that the negative effects of warming on productivity in an alpine meadow resulting from decreased soil moisture negated the stimulatory effects of earlier snowmelt. Clearly, developing a predictive understanding of the consequences of climate change for grassland biodiversity will involve assessing a network of responses rather than simply assessing the direction or magnitude of net responses.

Further complicating our understanding of these responses, interactive effects can result when factors impact the same indirect process. For example, both precipitation and warming can modify soil moisture (Bell *et al.*, 2010), and as would be expected, effects of warming can be highly dependent on precipitation availability (Hoeppner *et al.*, 2012). As well, grasslands are also most often highly managed landscapes (Gibson, 2009), and land use can further interact with environmental changes (Butof *et al.*, 2012). Livestock production in particular is a widespread and economically important activity on grasslands (Gibson, 2009), and grazing can influence the effects of warming on plant biodiversity (Klein *et al.*, 2004). The many factors that control grassland biodiversity and their potential for interactions necessitate that environmental factors likely to interact be assessed in multi-factorial experiments (Rustad, 2008).

Efforts to understand generalized biodiversity responses to climate change would also be more effective if experiments were replicated among sites, as variation in methodology can influence results. We assessed biodiversity in response to experimental climate change, and simulated grazing, in a field experiment replicated across three northern temperate grassland sites. Rather than restricting analysis to direct treatments effects on biodiversity, we use structural equation modelling (SEM) to assess both the direct effects of the environmental changes associated with the treatments, and their respective indirect effects. Finally, to move towards a broad understanding of biodiversity responses to climate change, we analyzed data using multi-group SEM, which allows us to differentiate generalized responses among sites from site-specific responses.

Research objectives

1) Is *biodiversity resistant or reduced* by the environmental drivers associated with reduced precipitation, warming, and clipping?
2) Are changes in *biodiversity* with reduced precipitation, warming and clipping driven by *indirect responses*?

3) Are *responses of biodiversity* to environmental drivers associated with reduced precipitation, warming, and clipping predominantly *site-specific?*

5.2 Methods

We established a fully factorial experiment manipulating precipitation (ambient and reduced), temperature (control and warming) and clipping intensity (none, low, high) at three sites across the Canadian prairies for three growing seasons (2007-2009). See Chapter 2 for description of sites and methodology. We present values from only the final year, 2009, of experimental manipulation and sampling, as these data are likely to represent the greatest cumulative responses and supersede any lag effects (Rustad, 2001). Clipping was used as an alternative to grazing as the experimental infrastructure precluded the presence of large herbivores within the plots. Plots were circular, 2 m in diameter, with at least 0.5 m spacing between adjacent plots. Although in the larger experiment the 12 treatment combinations were replicated five times at each site, we only analyze data from the subset of plots that have corresponding environmental conditions (72 plots total).

Model overview

To identify direct and indirect responses of biodiversity to the treatments, we used Structural Equation Modeling (SEM), a technique that is well-suited to assessing relationships among a network of variables (Grace, 2006)). Specifically, we used multi-group SEM (Grace, 2006), which allows us to identify relationships consistent across all sites, and those that are unique to certain sites. Unlike in traditional analyses such as regression techniques, variables are simultaneously able to act both as predictor and response variables in SEM (Grace, 2006). We used this modeling framework to assess consistent and unique biodiversity responses at the three sites in terms of direct effects of environmental drivers associated with the treatments, as well as the indirect effects of the environmental drivers via both environmental and vegetation intermediary variables. Environmental drivers, those measurements directly controlled by the treatments, included air temperature (°C), precipitation (mm), and clipping intensity (%). Environmental intermediary variables were soil moisture (% soil volumetric water content, VWC), soil temperature (°C), and soil nitrogen (mg total inorganic nitrogen/10 cm^2 ion-exchange membrane surface area/time of burial); litter biomass (g/m^2), shoot biomass (g/m^2), and root biomass (g/m^2) were intermediary vegetation variables. Species richness (# species/$0.25m^2$) and evenness (per $0.25m^2$) were the ultimate response variables.

We built an initial model linking these variables based on our expectation of biological processes likely to be important for biodiversity in northern temperate grasslands (Figure 5-1). The model was tested separately for richness and evenness. Paths with single headed arrows represent hypothesized causal relationships, while paths with double-headed curved arrows represent co-varying variables. Details on model development follow.

Sampling – Environmental drivers

Ambient precipitation was measured using two Davis Rain Collector II buckets (Davis Instruments, Hayward, CA, USA) at each site; total precipitation over the sampling period was averaged per site. At MB, rain gauge deployment failed until July; however, late season precipitation was in close accordance (i.e. within 1.5 mm accuracy) with data from a nearby (45 km) Environment Canada weather station, so values from the latter weather station were used. These ambient precipitation values were used for control plots of the 'Precipitation' variable; reduced precipitation levels of the 'Precipitation' variable were 40% of ambient precipitation for each site, based on average efficacy of the reduced treatment in preventing entry of precipitation (Chapter 3). Unless otherwise noted, all environmental measures, including environmental drivers and intermediary environmental variables, were calculated for the sampling period from initial experimental setup in mid-May until the date when species percent cover was estimated in late July (May 12th - July 19th, AB; May 17th – July 26th, SK; May 19th – July 28th, MB). Sampling of all variables is summarized in Table 5-1.

Air temperature was recorded every thirty minutes at low (7.5 cm) and high (25 cm) vegetation height, using Onset HOBO Pendant Temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) shielded by PVC pipes. To calculate 'Air temperature' for each plot, low and high air temperatures were averaged over the sampling period, and then averaged between heights.

Clipped vegetation from the central sampling subplot was dried and weighed. 'Clipping intensity' was calculated at the plot level by dividing clipped biomass by average ‘control’ (i.e. non-clipped) shoot biomass (described below) of the corresponding precipitation and warming treatment combination.

Sampling – Environmental intermediary variables

Each plot had two 10 cm long Decagon ECH2O EC-TM soil moisture and temperature probes (Decagon Devices Inc., Pullman, WA, USA) positioned at the top of the permanent sampling plots, one probe angled from 0-5 cm depth (shallow) and one placed at 7.5-17.5 cm depth (deep). Soil moisture (% soil VWC) and soil temperature (°C) were assessed every 30 minutes. We wanted soil moisture to represent moisture available to the plant separate from the effects of uptake and transpiration by the plant, and thus we used soil moisture measurements from 4-6 AM when plants would have less photosynthetic activity. Plot averages for ‘Soil moisture’ and ‘Soil temperature’ were calculated as for ‘Air temperature’. Plant available nitrogen was assessed from late June to early September using PRS^{tm} (Plant Root Simulator) probes (Western Ag Innovations, Saskatoon, SK). Each plot received four sets of PRS probes, positioned evenly around and within 10 cm of the permanent sampling subplot, consisting of both a cation (NH_4^+) and anion (NO_3^-) probe. Probes were pooled prior to elution and colourimetrical analysis by Western Ag; the variable ‘Nitrogen’ is based on total N inorganic as NH_4^+ and NO_3^- (mg total nitrogen/10 cm^2 ion-exchange membrane surface area/time of burial).

Sampling – Biomass

Vegetation was sampled in mid-July by clipping a 10 cm by 100 cm vegetation strip to ground level; a different strip was sampled each year. Previous years' litter, including standing dead biomass, was sorted from standing live vegetation, and both components were dried to constant mass and weighed. This litter is the variable "Litter biomass", and for non-clipped plots the standing live vegetation is "Shoot biomass". For plots receiving the June clipping treatment, July standing live vegetation was added to biomass from June clipping to determine total 'Shoot biomass'. Root mass was sampled in a 5 cm diameter, 20 cm deep core adjacent to the vegetation strip. Root samples were washed with a 2 mm sieve, dried and weighed; this constituted the variable 'Root biomass'.

Sampling – Diversity

The response variables, richness and evenness, were calculated from vascular plant species foliar percent cover visually estimated from the central 50 cm by 50 cm sampling subplot, estimated in late July to correspond with peak biomass (Steinaker *et al.*, 2008). Naming of species follows USDA (2011). To limit plot disturbance, the researcher used an elevated platform during estimation of plant cover. Richness was simply the number of species present in a plot. For evenness, we used the Shannon-Weiner evenness index, calculated using PC-ORD Version 5.10 (McCune, 2006).

Model specification

SEM requires that data meet assumptions of multivariate normality and absence of nonlinear relationships. To satisfy the normality assumption, we used a cubed transformation for evenness, a square root transformation for litter, and natural log transformations for richness, nitrogen, shoot biomass, and root biomass. To detect nonlinear relationships, we used curve estimation in PSAW

Statistics 18 Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com) to compare the fit of linear, quadratic, and cubic regressions for all bivariate relationships in both models. We found two significant ($p<0.05$) non-linear relationships: a cubic relationship between precipitation and litter, and a quadratic relationship between root biomass and evenness. As linear models described overall relationships between variables, and the models fit well, we decided it was unnecessary to explicitly model non-linear relationships. Amos 18.0 (Amos Development Corporation, Crawfordville, FL, USA) was used to compute models. The means and range of all variables, as well as bivariate correlations, included in the models are listed in Appendix D.

Data missing more than 10% of observations was considered missing for that plot. This resulted in soil measurements missing at random for two plots in AB and two plots in SK (all deep soil probes), and one in MB (missing shallow and deep soil probes). In SK high air temperature was also missing for one plot. We deleted plots with missing values before solving richness and evenness models.

We followed standard multi-group modelling methodology (Grace *et al.*, 1999), described below, to fit the final models. First, we confirmed that initial model fit was acceptable when all parameters were allowed to vary between the three sites (a χ^2 value associated with a high p-value, i.e. $p>0.05$, indicates acceptable fit). Initial model fit for richness and evenness was high ($\chi^2=4.0_6$, $p=0.671$, both). We then constrained regression weight, variance, and covariance values to be equal between the three sites. With these parameters constrained, fit was, as expected, unsatisfactory (richness, $\chi^2 = 180.7$, $p<0.001$; evenness, $\chi^2 = 188.6$, $p<0.001$). We then evaluated the three standardized residual matrices (one for each site), as a high standardized residual represents a parameter that differs from that described by the model. Using a step-wise approach we identified and relaxed the parameters associated with each subsequent variable with the highest residual, thus allowing parameters to differ for a particular site. This process was continued until all residual values were low and model fit was satisfactory. Final model fit was evaluated using three values: χ^2, Tucker Lewis Index (TFI), and Root Mean Square Error of Approximation (RMESA). Acceptable fit is indicated by non-significant ($p>0.05$) χ^2 values, TFI values >1, and low (<0.05) RMSEA values (Grace, 2006, Byrne, 2010).

We retained all paths in our final model, regardless of significance, because of our underlying assumption of the biological relevance of each path. In our calculation of total, direct, and indirect effects, we included only regression weights and correlations that had unstandardized path estimates that significantly ($p<0.05$) differed from zero, in order to avoid basing results on parameters which could not be accurately estimated by the model.

To evaluate the effects of missing plots on the models, we compared output with models based on estimated missing values. We based estimates of these missing average values on the corresponding shallow soil probe (for the missing plot in MB this was not possible and so it remained deleted) or low air temperature measurement, and found a high correlation, i.e. average soil temperature and moisture values were strongly driven by shallow measurements (linear regression r^2 values ranged from 0.68-0.96, p-values all <0.001). Estimating the values caused sufficient changes in standard errors to change significance of only one to three paths per model (Appendix D). As models were overall qualitatively and quantitatively similar, and as the paths usually gained rather than lost significance when based on the estimated values, we chose the conservative approach of list-wise deletion of plots with missing data.

Univariate analyses

To isolate treatment effects from associated environmental effects, we performed linear mixed models testing the main effects of air temperature, clipping intensity, and precipitation (as fixed factor covariates) and their interactions with site (AB, SK, and MB), on both richness and evenness response variables. These models were computed using PSAW Statistics 18 Release Version 18.0.0.

5.3 Results

The final models provided satisfactory fit to the data (richness, χ^2_{91} = 83.6, P=0.697, TLI=1.073, RMSEA = <0.001; evenness χ^2_{92}=85.1, p=0.683, TLI=1.070, RMSEA = <0.001). Standardized coefficients, unstandardized coefficients, and t-test results are summarized in Appendix D.

Richness

At all sites, through direct or indirect effects, richness increased with precipitation, and decreased with air temperature and clipping intensity (Figure 5-2, Table 5-2). Air temperature decreased richness through direct rather than indirect effects at all sites. The opposite was true of clipping intensity, with only indirect effects of clipping intensity on richness. At all sites, increased clipping intensity decreased litter, which in turn positively affected shoot biomass and finally richness. At AB (Figure 5-2a) and MB (Figure 5-2b), richness decreased with clipping intensity via an increase in air temperature, which negatively affects richness. At SK (Figure 5-2c), clipping intensity did not affect air temperature, with no associated indirect pathway to richness.

Precipitation increased richness through two indirect pathways both in AB (Figure 5-2a) and MB (Figure 5-2c): one involving increasing shoot biomass and the other decreasing air temperature. At SK (Figure 5-2b), precipitation similarly affected richness via air temperature, but not via shoot biomass, as shoot biomass did not respond to precipitation at this site. The total effect of precipitation on richness was strongest at MB and weakest at AB. Although not controlled by the environmental drivers except by clipping in SK, increased soil nitrogen decreased richness at all three sites.

Evenness

Evenness was less responsive to the environmental drivers than was richness (Table 5-3). Responses of evenness varied widely among sites, with no consistent effects between sites (Figure 5-3). At MB (Figure 5-3c), changes in evenness were related directly or indirectly to air temperature. Increased air temperature directly decreased evenness, while precipitation indirectly increased evenness via lower air temperature. In SK (Figure 5-3b), clipping intensity directly decreased evenness. There were no other effects of the environmental drivers on evenness at SK. There were no significant relationships for evenness at AB (Figure 5-3a).

Other relationships

In both richness (Figure 5-2) and evenness (Figure 5-3) models at all sites, greater precipitation increased soil moisture and decreased both air and soil temperatures. Increased air temperature also increased soil temperature, which decreased soil moisture. Clipping intensity had a consistent negative effect on litter, while litter correlated positively with shoot biomass, and decreased soil temperature.

At AB and MB but not SK, reduced precipitation decreased shoot biomass and clipping intensity increased air temperature, in both models of richness and evenness,. Root biomass increased evenness in SK only. Clipping intensity also increased nitrogen, but only in the SK evenness model.

Univariate analyses

Linear mixed models revealed a main negative effect of air temperature ($p=0.03$) and a clipping intensity by site interaction ($p=0.04$) on richness; clipping intensity decreased richness in AB and MB but not SK (Appendix D). There were no significant ($p<0.05$) treatment effects on evenness, although marginally significant effects of site ($p=0.10$) and interaction of air temperature by site ($p=0.08$) reflected a negative effect of air temperature on evenness at MB.

5.4 Discussion

Plant species richness was sensitive to environmental drivers associated with all three experimental treatments (climatic and clipping) across the sites. Moreover, details of the direct and indirect pathways influencing richness were also largely uniform across sites. Conversely, evenness showed more stability in response to environmental drivers, but also more variability in site-specific responses. Effects of air temperate on both biodiversity metrics were direct rather than indirect, while clipping intensity and precipitation generally acted indirectly. Univariate statistical results were consistent to those of the SEM models; however, as expected, the SEM approach revealed additional relationships (Grace, 2006).

The negative effect of air temperature on richness at all three sites is consistent with a number of grassland warming studies (Klein *et al.*, 2004, Yang *et al.*, 2011). Meta-analysis of tundra responses also reveals that warming generally decreases both richness and evenness (Walker *et al.*, 2006). Compared to tundra, biodiversity responses to warming in grassland have been understudied, and we know of no equivalent meta-analysis for grasslands. We found the relative strengths of effects due to air temperature on richness were equal to or greater than the effects of precipitation or clipping intensity, across all sites. The implications of biodiversity loss with warming are particularly important as there is less variability in the future forecast of global temperatures than for precipitation (IPCC, 2007), and unlike grazing, air temperature cannot be modified by resource managers.

As observed in our study, precipitation generally stimulates richness in grasslands (Yang *et al.*, 2011, Zavaleta *et al.*, 2003, Hoeppner *et al.*, 2012) . However, at SK, the driest site, the effect of precipitation on richness occurred only via air temperature, suggesting the community at site is largely resistant to low precipitation. Clipping intensity consistently decreased biodiversity at all sites. In contrast, many studies simulating grazing have shown that clipping increases plant diversity (Harmens *et al.*, 2004, Collins *et al.*, 1998, Klein *et al.*, 2004), with similar results found in field studies using grazing (Bai *et al.*, 2001). However, Vujnovic *et al.* (2002) studied the effects of grazing on Aspen Parkland grasslands across central AB, finding that diversity peaked under moderate grazing, results consistent with the Intermediate Disturbance Hypothesis (Connell, 1979). Our clipping treatments were sufficiently intense to reduce shoot biomass, a major control of richness at our sites, and thus decrease richness.

The relatively consistent responses and sensitivity of richness across the three sites contrasted with the inconsistent responses and stability of evenness. Indeed, evenness at AB did not respond to any of the variables examined. Decreases in evenness are often the first step towards species loss (Hoeppner *et al.*, 2012, Chapin *et al.*, 2000). However, the only examples of parallel decreases in both richness and evenness were at MB and involved air temperature. It is possible that increased air temperature caused dominance of one or more species at this site. As opposed to shifts in cover of certain dominant species affecting diversity (Engel *et al.*, 2009), change in richness, but not evenness, suggest that species' climatic niches (Pearson and Dawson 2003) did not correspond to the new climate conditions.

We also explicitly tested whether environmental drivers associated with the treatments acted directly or indirectly on biodiversity. Effects of air temperature on biodiversity were direct rather than indirect, inconsistent with expected impacts of warming on plants (Shaver *et al.*, 2000). Although the effects of warming on biodiversity have been attributed to indirect mechanisms (Yang *et al.*, 2011), without explicitly addressing these questions in the experimental design (De Valpine *et al.*, 2001) or statistical framework, it can be difficult to separate indirect from direct effects (Didham *et al.*, 2005). There are many direct pathways by which air temperature can affect plants, including through physiology, such as direct heat stress and changes to photosynthesis or growth, or life history, such as changes to germination, phenology, or reproductive output (Walther, 2004, Walck *et al.*, 2011). These effects could, in turn, lead to altered species interactions, another mechanism by which climate change impacts diversity (Suttle *et al.*, 2007).

Conversely, precipitation and clipping generally acted indirectly on biodiversity; the exception being a direct effect of clipping intensity on evenness at SK. Precipitation acted mainly indirectly through changes in shoot biomass. Compared to other factors, biomass has been shown to be a strong determinant of richness (Grace, 1999), which in our case was a positive relationship. Although highly debated, the relationship between productivity and species richness has often been described as unimodal, i.e. "hump-shaped", reversing from positive to negative as productivity increases (Mittelbach *et al.*, 2001, Waide *et al.*, 1999). As our precipitation treatment reduced rather than added resources, and because our grasslands were initially moderate-to-low in productivity (Gibson, 2009), this likely accounts for the positive relationship between shoot biomass and richness, consistent with expectations under lower productivity. At all sites, an indirect negative effect of precipitation on air temperature had a weak contribution to the positive effect of precipitation on biodiversity. This increase in air temperature with reduced precipitation could either be due to the experimental infrastructure (Beier *et al.*, 2012), or modified vegetation structure associated with reduced shoot biomass.

In the predominant pathway whereby clipping intensity indirectly decreased richness, decreases in litter due to clipping were associated with decreased shoot biomass and, finally, richness. Although not assessing richness, Willms *et al.* (1986) also found that litter increased biomass in native AB fescue prairie. Lamb (2008), also using SEM, showed that litter had a direct, negative effect on diversity in Aspen Parkland grasslands, but did not assess an equivalent indirect pathway via shoot biomass.

Clipping intensity also increased air temperature in AB and MB, leading to decreased richness, as discussed above. However, this relationship remained weaker than that involving litter and shoot biomass. The absence of any relationship between clipping intensity and air temperature at SK may be due to the low-statured vegetation and limited shoot biomass at that site, which would limit the extent of change to the plant canopy and associated air temperatures under clipping.

By identifying the indirect pathways by which these environmental drivers act, we can identify potential interacting mechanisms. In our study systems, richness decreased with warming, both as a result of the clipping treatment indirectly, and the warming treatment itself; in MB, these effects were further extended to include evenness. This suggests that clipping and warming may have combined effects on richness greater than what their single-factor responses would indicate. Indeed, Klein *et al.* (2004) found that clipping and warming had interactive effects on richness, although clipping weakened, rather than exacerbated, the effect of warming on richness. In addition, both reduced precipitation and clipping intensity decreased richness via shoot biomass reductions in MB and AB, and thus have the potential to interact to exacerbate species loss. In no model did we found an instance of effects operating in opposing directions so that they might offset each other.

What are the implications of biodiversity loss for grasslands? Craine *et al.* (2011) showed that despite projected species loss with climate change, without congruent loss of functional traits, ecosystem functioning need not be impacted. Although a host of ecosystem functions are dependent on or correlated with species richness (Chapin *et al.*, 1997), to make accurate predictions we need to understand which individual species are shifting (including disappearing and appearing) and relate this to species function (Sandel *et al.*, 2010). For example, invasive species are expected to be well-poised to take advantage of climate change, but an increase in diversity due to invasive species is likely not desired (Dukes *et al.*, 1999).

In this investigation we demonstrate negative effects of the environmental drivers associated with reduced precipitation, warming, and clipping on species richness at three grassland sites. Not only was the effect of air temperature on richness consistent in direction and mechanism across sites, but this finding is in line with concerns about declining biodiversity under climate change (Bellard *et al.*, 2012). There was, however, variability in site responses to evenness, suggesting that systems vary by response variable in terms of stability. We suggest that either a meta-analytic or large-scale experimental approach, such as the Coordinated Distributed Experiments described in Fraser (2012), be used to further evaluate whether these biodiversity responses, especially to warming, are broadly consistent across a certain grassland type, and if not, to identify characteristics of systems resistant to climate change.

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Table 5-1. Summary of derivation of variables in structural equation models of precipitation, air temperature, and clipping intensity on richness and evenness.

Type of variable	Variable name	Source	Units	Sampling period
Environmental drivers	Precipitation	Average of two rain gauges at each site (in MB, based on local weather station)	mm	Mid-May until late July
	Air temperature	Average of low and high data loggers	°C	Mid-May until late July
	Clipping intensity	Clipped biomass per plot divided by average non-clipped shoot biomass for that temperature and precipitation treatment	%	Clipped biomass (June); Shoot biomass (late July)
Intermediary environmental variables	Soil moisture	Average of shallow and deep soil probes	% VWC	Mid-May until late July
	Soil temperature	Average of shallow and deep soil probes (04:00 AM – 06:00 AM only)	°C	Mid-May until late July
	Soil nitrogen	PRS probes	mg total nitrogen/10 cm² ion-exchange membrane surface area/time of burial	Late June – early September
Intermediary vegetation variables	Litter biomass	10 cm by 100 cm vegetation strip	g/m^2	Late July
	Shoot biomass	10 cm by 100 cm vegetation strip (non-clipped plots), and 50 cm by 50 cm sampling plot (clipped plots)	g/m^2	Late July (non-clipped plots); Late July & June (clipped plots)
	Root biomass	5 cm diameter, 20 cm deep core	g/m^2	Late July
Response variables	Richness	% cover in 50 cm by 50 cm sampling plot	# species/0.25m^2	Late July
	Evenness	% cover in 50 cm by 50 cm sampling plot	No unit (index)	Late July

Table 5-2. Direct, indirect and total effects of precipitation, air temperature, and clipping intensity on plant species richness at AB, SK, and MB, based on standardized values of statistically significant (p<0.05) paths. Only paths originating with the environmental drivers and terminating with richness included here.

Environmental Driver	Effect	AB	MB	SK
Precipitation	Direct	—	—	—
	Indirect	Precipitation → Air temperature → Richness (0.04)	Precipitation →Air temperature →Richness (0.09)	Precipitation → Air temperature → Richness (0.06)
		Precipitation → Shoot biomass → Richness (0.10)	Precipitation →Shoot biomass → Richness (0.19)	
	Total	0.14	0.28	0.06
Air temperature	Direct	Air temperature → Richness (-0.37)	Air temperature → Richness (-0.27)	Air temperature → Richness (-0.27)
	Indirect	—	—	—
	Total	-0.37	-0.27	-0.27
Clipping intensity	Direct	—	—	—
	Indirect	Clipping intensity → Litter → Shoot biomass → Richness (-0.07)	Clipping intensity → Litter → Shoot biomass → Richness (-0.12)	Clipping intensity → Litter → Shoot biomass → Richness (-0.12)
		Clipping intensity → Air temperature → Richness (-0.07)	Clipping intensity → Air temperature → Richness (-0.09)	
	Total	-0.15	-0.21	-0.12

Table 5-3. Direct, indirect and total effects of precipitation, air temperature, and clipping intensity on species evenness at SK and MB based on standardized values of statistically significant (p<0.05) paths. Only paths originating with the environmental drivers and terminating with evenness are included here.

Environmental drivers	Effect	AB	MB	SK
Precipitation	Direct	—	—	—
	Indirect		Precipitation → Air temperature → Evenness (0.175)	—
	Total	—	0.175	—
Air temperature	Direct	—	Air temperature → Evenness (-0.555)	—
	Indirect		—	—
	Total	—	-0.555	—
Clipping intensity	Direct	—	—	Clipping intensity → Evenness (-0.482)
	Indirect	—	Air temperature → Evenness (-0.185)	—
	Total	—	-0.185	-0.482

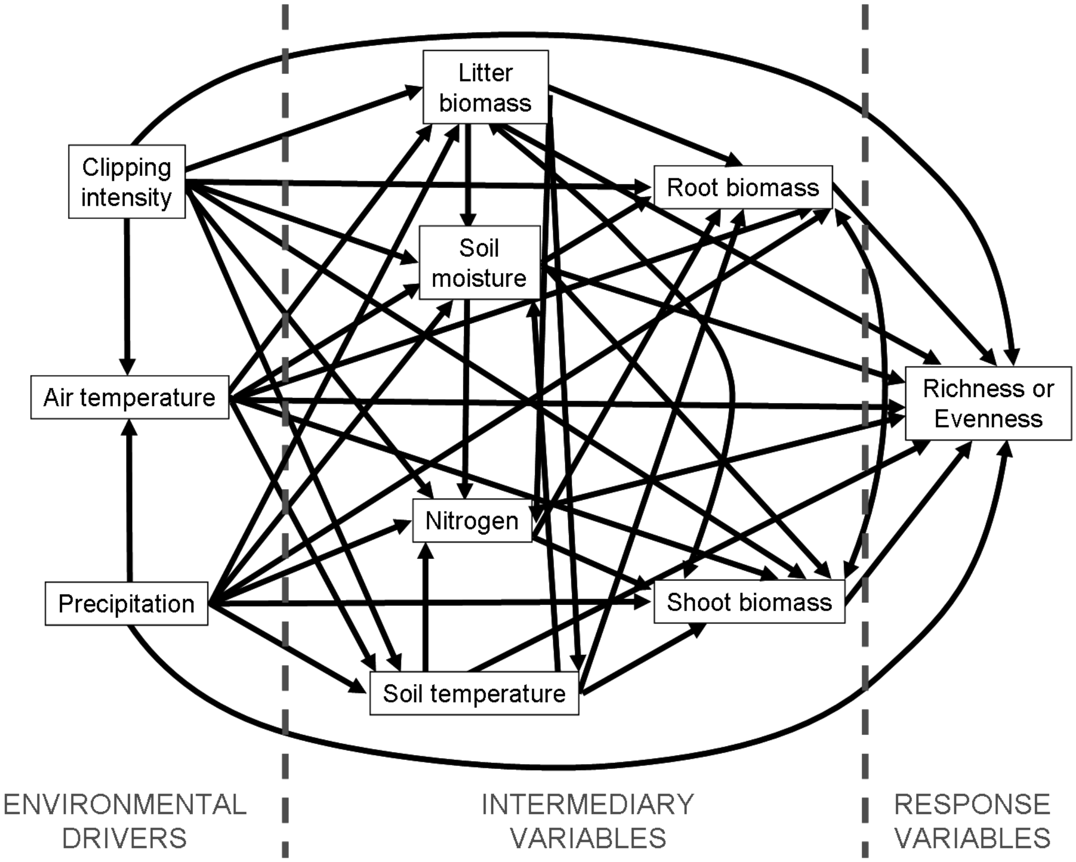
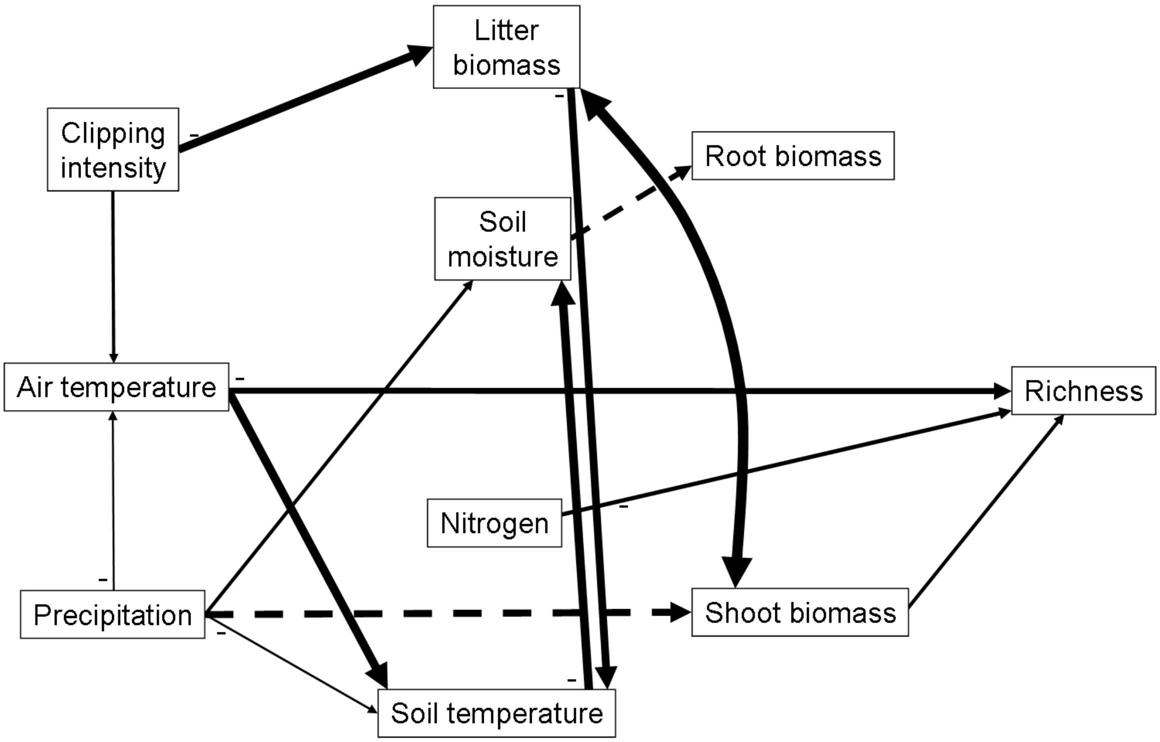
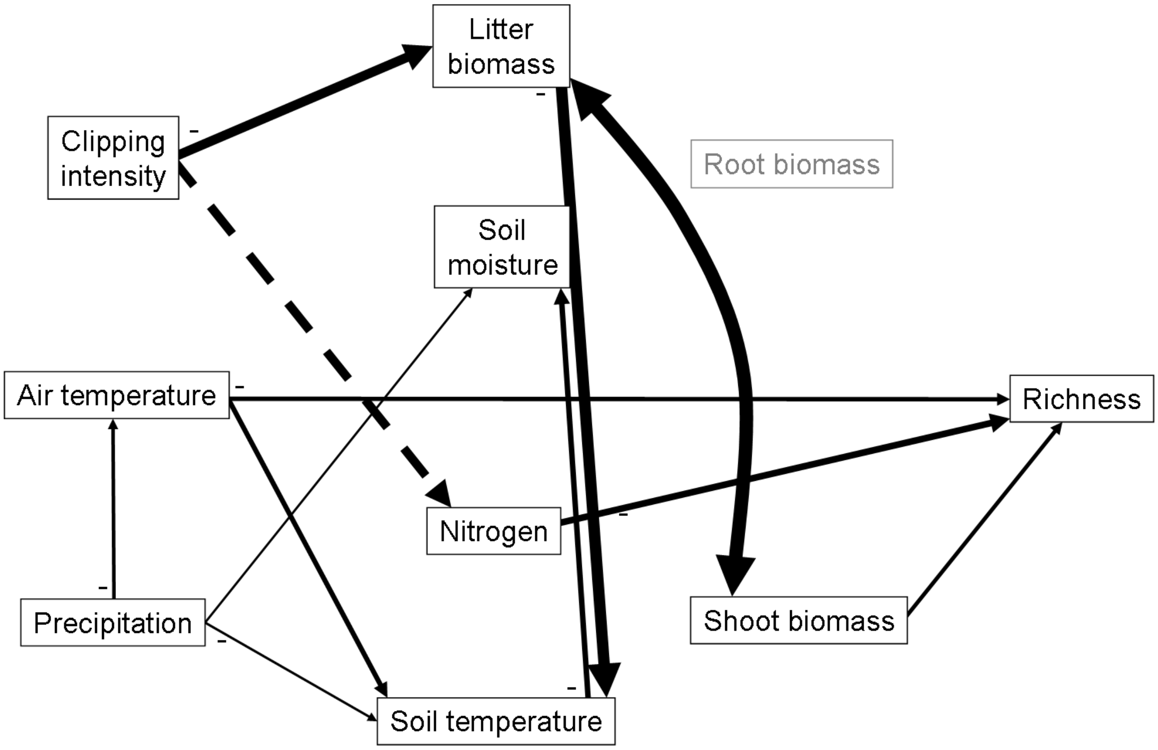


Figure 5-1. Arrows indicate a-priori hypothesized relationships tested in initial multi-group structural equation model of the effects of environmental variables on intermediary environmental and vegetation variables, and finally, the response variables plant species richness or evenness. Single-headed arrows represent causal relationships between variables, and double-head arrows indicate co-varying variables.

a) AB


Litter biomass
Clipping intensity
Root biomass
Soil moisture
Air temperature
Richness
Nitrogen
Precipitation
Shoot biomass
Soil temperature
-

b) SK


Litter biomass
Clipping intensity
Root biomass
Soil moisture
Air temperature
Richness
Nitrogen
Precipitation
Shoot biomass
Soil temperature
-

c) MB

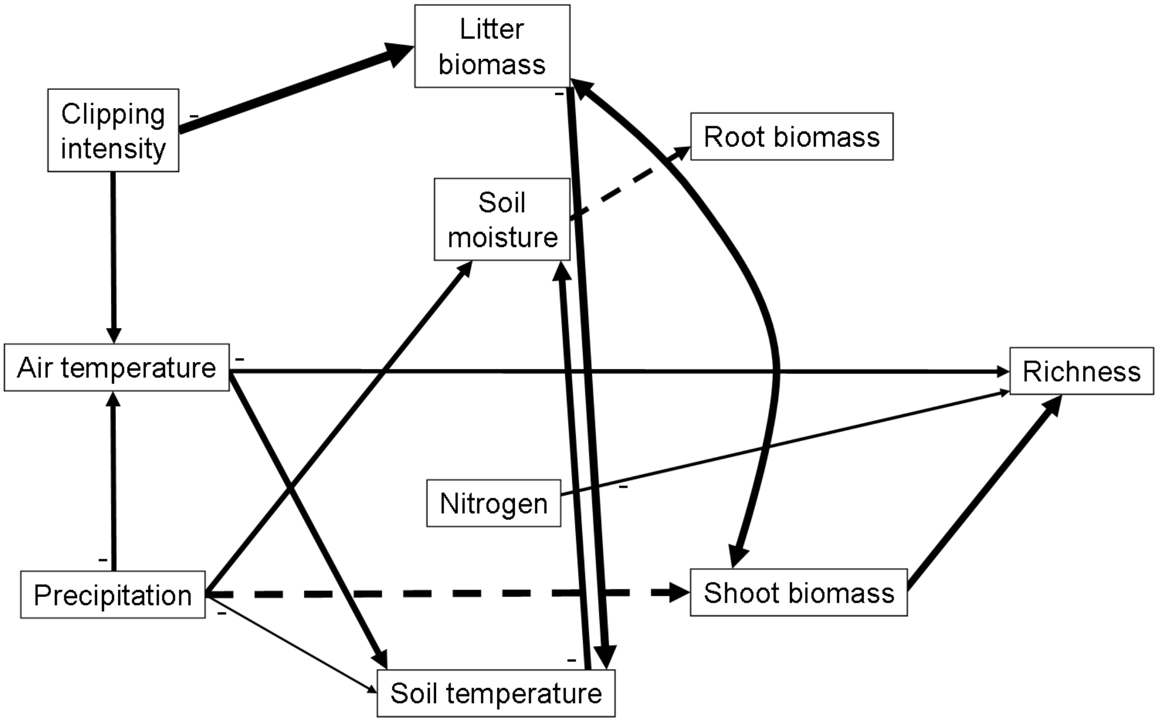
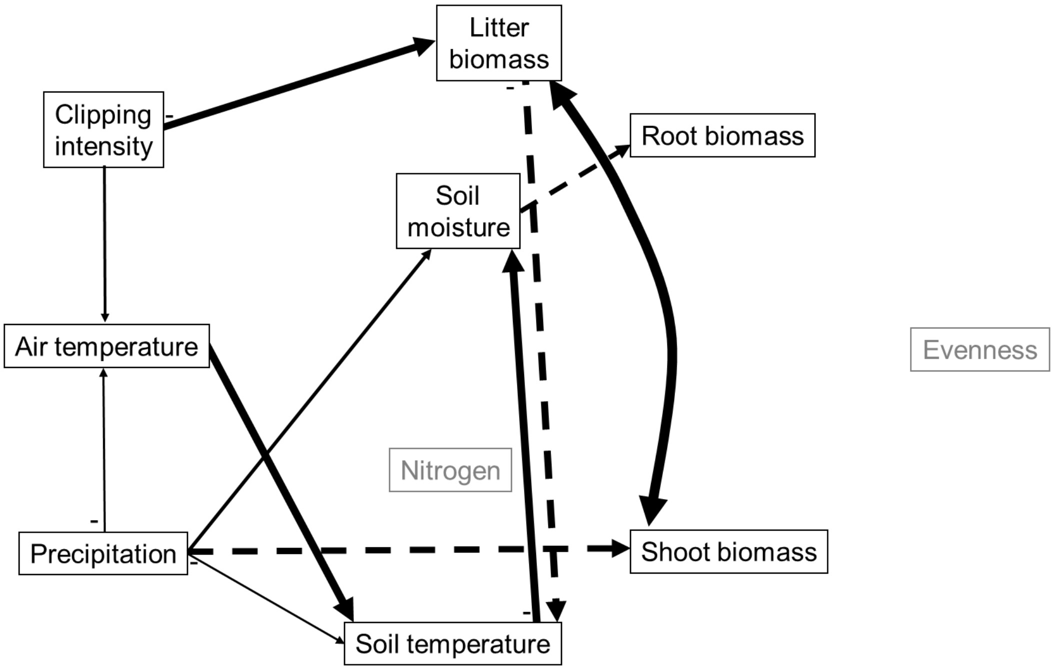
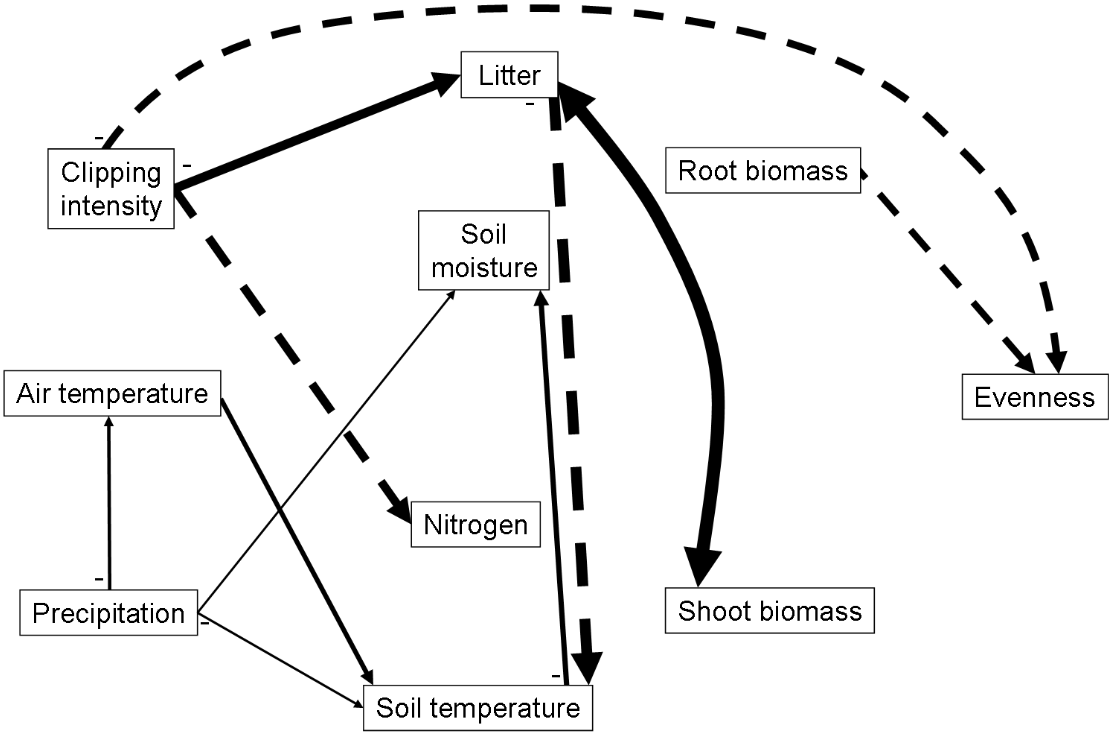


Figure 5-2. Arrows represent significant ($p < 0.05$) paths in a fitted multi-group structural equation model depicting the effects of environmental drivers on intermediary environmental and biological variables, and ultimately species richness for (a) AB, (b) SK, and (c) MB. Bold arrows indicate paths consistent across all sites; dashed arrows indicate paths that varied among sites. Single-headed arrows represent causal relationships, while double-headed arrows represent co-varying variables. Arrow width corresponds to standardized path strength. A minus sign ("-") indicates the relationship is negative. Variables lacking significant relationships with other variables in the model are presented in grey.

a) AB


Litter biomass
Clipping intensity
Root biomass
Soil moisture
Air temperature
Evenness
Nitrogen
Precipitation
Shoot biomass
Soil temperature
-
-
-
-
-

b) SK


Litter
Clipping intensity
Root biomass
Soil moisture
Air temperature
Evenness
Nitrogen
Precipitation
Shoot biomass
Soil temperature
-
-
-
-
-
-

c) MB

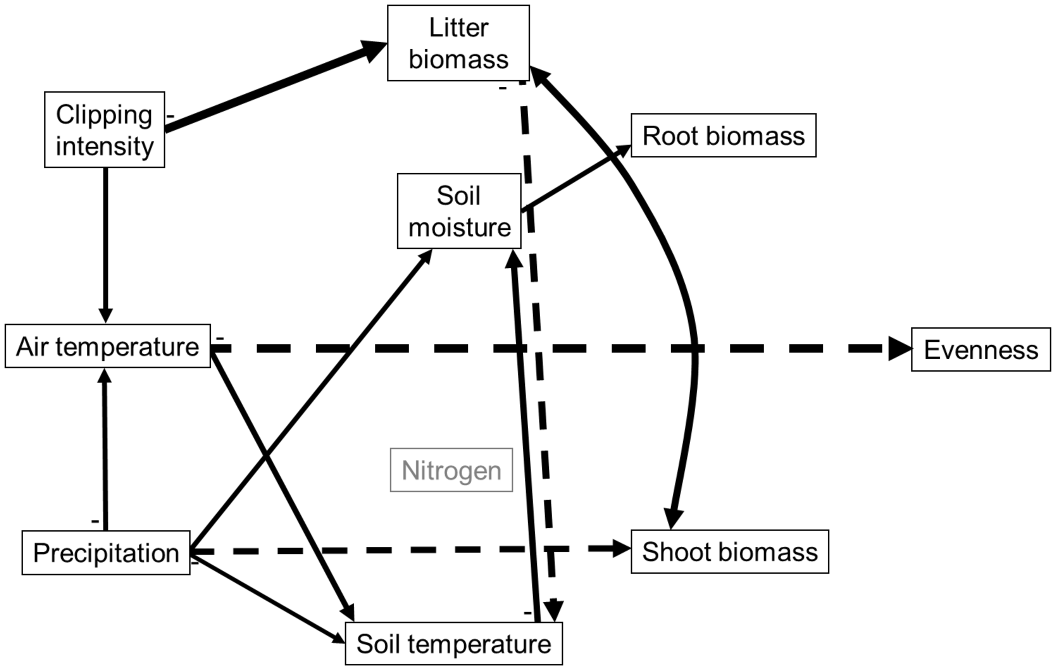


Figure 5-3. Arrows represent significant ($p < 0.05$) paths in a fitted multi-group structural equation model depicting the effects of environmental drivers on intermediary environmental and biological variables, and finally species evenness, for (a) AB, (b) SK, and (c) MB. Bold arrows indicates paths consistent across all sites; dashed arrows indicate paths that varied by site. Single-headed arrows represent causal relationships, while double-headed arrows represent co-varying variables. Arrow width corresponds to standardized path strength. A minus sign (“-“) indicates the relationship is negative. Variables lacking significant relationships with variables in the model are presented in grey.

6. CLIMATE CHANGE EXPERIMENTS IN TEMPERATE GRASSLANDS: SYNTHESIS AND FUTURE DIRECTIONS[2]

6.1 Introduction

Temperate grasslands are important as major components of terrestrial land-cover and biodiversity and for the ecosystem services they provide, including livestock forage and carbon sequestration. Not only is the extent of this ecosystem decreasing as it is converted for development and farmland, but remaining grasslands are increasingly under pressure from climate change and increased grazing with larger livestock herds. The immediate need to understand the responses of grasslands to these changes, to ensure food supplies and to mitigate future climate change through carbon sequestration, necessitate a global, synthesized approach.

Over the last century, precipitation patterns have changed and average global temperatures have increased, as has the occurrence of extreme weather events; these climate change trends are expected to continue (Solomon, 2007). Grasslands are dynamic systems that are responsive to several dominant processes: grazing, climate, and fire. Thus, climate change, especially in conjunction with increased grazing pressure, might be expected to have long-term impacts on sustainability of these ecosystems. Already, observed responses of terrestrial ecosystems to climate change include changes to plant community structure (Parmesan *et al.*, 2003) and productivity (Nemani *et al.*, 2003).

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[2] A version of this chapter has been published: White SR, Carlyle CN, Fraser LH, and Cahill JF. 2012. *Biology Letters* 13(2):484-487.

Experiments manipulating precipitation and temperature have been conducted in temperate grasslands to explore responses and mechanisms of change. The next step remains to evaluate whether these experiments can be translated into a broad understanding of how temperate grasslands will respond to these pressures.

It was with the aim of discussing and synthesizing climate change experiments in temperate grasslands that the session 'Climate change experiments in temperate grasslands' was convened on 20 and 21 June 2011 in Lyon, France, during the 54th Annual Symposium of the International Association for Vegetation Science (IAVS). The session attracted many of the leading researchers in the field, with 19 oral presentations and poster contributions from 14 field experiments (see Appendix E for complete list of contributions). Presenters came from six European and North American countries and discussed research from three continents. Owing to high participation, the session had to be moved to a larger lecture theatre than originally planned. Following the session, we recognized the call for a more casual and interactive forum for discussion, and held a well-attended additional workshop.

6.2 Experimental design

Although all research addressed temperate grassland responses to climate change, a variety of methodologies was used. Manipulations of either average temperature or precipitation, based on regional climate model projections, were applied in 10 of the 14 experiments described (Table 6-1). Warming was achieved by a variety of methods: open-top chambers (OTCs), open-sided chambers

(OSCs), overhead infrared heaters and heating cables, which all differ in their specific and unintended effects. Precipitation was decreased using either permanent or automatic rain-out shelters, and increased via manual water addition. These precipitation methods also were varied, limiting our ability to separate treatment effects from those of inconsistent methodology. For example, some of the rain-out shelter designs included controls with sham structures, while others did not.

Increased frequencies of heat waves, droughts and heavy precipitation events are associated with climate change; suitably, four experiments imposed extreme weather events (Table 6-1). Only once was an extreme event (heat waves) investigated in combination with manipulation of average conditions (decreased precipitation). Although the different ways in which temperature and precipitation may change to affect grasslands were well represented, it was rare that more than two studies used similar methodology, possibly limiting the generality of any finding.

There is a potential for important interactions between temperature, precipitation and other treatments (Wu *et al.*, 2011). Six experiments included interactions between any temperature or precipitation treatment. A number of additional factors were also manipulated in conjunction with temperature or precipitation, especially those related to grazing. Grazing by animals was sometimes logistically precluded; in these cases, mowing, defoliation or clipping was used as a proxy for grazing. Other interacting factors included nitrogen, removal of subordinate species and CO_2 addition. Most of the treatments were applied for a relatively short (1–3 years) duration. A notable exception was the Buxton experiment reported by Jason Fridley (Syracuse University), which has been running for almost two decades.

In addition to variability in treatments applied, comparison among experiments is also complicated by the lack of consistency in response variables measured (Table 6-1). Although the majority of the presentations discussed some aspect of above-ground productivity, less than half monitored plant community composition or species diversity. Despite the sensitivity of below-ground responses to climate change (Pritchard, 2011), and their importance in ecosystem responses (Wardle *et al.*, 2004), the experiments focused on above-ground responses. Within individual experiments, a breadth of response variables were monitored, including phenology, plant physiology, nutrient cycling, focal species, soil properties and micro-organisms. Although this provides insight into which grassland properties can be sensitive to climate change, because many of these response variables were included in only one or two experiments, conclusions can only be site-specific.

6.3 Analytical approaches

Traditional General Linear Model (GLM)-type analyses were commonly used and are important in determining responses to treatments. However, researchers also used a diversity of analytical techniques to identify mechanisms of response. Instead of simply reporting how flowering phenology responded to treatments, Elsa Cleland (University of California- San Diego) differentiated changes between the contributions of inter-specific plasticity and intra-specific abundances. Likewise, Pierre Mariotte (Ecole Polytechnique Fédérale de Lausanne) explored the involvement of subordinate versus dominant species in drought resistance. Both James F. Cahill (University of Alberta) and Anke Jentsch (University of Bayreuth) explored the contributions of multiple mechanisms to observed responses. Cahill accomplished this through a systems-level analysis, and Jentsch extensively surveyed plant physiological processes, linking them to ecosystem productivity.

Methods such as these provide insight into mechanisms that would be concealed with traditional methods. However, because there was little overlap in innovative analytical approaches, the opportunity to identify mechanisms at play in multiple experiments may have been missed.

6.4 Understanding responses

Results from these experiments were largely mixed and complex; thus, a broad understanding of temperate grassland response to climate change remains elusive. In some cases, grasslands were resistant to changes in both temperature and precipitation. Generally, most grassland response variables were more sensitive to changes in precipitation than temperature, though exceptions to this abounded, especially when extreme heat waves, rather than smaller changes in average temperature, were imposed. There were also a number of experiments which manipulated only temperature or precipitation, limiting the robustness of this conclusion. Further complicating the results, a number of responses were dependent upon interactions between the treatments. This was true particularly for temperature and precipitation, as well as CO_2 in combination with other treatments, but not found for nitrogen. There were few interactions with the management treatments (grazing, clipping, etc.), although main effects of management treatments were usually, though not always, substantial.

The direction of productivity response to both precipitation and warming was highly variable, switching between and within sites, and over the duration of the experiments. Lauchlan Fraser (Thompson Rivers University) found that effects of decreased water on plant biomass surprisingly switched in direction based on the productivity gradient between experimental sites. To provide a more in-depth understanding of precipitation responses, Scott Collins (University of New Mexico) and Alan Knapp (Colorado State University) manipulated only size of rainfall events, rather than total precipitation, under different hydrological regimes.

6.5 Future directions

From the many experiments that have been performed, it is clear that grasslands are responsive to climate change in general, although the magnitude and direction of response varies highly with both treatment and site. The research discussed represented a great variety of both methodological and analytical approaches, although perhaps at the expense of cohesiveness. Thus, we still remain largely unable to identify any site-specific conditions or mechanisms that may lead to predictable responses.

To identify general patterns and mechanisms of response, the workshop attendees identified the importance of a broader, coordinated experimental approach to determine temperate grassland responses to climate change. A meta-experiment approach will be pursued, rather than meta-analysis, as the current disparate methodology prohibits an informative meta-analysis. A standardized treatment methodology and sampling protocol with minimal financial cost will be developed, potentially modelled-off of the Nutrient Network (Firn *et al.*, 2011) Or International Tundra Experiment (Arft *et al.*, 1999), in which there is a small investment per investigator, but large scientific returns. The workshop attendees briefly discussed the treatments that would be used, probably precipitation removal and addition, and discussed costs, but identified the need to work on a synthesis paper and conduct a further workshop to determine details.

The workshop attendees also identified the need for increased research in overlooked geographical areas, and the critical need for funding opportunities for long-term research. Research was predominantly in North America or Europe, with the notable exception of Brenda Casper (University of Pennsylvania) in Mongolia. Although containing sizable temperate grasslands, Africa, Oceania and South America were missing from our session and have previously been identified as areas lacking research into the effects of climate change (Wu *et al.*, 2011). Similarly, though long-term studies have been identified as important for identifying and understanding responses (Rustad *et al.*, 2001), the majority of research presented was from short-term studies.

A collaboration based on a set of globally dispersed, inexpensive experiments with consistent methodology will provide the data needed to understand responses of temperate grassland to climate change. The high level of support and interest in future collaboration illustrates the importance of international meetings in fostering communication of similar, yet geographically dispersed, research. The session was a very successful first forum for discussion, and an impetus to develop experiments where this dialogue can be continued quantitatively.

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Table 6-1. Experiments presented at the session “Climate change experiments in temperate grasslands” at the 2011 IAVS symposium, by lead author, location, treatments, and response variables. Treatments are either described by methodology type or, in the case of precipitation, whether the resource was increased or decreased, as indicated by arrows. Double-headed arrows indicate precipitation was both increased and decreased. A “-“ indicates the treatment or response variable was not included in the experiment. Aboveground and belowground productivity is referred to as “AG” and “BG”.

Lead author	**Location**	**Treatments**				**Response variables**			
		Warming	**Precipitation**	**Management**	**Others**	**Productivity**	**Community**	**Focal species**	**Others**
Fraser	British Columbia, Canada	OTCs	↕	Clipping	Productivity gradient	-	Composition	-	Community function
Cahill	Alberta, Canada	OTCs	↕	Defoliation	-	AG/BG	Composition diversity	-	Nutrient cycling, soil arthropds, etc.
Collins	Arid grasslands, USA	-	Frequency	Grazing	Nitrogen addition	AG	-	Function and physiology	-
Knapp	Central plains, USA	-	Timing	-	Arid and mesic sites	AG	-	-	-
Smith	Tallgrass prairie, USA	Heat wave	↓	-	-	AG	-	Physiology and biomass	-
Fridley	Calcareous grassland, England	Cables	↕	-	-	-	Composition	-	Species introduction
Cleland	California, USA	Overhead heaters	↑	-	CO_2, Nitrogen addition	-	Composition	Flower phenology	Decomposition
Casper	Mongolian Steppe	OTCs	↑	Grazing	Slope	-	-	-	Flower phenology and production
Henry	Old field, Canada	Overhead heaters	-	-	Nitrogen addition, Winter warming	AG	-	-	Soil responses
Soussana	Montpellier, France	Yes	↓	-	CO_2, Mesic and alpine sites	AG	Functional group	-	Leaf traits
Jentsch	Central Europe	-	Drought events	-	-	AG/BG	Stability	-	Physiological processes, nutrient cycling, phenology, etc.
Maalouf	Calcareous grassland, Europe	-	Drought	Mowing	-	AG	Richness	Transplant survival	Litter decay
Mariotte	Alpine grassland, Europe	-	↓	-	Subordinate species removal	AG	-	-	Litter decomposition
Dutoit	Mediterranean steppe	-	↓	Grazing	-	-	-	-	Tree colonization

7. GENERAL DISCUSSION AND CONCLUSIONS

7.1 Review and synthesis

In this thesis I asked how productivity, grazing resources, and biodiversity in northern temperate grassland respond to the main and interactive effects of warming, reduced precipitation, and clipping. Below, I summarize the main research questions/objectives addressed in this thesis and present a short synthesis of the findings.

Research questions/objectives

Chapter 2: How does *total plant biomass, shoot biomass,* and *root biomass* respond to reduced precipitation, warming, and clipping?

Chapter 3*:* What is the influence of growing conditions (warming and altered precipitation) on *accumulated herbage production*, *regrowth biomass,* and *herbage quality,* including (if applicable) contributions from graminoids and forbs, and does this vary with clipping intensity?

Chapter 4: What are the main, interactive, and relative effects of reduced precipitation, warming, and clipping on *similarity between aboveground vegetation and seed bank composition* at a northern temperate grassland site?

Chapter 5: Determine *resistance versus responsiveness, direct versus indirect responses,* and *general versus site-specific responses of biodiversity* in response to environmental drivers associated with reduced precipitation, warming, and clipping

Chapter 6*:* What are *current approaches* and *future directions* of experimental climate change research in temperate grasslands?

Overall, aboveground productivity at these sites tended to decrease with warming, although the magnitude of this response was limited compared to effects of reduced precipitation and clipping, both of which strongly reduced shoot biomass. However, total plant biomass was generally unresponsive to all three treatments. Stability in total plant biomass in response to experimental climate change and clipping, enabled by shifts in root: shoot ratio, suggests overall resistance of ecosystem function within these northern grasslands. Despite this stability, decreases in herbage biomass, regrowth potential, and herbage quality under reduced precipitation and warming, as observed in Chapter 3, will have ramifications for agricultural producers.

The seed bank can provide a diverse repository of propagules influencing composition under future climate conditions if seedlings and adults are affected by conditions aboveground. Clipping increased similarity between the seed bank and aboveground vegetation, suggesting the seed bank may help maintain species composition under grazing. However, reduced precipitation led to a community more dissimilar to the seed bank; thus, the seed bank did not serve as a repository under these conditions. Despite no shift in similarity between the seed bank and aboveground vegetation in response to warming (Chapter 4), we observed a decline in aboveground vegetation species richness with increased air temperature at all sites (Chapter 5). A concomitant decline in evenness was not observed. On the whole, our results show complicated diversity responses to climate change, with overall negative responses of richness to environmental factors associated with the three treatments.

7.2 Implications

Producers who derive their livelihood from cattle produced on rangeland will be among those directly impacted by climate change (Izaurralde *et al.*, 2011). A full economic analysis is beyond the scope of this thesis, although my data might be well-suited to contribute to such a project. Even seemingly small changes in herbage production, and certainly the changes I observed in my experiment, might require managers to adjust animal numbers, purchase supplemental feed, alter grazing schedules, or produce livestock with lower nutrients requirements, all actions with economic repercussions (Craine *et al.*, 2010, Morgan, 2008). Although rangeland managers are accustomed to operating in variable conditions, the extent of projected climate change implies managers will be challenged at unprecedented levels in the future (Brown, 2008). Not only does research have to be applicable at the scale of management, but managers have to be responsive (Brown, 2008), especially in the face of environmental uncertainty (Campbell *et al.*, 2000).

Management can be used as a tool to mitigate grassland responses to environmental conditions (Klein *et al.*, 2004, Butof *et al.*, 2012) or, alternatively, can exacerbate these responses (Frank, 2007). Although analysis of direct and indirect responses using SEM did reveal some mechanisms by which clipping could interact with the climatic treatments, I did not identify any consistent interactions using conventional analyses, suggesting little on which to base future management. Using five replicates is typical for these types of experiments. Although greater replication might have revealed additional significant interactions, the five replicates were sufficient to identify strong, and arguably biologically relevant, main and interactive responses.

Negative effects of climate change on both biodiversity and productivity have implications for grassland conservation and the associated management of rare and threatened fauna that rely on those habitats. Only 4.6% of temperate grasslands are protected, resulting in a higher ratio of habitat converted to habitat protected than that of any other terrestrial biome (Hoekstra *et al.*, 2005). Grasslands are also highly threatened by invasive species (Gibson, 2009).

Another implication of changes to productivity is feedback into the carbon cycle (Lashof *et al.*, 1997). Climatic warming is predicted to increase release of terrestrial CO_2 through decomposition, leading to positive feedback and further climate change (Cox *et al.*, 2000). Although increased productivity is identified as a potential response that could contribute negative feedback and therefore mitigate climate change (Luo, 2007), my results suggest that any productivity changes under warming and reduced precipitation at these sites would translate into increased, rather than decreased, climate warming.

7.3 Caveats

Like our experiment, the majority of climate change studies are short term (1-5 years). There are ample data to suggest that extrapolating short-term responses to the long-term, which are ultimately of greater interest, can be misleading (Rustad, 2001, Elmendorf *et al.*, 2012). For species composition, response time is known to increase from the individual species level, through species reordering, and ultimately to species immigration (Smith *et al.*, 2009); indeed, several years of warming are required before compositional shifts are expected (Shaver *et al.*, 2000). Elmendorf *et al.* (2012) found that tundra vegetation responses to warming were highly dependent on experimental duration. Alternatively, Grime *et al.* (2008), working in grassland, observed short-term but minor responses to altered precipitation, and resistance to warming; these results did not exacerbate over time. Although I observed short-term responses to warming, altered precipitation, and clipping, I cannot conclude whether these responses are transient, permanent, or apt to fluctuate.

Also integral to informative climate change experiments is choosing appropriate manipulations of environmental conditions. Assessing responses to both reduced and added precipitation as I did, albeit at only one site, is one way to deal with unknown precipitation projections. Biological processes can have nonlinear responses to climatic factors, which can be observed only with experiments testing multiple levels of single factors (Hoeppner *et al.*, 2012, Bradford *et al.*, 2012). By using only two levels of temperature, and two levels of precipitation at SK and MB, I may have missed an opportunity to discover nonlinear effects of these factors. Another component of climate change lacking in my experiment is extreme weather events; ecological responses to these events are generally understudied and poorly understood (Smith, 2011). As well, studies show that variation in precipitation can be more important than overall precipitation amount in altering ecosystem processes (Knapp *et al.*, 2008).

There are also general limitations to the manipulative experimental approach used in my experiment. Caution needs to be taken when translating experiments conducted at the small plot scale into whole ecosystem responses (Rustad *et al.*, 2001, Norby *et al.*, 2004). As well, a characteristic of these experiments is the unintended and unknown consequences of treatment infrastructure influencing an array of factors, such as shading, spectral composition, and wind (Beier *et al.*, 2012, Shaver *et al.*, 2000). There is an advantage to explicitly addressing unintended effects of these treatments as we did in Chapter 5, but many more response variables would be needed to capture all potential effects of infrastructure. Additionally, trampling by researchers and intensive sampling of relatively small plots (Cahill *et al.*, 2001, Beier *et al.*, 2012) can further influence results. Efforts to reduce these effects in my experiment were made by the use of elevated sampling platforms and a stratified sampling design, combined with a permanent sampling plot wherein disturbance was minimized.

In particular, using clipping as a substitute for grazing artificially limited associated effects of the latter. Clipping essentially imposes isolated direct and indirect effects of biomass removal, while the effects of true herbivory are much more extensive, including trampling, deposition of nutrients, species-selection, and patch grazing (Hobbs, 1996).

7.4 Future directions

Due to their daunting complexity, experiments with three interacting treatments are still somewhat rare (Rustad, 2008), and four interacting factors even rarer (but see Shaw *et al.*, 2002). Nevertheless, there are many global change factors in addition to temperature and precipitation that impact grasslands. Nitrogen deposition is a largely overlooked global change factor (Vitousek, 1994), and nitrogen is known to limit production in northern temperate grassland (Lamb *et al.*, 2007), but is lacking as a treatment in my experiment. Increased CO_2 concentrations are another global change factor that can affect systems independently or modify responses to other factors (Norby *et al.*, 2004). Higher CO_2 concentrations are expected to favour C_3 species (Chamaille-Jammes *et al.*, 2010), of which my three sites are mainly comprised of, but it is unknown how the few C_4 species at these sites will respond. Grassland systems are also known to be responsive to fire (Vermeire *et al*., 2011) but this factor is rarely included in experiments.

As all these factors simultaneously affect grassland systems, manipulative experiments integrating multiple factors are valuable in understanding responses to global change. Gradient studies can also be used to investigate potential interactions when additional factors are not feasible (Dunne *et al.*, 2004), and I did incorporate some of this in my approach as the study sites used here encompass some variability in mean annual temperature and precipitation.

Developing an understanding of the effects of climate on grasslands will involve identifying attributes of resistant versus sensitive systems. Although grasslands can be resistant to warming in terms of ecosystem function or biodiversity (Grime *et al.*, 2008), both were somewhat sensitive to warming at all three sites. Exposure to past extreme conditions can make a system less sensitive to altered climate (Pollock, 1990, Levitt, 1980). However, a number of other hypotheses have been proposed to explain resistance to climate change. Grime *et al.* (2000) found large differences between two grasslands in resistance to warming and reduced precipitation, and attributed this to successional age and fertility: the older and less fertile community being more resistant. Another mechanism providing resilience for biodiversity in response to environmental change is regeneration from the soil seed bank (Harper, 1977).

Specifically in response to precipitation, drier sites within a biome might be expected to be water-limited, and thus more sensitive to water availability (Cherwin *et al.*, 2012). However, I found the driest site, SK, was most resistant to the effects of reduced precipitation. New research suggests responses to precipitation are more nuanced, with the effect of altered precipitation depending on whether rainfall events are characterized by small or large rainfall events (Cherwin *et al.*, 2012). Clearly, ecologists are only beginning to understand what drives resistance or sensitivity to climatic changes.

7.5 Conclusion

With so much information, and so little synthesis, where do ecologists interested in climate change go from here? Conducting more geographically distributed experiments using consistent methodology, such as the experiments that form the base for this thesis, and at a larger scale (Fraser, 2012), is a move in the right direction (Chapter 6). Nevertheless, even synthesis of years of data at multiple climate change experiments seems to return to a common theme: responses are variable (Elmendorf *et al.*, 2012). The science of ecology, however, has changed dramatically in the last thirty years, and I believe these changes will continue, partially to address the need to better understand human impacts on natural systems. Approaches involving system-based understanding, such as those advocated by Grace (2006), have potential to be pivotal in developing ecological theory in the context of climate change. If it is true that community dynamics and underlying processes differ markedly from system to system (Lawton, 1999), developing a comprehensive understanding of biological responses to climate change will be a daunting challenge, although a worthy one.

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8. APPENDIX A: Supplemental material for Chapter 2

Figure A-1. Rain-out shelter design

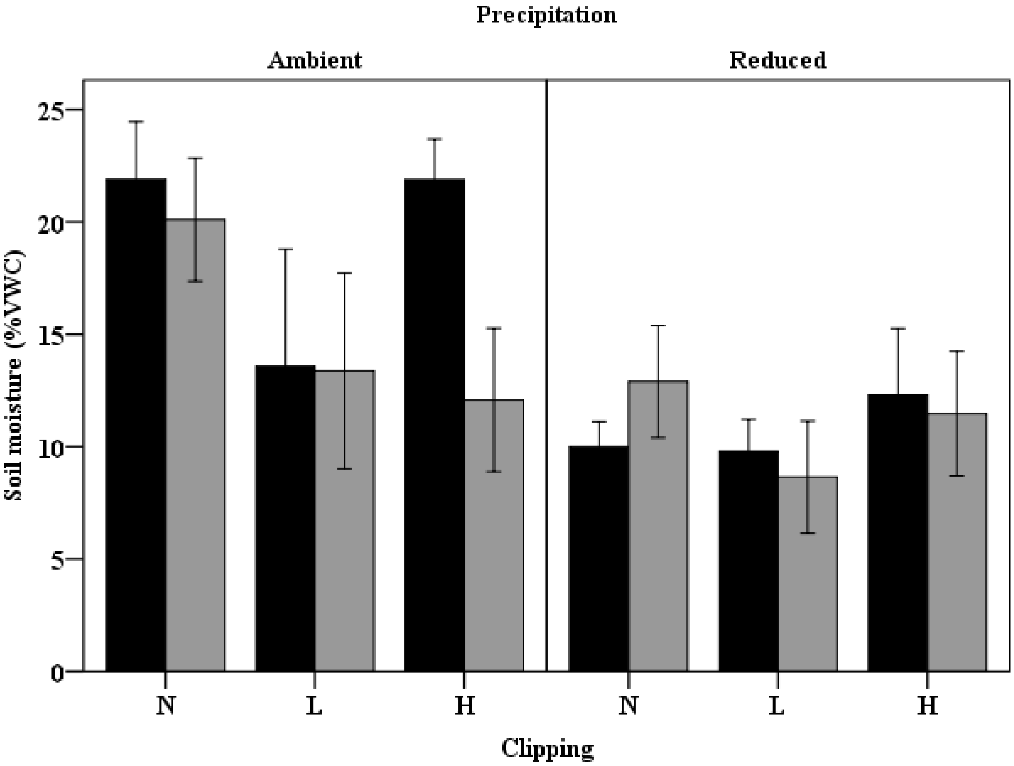


Figure A-2. Effects of precipitation (ambient and reduced), warming (black bars, control; grey bars, warmed), and clipping (no clipping, “N”; low intensity, “L”; high intensity, “H”) across all sites on 2009 soil moisture. Error bars represent ±1SE.

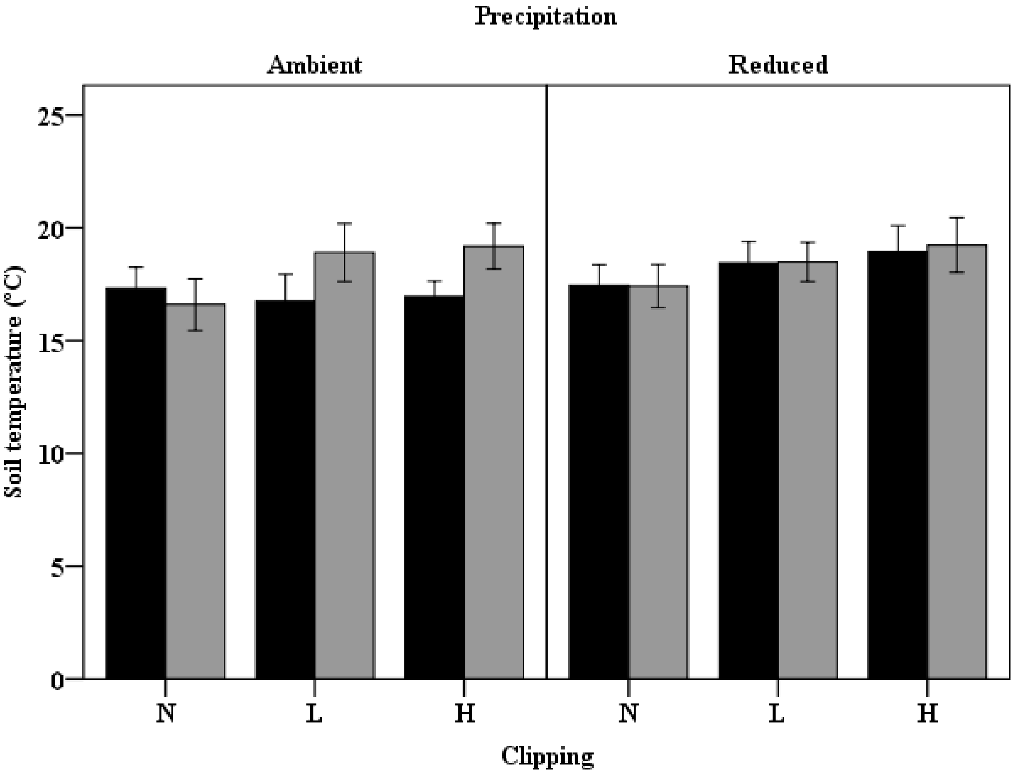


Figure A-3. Effects of precipitation (ambient and reduced), warming (black bars, control; grey bars, warmed), and clipping (no clipping, “N”; low intensity, “L”; high intensity, “H”) across all sites on 2009 soil temperature. Error bars represent ±1SE.

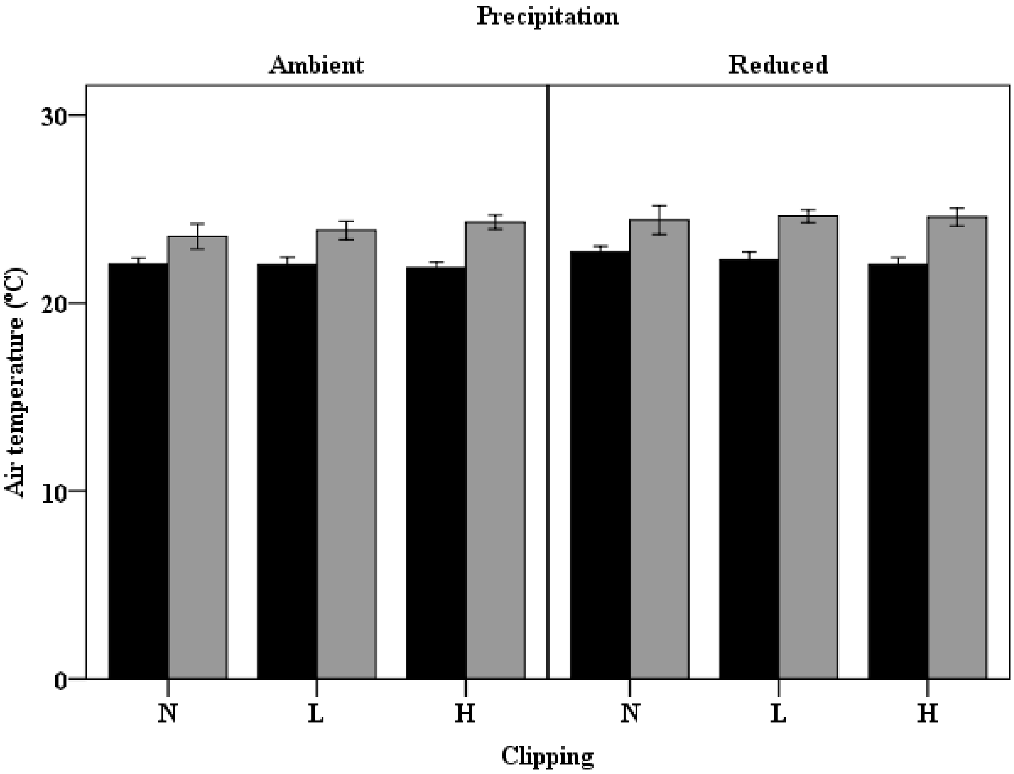


Figure A-4. Effects of precipitation (ambient and reduced), warming (black bars, control; grey bars, warmed), and clipping (no clipping, "N"; low intensity, "L"; high intensity, "H") across all sites on 2009 air temperature. Error bars represent ±1SE.

Table A-1. Mean and standard error for plant biomass, ("shoot biomass" in Chapter ; "accumulated biomass" in Chapter) by plant group (graminoid, forb, or shrub), clipping treatment (H, high intensity; L, low intensity, and N, no clipping), precipitation (precip) treatment ('-', reduced; 'A', ambient; '+', added), site, and year.

Plant group	Clipping	Precip	Warming	2007						2008						2009					
				AB		SK		MB		AB		SK		MB		AB		SK		MB	
				Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Graminoid	H	-	C	135	21	173	14	100	43	59	12	94	7	109	18	67	22	73	23	135	9
Forb	H	-	W	54	11	7	12	56	1	22	6	19	4	20	7	28	11	8	5	13	2
Shrub	H	+	C	0	0	0	55	69	0	0	0	0	20	39	0	0	0	0	23	39	0
Graminoid	H	+	W	125	21	146	24	105	25	58	16	73	9	84	16	55	23	86	30	148	9
Forb	H	A	C	64	11	10	20	99	2	33	8	11	17	52	4	35	11	4	4	21	1
Shrub	H	A	W	0	0	0	14	45	0	0	0	0	7	23	0	0	0	0	11	26	0
Graminoid	L	-	C	206	18		0		0	156	11		0		0	264	54		0		0
Forb	L	-	W	65	15		0		0	35	3		0		0	17	3		0		0
Shrub	L	+	C	0	0		0		0	0	0		0		0	1	1		0		0
Graminoid	L	+	W	221	32		0		0	189	35		0		0	300	74		0		0
Forb	L	A	C	47	18		0		0	23	5		0		0	17	6		0		0
Shrub	L	A	W	0	0		0		0	0	0		0		0	0	0		0		0
Graminoid	N	-	C	172	29	148	20	86	31	96	14	96	18	115	19	129	29	96	10	125	19

Forb	N	-	W	65	13	8	19	87	3	44	8	10	18	69	4	26	5	12	15	69	5
Shrub	N	+	C	5	5	0	39	64	0	3	3	0	7	9	0	1	1	0	7	12	0
Graminoid	N	+	W	143	18	99	8	65	32	110	11	80	15	110	25	95	12	57	19	112	14
Forb	N	A	C	48	19	11	22	77	5	21	9	25	14	85	10	14	5	14	15	54	4
Shrub	N	A	W	1	1	0	41	57	0	1	1	0	17	23	0	4	3	0	15	27	0
Graminoid	H	-	C	119	20	179	19	102	17	56	10	100	27	131	13	70	21	103	30	158	9
Forb	H	-	W	44	8	14	12	63	4	19	7	17	11	35	8	15	4	11	5	28	3
Shrub	H	+	C	0	0	0	83	92	0	0	0	0	8	15	0	0	0	0	14	20	0
Graminoid	H	+	W	100	19	150	18	96	32	56	12	79	7	112	22	66	20	113	39	149	14
Forb	H	A	C	66	9	24	12	83	10	25	8	7	7	32	1	43	24	29	10	25	9
Shrub	H	A	W	0	0	0	28	36	0	0	0	0	38	62	0	0	0	0	5	8	0
Graminoid	L	-	C	180	29		0		0	187	24		0		0	340	38		0		0
Forb	L	-	W	65	20		0		0	45	11		0		0	35	17		0		0
Shrub	L	+	C	0	0		0		0	0	0		0		0	0	0		0		0
Graminoid	L	+	W	133	17		0		0	129	13		0		0	244	48		0		0
Forb	L	A	C	53	9		0		0	37	7		0		0	44	11		0		0
Shrub	L	A	W	1	1		0		0	5	5		0		0	2	2		0		0
Graminoid	N	-	C	146	16	253	12	158	74	131	11	119	21	207	7	175	12	127	23	206	21
Forb	N	-	W	51	11	10	7	69	2	38	8	13	8	46	5	43	12	42	26	56	16
Shrub	N	+	C	0	0	0	16	21	0	0	0	0	7	20	0	0	0	0	12	36	0

Graminoid	N	+	W	166	35	111	46	158	12	135	34	82	54	187	11	159	39	97	24	145	14
Forb	N	A	C	86	19	17	20	61	4	32	10	23	15	52	8	29	9	12	83	153	4
Shrub	N	A	W	0	0	0	17	36	0	1	1	0	6	16	0	4	3	0	9	20	0
Graminoid	H	-	C	105	25	109	9	80	20	70	23	117	4	94	37	119	36	143	30	166	19
Forb	H	-	W	47	12	33	12	47	13	33	11	24	37	100	10	43	16	33	91	135	13
Shrub	H	+	C	0	0	0	25	44	0	0	0	0	12	42	0	0	0	0	52	70	0
Graminoid	H	+	W	71	6	132	14	93	36	73	27	84	21	99	14	69	27	128	34	186	20
Forb	H	A	C	61	14	1	19	95	1	47	8	15	18	82	11	68	21	9	14	49	4
Shrub	H	A	W	0	0	0	7	8	0	0	0	0	4	6	0	0	0	0	40	46	0
Graminoid	L	-	C	120	22		0		0	188	40		0		0	220	37		0		0
Forb	L	-	W	52	13		0		0	66	25		0		0	137	37		0		0
Shrub	L	+	C	0	0		0		0	0	0		0		0	0	0		0		0
Graminoid	L	+	W	60	8		0		0	169	22		0		0	217	42		0		0
Forb	L	A	C	105	36		0		0	54	20		0		0	61	23		0		0
Shrub	L	A	W	1	1		0		0	7	7		0		0	4	4		0		0
Graminoid	N	-	C	97	21	77	14	79	17	166	14	93	27	119	14	155	35	133	21	110	26
Forb	N	-	W	42	5	20	6	79	10	65	11	15	40	88	6	123	46	23	53	131	2
Shrub	N	+	C	0	0	0	8	12	0	0	0	0	140	180	0	0	0	0	71	136	0
Graminoid	N	+	W	97	5	99	9	86	30	119	14	108	13	129	11	163	24	154	15	123	37
Forb	N	A	C	43	10	20	14	63	9	38	12	13	16	81	6	35	13	35	62	131	13

Shrub	N	A	W	0	0	0	9	22	0	1	1	0	22	56	0	0	0	0	22	66	0

9. APPENDIX B: Supplemental material for Chapter 3

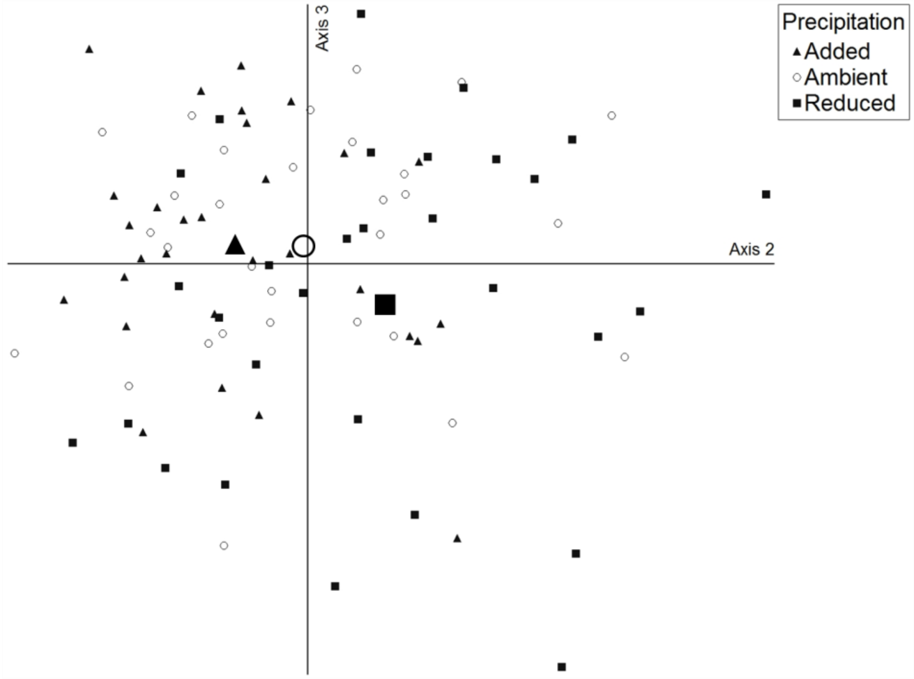
Table B-1. Transformations (either square root or natural log) performed on data to meet ANOVA assumptions.

Response variables not indicated were not transformed.

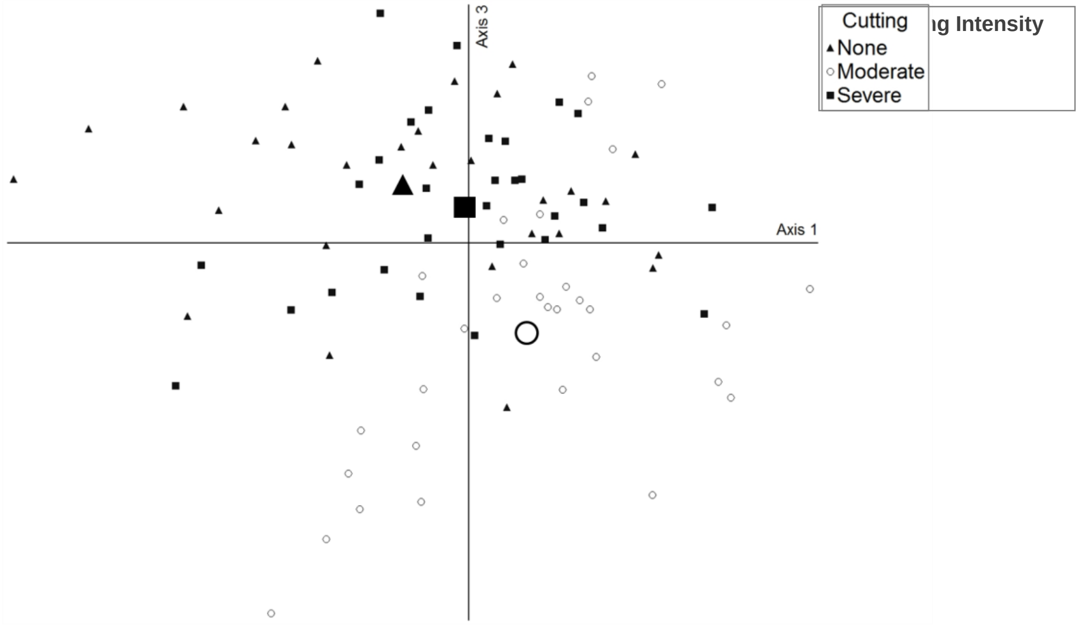
Transformation	Total biomass	Total biomass (AB only)	Regrowth biomass	Regrowth biomass (AB only)	CP (clipped and non-clipped)	CP (AB only)
Square root	Forb	Forb, Graminoid	Herbage, Graminoid	Herbage, Graminoid	Graminoid, Forb	Forb, Graminoid (Clipped)
Natural log	Herbage	Herbage	Forb	Forb	-	Graminoid (Non-clipped)

10. APPENDIX C: Supplemental material for Chapter 4

a)



b)



c)

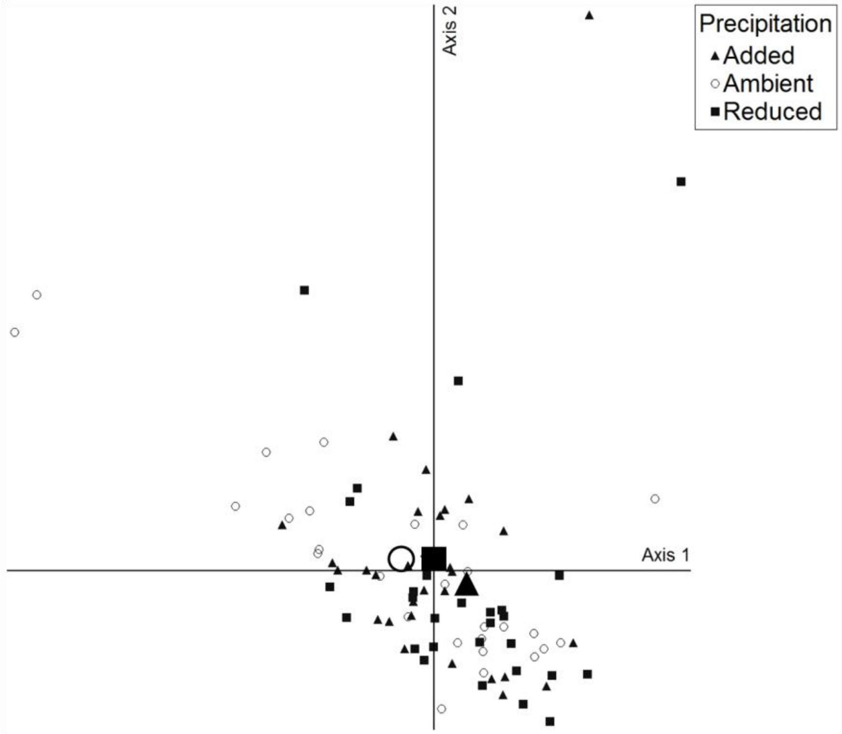


Figure C-1. Precipitation (a) and clipping (b) treatments overlaid on NMS ordination of AB aboveground plant community; precipitation (c) overlaid on NMS ordination of seed bank community. The vegetation ordination resulted in a final stress of 17.8 and cumulative explained variance of 78.9%, with Axis 1 explaining 26.3%, Axis 2 explaining 25.1%, and Axis 3 explaining 27.6% of variance. The seed bank ordination resulted in a final stress of 15.9 and cumulative explained variance of 79.9%, with Axis 1 explaining 54.8% and Axis 2 explaining 25.1% of seed bank variance

Table C-1. Results of Multi-Response Permutation Procedures (MRPP) comparisons of effects of precipitation, clipping and warming on seed bank and aboveground vegetation composition in AB. Significant differences between precipitation and defoliation levels are shown using multiple comparisons among all three treatments (T, test statistic; A, Chance-corrected within-group agreement; P, Probability of a smaller or equal delta)

Treatment	Seed bank			Aboveground vegetation		
	T	A	P	T	A	P
Precipitation	-1.91	0.02	0.05	-10.0	0.04	<0.001
added vs. ambient	-0.39	0.00	0.27	-3.6	0.02	0.004
added vs. reduced	-2.7	0.03	0.02	-13.9	0.07	<0.001
ambient vs. reduced	-1.2	0.01	0.12	-3.5	0.02	0.005
Clipping intensity	-0.57	0.00	0.24	-10.0	0.04	<0.001
none vs. high				-10.1	0.05	<0.001
none vs. low				-8.04	0.04	<0.001
low vs. high				-2.42	0.01	0.03
Warming	-0.54	0.00	0.23	1.02	-0.003	0.86

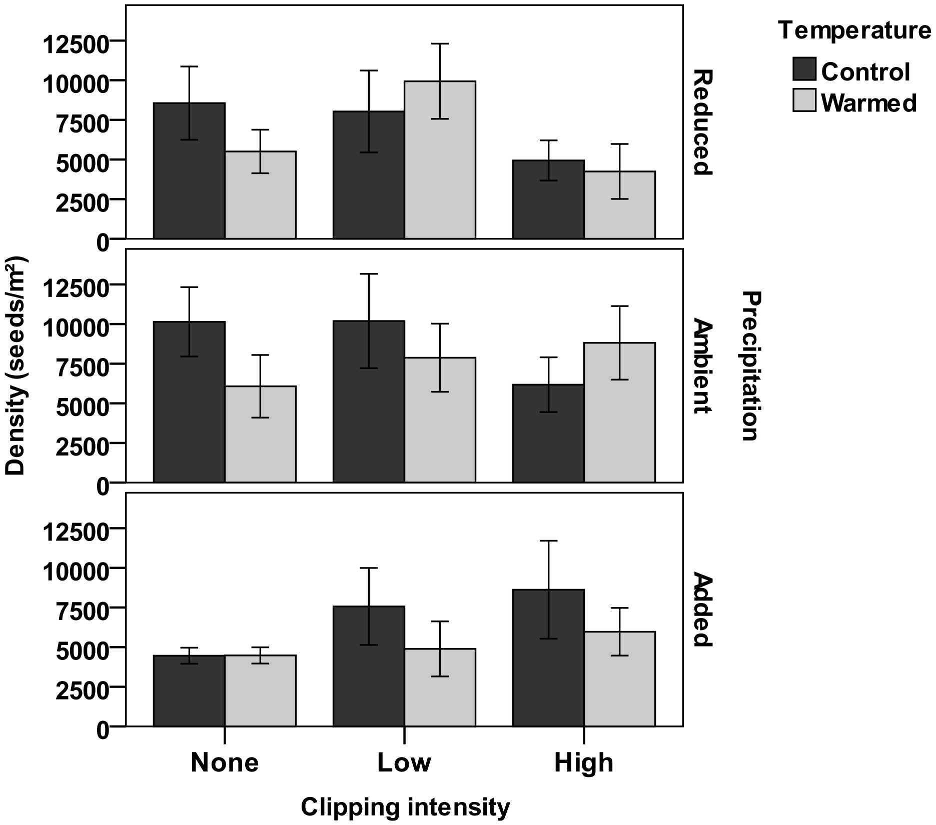


Figure C-2. Effect of precipitation, clipping, and warming on 2008 germinable seed bank density (seeds/m2). Error bars are ±1 SE. We tested the response of seed density to the treatments, using a mixed model with precipitation, defoliation, and warming as fixed factors, and block as a random factor. Seed density was square-root transformed to meet assumptions. Seed bank density was unaffected by the treatments (minimum $p > 0.147$).

11. APPENDIX D: Supplemental material for Chapter 5

Table D-1. Means ±1SE, data ranges, and bivariate correlations among variables in structural equation model of environmental drivers and intermediary variables on richness. Means, SE, and data ranges all from untransformed data; bivariate correlations are based on transformed values. *0.01<P<0.05; **0.001<P<0.01; ***<0.001P<0.001.

Variable	Precip	Air temp	Clipping intensity	Soil moisture	Soil temp	Soil nitrogen	Litter biomass	Shoot biomass	Root biomass	Richness	Evenness
Means±1 SE	58.1±3.4	22.8±0.2	23.8±2.7	13.9±0.8	15.4±0.3	40.8±5.6	277.0±21.5	196.9±16.6	659.8±4.05	7.83±0.3	0.83±0.0
Data range	26.7-107.4	20.1-24.8	0-88.9	-1.1-29.0	11.7-20.4	4.8-262.6	19.3-640.1	54.6-772.8	194.6-1876.5	4-16	0.6-1.0
Precip	1										
Air temp		1									
Clipping intensity	-	0.13	1								
Soil moisture	0.50<0.001	-0.17	-0.14	1							
Soil temp	-0.38**	0.34**	0.10	-0.61<0.001	1						
Soil nitrogen	-0.38**	-	0.19	-0.38**	0.46<0.001	1					
Litter biomass	0.12	-0.00	-0.47<0.001	0.40<0.001	-0.65<0.001	-0.44<0.001	1				
Shoot biomass	0.39**	-0.17	0.03	0.36**	-0.65<0.001	-0.31**	0.56<0.001	1			
Root biomass	0.36**	-0.04	0.07	0.52<0.001	-0.68<0.001	-0.50<0.001	0.41<0.001	0.46<0.001	1		
Richness	0.27*	-0.30**	0.01	0.46<0.001	-0.58<0.001	-0.57<0.001	0.30**	0.49<0.001	0.56<0.001	1	
Evenness	-0.17	-0.16	-0.00	-0.21**	0.18	0.27*	-0.18	-0.19	-0.07	-	1

Table D-2. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for richness response variable at AB. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.222	0.169	0.068	2.476	0.222
Air temperature to Shoot biomass	-0.239	-0.057	0.038	-1.502	-0.239
Air temperature to Litter biomass	0.038	0.119	0.403	0.296	0.038
Air temperature to Root biomass	-0.147	-0.018	0.027	-0.681	-0.147
Air temperature to Richness	-0.366	-0.067	0.027	-2.485	-0.366
Air temperature to Soil temperature	0.527	0.409	0.092	4.456	0.527
Air temperature to Soil moisture	-0.072	-0.191	0.45	-0.424	-0.072
Clipping to Shoot biomass	0.141	0.251	0.212	1.185	0.141
Clipping to Air temperature	0.203	1.513	0.756	2.002	0.203
Clipping to Litter biomass	-0.501	-11.553	2.071	-5.579	-0.501
Clipping to Root biomass	0.076	0.071	0.151	0.47	0.076
Clipping to Richness	0.253	0.344	0.262	1.314	0.253
Clipping to Soil temperature	-0.125	-0.723	0.595	-1.216	-0.125
Clipping to Soil moisture	-0.148	-2.914	2.685	-1.085	-0.148
Clipping to Nitrogen	0.057	0.163	0.374	0.436	0.057
Precipitation to Shoot biomass	0.443	0.008	0.003	3.143	0.443
Precipitation to Air temperature	-0.12	-0.009	0.005	-2.016	-0.12
Precipitation to Litter biomass	0.111	0.027	0.042	0.646	0.111
Precipitation to Root biomass	0.164	0.002	0.001	1.362	0.164
Precipitation to Richness	-0.331	-0.005	0.003	-1.844	-0.331
Precipitation to Soil temperature	-0.141	-0.009	0.004	-2.041	-0.141
Precipitation to Soil moisture	0.216	0.045	0.019	2.377	0.216
Precipitation to Nitrogen	-0.134	-0.004	0.002	-1.638	-0.134
Litter biomass to Root biomass	0.026	0.001	0.008	0.13	0.026
Litter biomass to Richness	-0.187	-0.011	0.01	-1.151	-0.187
Litter biomass to Soil temperature	-0.48	-0.12	0.031	-3.868	-0.48
Litter biomass to Soil moisture	-0.209	-0.179	0.143	-1.252	-0.209
Litter biomass to Nitrogen	-0.073	-0.009	0.019	-0.469	-0.073
Root biomass to Richness	0.106	0.154	0.103	1.494	0.106
Soil temperature to Shoot biomass	-0.031	-0.009	0.036	-0.262	-0.031
Soil temperature to Root biomass	0.209	0.034	0.033	1.014	0.209
Soil temperature to Richness	-0.034	-0.008	0.032	-0.248	-0.034
Soil temperature to Soil moisture	-0.529	-1.804	0.564	-3.2	-0.529
Soil temperature to Nitrogen	-0.087	-0.043	0.071	-0.602	-0.087
Soil moisture to Shoot biomass	-0.007	-0.001	0.006	-0.102	-0.007
Soil moisture to Root biomass	0.336	0.016	0.008	1.931	0.336
Soil moisture to Richness	0.038	0.003	0.006	0.451	0.038
Soil moisture to Nitrogen	0.046	0.007	0.015	0.435	0.046
Nitrogen to Shoot biomass	-0.044	-0.027	0.049	-0.564	-0.044
Nitrogen to Root biomass	0.131	0.043	0.048	0.888	0.131
Nitrogen to Richness	-0.262	-0.125	0.048	-2.574	-0.262

Table D-3. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for richness response variable at SK. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.251	0.169	0.068	2.476	0.013
Air temperature to Shoot biomass	-0.153	-0.057	0.038	-1.502	0.133
Air temperature to Litter biomass	0.025	0.119	0.403	0.296	0.767
Air temperature to Root biomass	-0.051	-0.018	0.027	-0.681	0.496
Air temperature to Richness	-0.265	-0.067	0.027	-2.485	0.013
Air temperature to Soil temperature	0.309	0.409	0.092	4.456	<0.001
Air temperature to Soil moisture	-0.027	-0.191	0.45	-0.424	0.672
Clipping to Shoot biomass	0.162	0.251	0.212	1.185	0.236
Clipping to Air temperature	-0.07	-0.288	0.901	-0.32	0.749
Clipping to Litter biomass	-0.589	-11.553	2.071	-5.579	<0.001
Clipping to Root biomass	0.048	0.071	0.151	0.47	0.638
Clipping to Richness	-0.075	-0.078	0.244	-0.32	0.749
Clipping to Soil temperature	-0.132	-0.723	0.595	-1.216	0.224
Clipping to Soil moisture	-0.099	-2.914	2.685	-1.085	0.278
Clipping to Nitrogen	0.55	1.881	0.66	2.85	0.004
Precipitation to Shoot biomass	-0.183	-0.003	0.002	-1.228	0.22
Precipitation to Air temperature	-0.216	-0.009	0.005	-2.016	0.044
Precipitation to Litter biomass	-0.168	-0.035	0.018	-1.897	0.058
Precipitation to Root biomass	-0.159	-0.003	0.003	-0.753	0.451
Precipitation to Richness	-0.111	-0.001	0.001	-0.955	0.339
Precipitation to Soil temperature	-0.148	-0.009	0.004	-2.041	0.041
Precipitation to Soil moisture	0.144	0.045	0.019	2.377	0.017
Precipitation to Nitrogen	-0.111	-0.004	0.002	-1.638	0.101
Litter biomass to Root biomass	0.014	0.001	0.008	0.13	0.896
Litter biomass to Richness	-0.207	-0.011	0.01	-1.151	0.25
Litter biomass to Soil temperature	-0.713	-0.199	0.049	-4.07	<0.001
Litter biomass to Soil moisture	-0.119	-0.179	0.143	-1.252	0.21
Litter biomass to Nitrogen	-0.052	-0.009	0.019	-0.469	0.639
Root biomass to Richness	0.22	0.154	0.103	1.494	0.135
Soil temperature to Shoot biomass	-0.033	-0.009	0.036	-0.262	0.793
Soil temperature to Root biomass	0.124	0.034	0.033	1.014	0.311
Soil temperature to Richness	-0.042	-0.008	0.032	-0.248	0.804
Soil temperature to Soil moisture	-0.335	-1.804	0.564	-3.2	0.001
Soil temperature to Nitrogen	-0.069	-0.043	0.071	-0.602	0.547
Soil moisture to Shoot biomass	-0.012	-0.001	0.006	-0.102	0.919
Soil moisture to Root biomass	-0.244	-0.012	0.011	-1.153	0.249
Soil moisture to Richness	0.074	0.003	0.006	0.451	0.652
Soil moisture to Nitrogen	0.057	0.007	0.015	0.435	0.663
Nitrogen to Shoot biomass	-0.061	-0.027	0.049	-0.564	0.572
Nitrogen to Root biomass	0.098	0.043	0.048	0.888	0.375
Nitrogen to Richness	-0.409	-0.125	0.048	-2.574	0.01

Table D-4. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for richness response variable at MB. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.443	0.169	0.068	2.476	0.013
Air temperature to Shoot biomass	-0.09	-0.057	0.038	-1.502	0.133
Air temperature to Litter biomass	0.027	0.119	0.403	0.296	0.767
Air temperature to Root biomass	0.321	0.069	0.045	1.546	0.122
Air temperature to Richness	-0.274	-0.067	0.027	-2.485	0.013
Air temperature to Soil temperature	0.39	0.409	0.092	4.456	<0.001
Air temperature to Soil moisture	-0.043	-0.191	0.45	-0.424	0.672
Clipping to Shoot biomass	0.087	0.251	0.212	1.185	0.236
Clipping to Air temperature	0.333	1.513	0.756	2.002	0.045
Clipping to Litter biomass	-0.579	-11.553	2.071	-5.579	<0.001
Clipping to Root biomass	0.073	0.071	0.151	0.47	0.638
Clipping to Richness	-0.282	-0.313	0.226	-1.384	0.166
Clipping to Soil temperature	-0.152	-0.723	0.595	-1.216	0.224
Clipping to Soil moisture	-0.144	-2.914	2.685	-1.085	0.278
Clipping to Nitrogen	0.09	0.163	0.374	0.436	0.663
Precipitation to Shoot biomass	0.436	0.008	0.003	3.143	0.002
Precipitation to Air temperature	-0.315	-0.009	0.005	-2.016	0.044
Precipitation to Litter biomass	-0.264	-0.035	0.018	-1.897	0.058
Precipitation to Root biomass	0.252	0.002	0.001	1.362	0.173
Precipitation to Richness	-0.168	-0.001	0.001	-0.955	0.339
Precipitation to Soil temperature	-0.273	-0.009	0.004	-2.041	0.041
Precipitation to Soil moisture	0.335	0.045	0.019	2.377	0.017
Precipitation to Nitrogen	-0.336	-0.004	0.002	-1.638	0.101
Litter biomass to Root biomass	0.021	0.001	0.008	0.13	0.896
Litter biomass to Richness	-0.199	-0.011	0.01	-1.151	0.25
Litter biomass to Soil temperature	-0.504	-0.12	0.031	-3.868	<0.001
Litter biomass to Soil moisture	-0.176	-0.179	0.143	-1.252	0.21
Litter biomass to Nitrogen	-0.099	-0.009	0.019	-0.469	0.639
Root biomass to Richness	0.135	0.154	0.103	1.494	0.135
Soil temperature to Shoot biomass	-0.015	-0.009	0.036	-0.262	0.793
Soil temperature to Root biomass	0.165	0.034	0.033	1.014	0.311
Soil temperature to Richness	-0.034	-0.008	0.032	-0.248	0.804
Soil temperature to Soil moisture	-0.424	-1.804	0.564	-3.2	0.001
Soil temperature to Nitrogen	-0.113	-0.043	0.071	-0.602	0.547
Soil moisture to Shoot biomass	-0.194	-0.028	0.027	-1.038	0.299
Soil moisture to Root biomass	0.332	0.016	0.008	1.931	0.053
Soil moisture to Richness	0.048	0.003	0.006	0.451	0.652
Soil moisture to Nitrogen	0.075	0.007	0.015	0.435	0.663
Nitrogen to Shoot biomass	-0.017	-0.027	0.049	-0.564	0.572
Nitrogen to Root biomass	0.08	0.043	0.048	0.888	0.375
Nitrogen to Richness	-0.204	-0.125	0.048	-2.574	0.01

Table D-5. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at AB. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.068	-0.028	0.052	-0.546	0.585
Air temperature to Shoot biomass	-0.239	-0.057	0.038	-1.502	0.133
Air temperature to Litter biomass	0.038	0.119	0.403	0.296	0.767
Air temperature to Root biomass	-0.147	-0.018	0.027	-0.681	0.496
Air temperature to Evenness	-0.273	-0.027	0.021	-1.317	0.188
Air temperature to Soil temperature	0.527	0.409	0.092	4.456	<0.001
Air temperature to Soil moisture	-0.072	-0.191	0.45	-0.424	0.672
Clipping to Shoot biomass	0.141	0.251	0.212	1.185	0.236
Clipping to Air temperature	0.203	1.513	0.756	2.002	0.045
Clipping to Litter biomass	-0.501	-11.553	2.071	-5.579	<0.001
Clipping to Root biomass	0.076	0.071	0.151	0.47	0.638
Clipping to Evenness	0.217	0.16	0.138	1.158	0.247
Clipping to Soil temperature	-0.125	-0.723	0.595	-1.216	0.224
Clipping to Soil moisture	-0.148	-2.914	2.685	-1.085	0.278
Clipping to Nitrogen	0.057	0.163	0.374	0.436	0.663
Precipitation to Shoot biomass	0.443	0.008	0.003	3.143	0.002
Precipitation to Air temperature	-0.12	-0.009	0.005	-2.016	0.044
Precipitation to Litter biomass	0.111	0.027	0.042	0.646	0.519
Precipitation to Root biomass	0.164	0.002	0.001	1.362	0.173
Precipitation to Evenness	-0.055	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.141	-0.009	0.004	-2.041	0.041
Precipitation to Soil moisture	0.216	0.045	0.019	2.377	0.017
Precipitation to Nitrogen	-0.134	-0.004	0.002	-1.638	0.101
Litter biomass to Root biomass	0.026	0.001	0.008	0.13	0.896
Litter biomass to Evenness	0.104	0.003	0.007	0.483	0.629
Litter biomass to Soil temperature	-0.48	-0.12	0.031	-3.868	<0.001
Litter biomass to Soil moisture	-0.209	-0.179	0.143	-1.252	0.21
Litter biomass to Nitrogen	-0.073	-0.009	0.019	-0.469	0.639
Root biomass to Evenness	-0.011	-0.009	0.119	-0.074	0.941
Soil temperature to Shoot biomass	-0.031	-0.009	0.036	-0.262	0.793
Soil temperature to Root biomass	0.209	0.034	0.033	1.014	0.311
Soil temperature to Evenness	0.286	0.037	0.024	1.545	0.122
Soil temperature to Soil moisture	-0.529	-1.804	0.564	-3.2	0.001
Soil temperature to Nitrogen	-0.087	-0.043	0.071	-0.602	0.547
Soil moisture to Shoot biomass	-0.007	-0.001	0.006	-0.102	0.919
Soil moisture to Root biomass	0.336	0.016	0.008	1.931	0.053
Soil moisture to Evenness	0	0	0.005	0	1
Soil moisture to Nitrogen	0.046	0.007	0.015	0.435	0.663
Nitrogen to Shoot biomass	-0.044	-0.027	0.049	-0.564	0.572
Nitrogen to Root biomass	0.131	0.043	0.048	0.888	0.375
Nitrogen to Evenness	0.141	0.037	0.035	1.039	0.299

Table D-6. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at SK. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.05	-0.028	0.052	-0.546	0.585
Air temperature to Shoot biomass	-0.153	-0.057	0.038	-1.502	0.133
Air temperature to Litter biomass	0.025	0.119	0.403	0.296	0.767
Air temperature to Root biomass	-0.051	-0.018	0.027	-0.681	0.496
Air temperature to Evenness	-0.127	-0.027	0.021	-1.317	0.188
Air temperature to Soil temperature	0.309	0.409	0.092	4.456	<0.001
Air temperature to Soil moisture	-0.027	-0.191	0.45	-0.424	0.672
Clipping to Shoot biomass	0.162	0.251	0.212	1.185	0.236
Clipping to Air temperature	-0.07	-0.288	0.901	-0.32	0.749
Clipping to Litter biomass	-0.589	-11.553	2.071	-5.579	<0.001
Clipping to Root biomass	0.048	0.071	0.151	0.47	0.638
Clipping to Evenness	-0.482	-0.425	0.186	-2.284	0.022
Clipping to Soil temperature	-0.132	-0.723	0.595	-1.216	0.224
Clipping to Soil moisture	-0.099	-2.914	2.685	-1.085	0.278
Clipping to Nitrogen	0.55	1.881	0.66	2.85	0.004
Precipitation to Shoot biomass	-0.183	-0.003	0.002	-1.228	0.22
Precipitation to Air temperature	-0.216	-0.009	0.005	-2.016	0.044
Precipitation to Litter biomass	-0.168	-0.035	0.018	-1.897	0.058
Precipitation to Root biomass	-0.159	-0.003	0.003	-0.753	0.451
Precipitation to Evenness	-0.046	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.148	-0.009	0.004	-2.041	0.041
Precipitation to Soil moisture	0.144	0.045	0.019	2.377	0.017
Precipitation to Nitrogen	-0.111	-0.004	0.002	-1.638	0.101
Litter biomass to Root biomass	0.014	0.001	0.008	0.13	0.896
Litter biomass to Evenness	0.074	0.003	0.007	0.483	0.629
Litter biomass to Soil temperature	-0.713	-0.199	0.049	-4.07	<0.001
Litter biomass to Soil moisture	-0.119	-0.179	0.143	-1.252	0.21
Litter biomass to Nitrogen	-0.052	-0.009	0.019	-0.469	0.639
Root biomass to Evenness	0.437	0.258	0.108	2.396	0.017
Soil temperature to Shoot biomass	-0.033	-0.009	0.036	-0.262	0.793
Soil temperature to Root biomass	0.124	0.034	0.033	1.014	0.311
Soil temperature to Evenness	0.227	0.037	0.024	1.545	0.122
Soil temperature to Soil moisture	-0.335	-1.804	0.564	-3.2	0.001
Soil temperature to Nitrogen	-0.069	-0.043	0.071	-0.602	0.547
Soil moisture to Shoot biomass	-0.012	-0.001	0.006	-0.102	0.919
Soil moisture to Root biomass	-0.244	-0.012	0.011	-1.153	0.249
Soil moisture to Evenness	0	0	0.005	0	1
Soil moisture to Nitrogen	0.057	0.007	0.015	0.435	0.663
Nitrogen to Shoot biomass	-0.061	-0.027	0.049	-0.564	0.572
Nitrogen to Root biomass	0.098	0.043	0.048	0.888	0.375
Nitrogen to Evenness	0.142	0.037	0.035	1.039	0.299

Table D-7. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at MB. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.105	-0.028	0.052	-0.546	0.585
Air temperature to Shoot biomass	-0.09	-0.057	0.038	-1.502	0.133
Air temperature to Litter biomass	0.027	0.119	0.403	0.296	0.767
Air temperature to Root biomass	0.321	0.069	0.045	1.546	0.122
Air temperature to Evenness	-0.555	-0.096	0.037	-2.602	0.009
Air temperature to Soil temperature	0.39	0.409	0.092	4.456	<0.001
Air temperature to Soil moisture	-0.043	-0.191	0.45	-0.424	0.672
Clipping to Shoot biomass	0.087	0.251	0.212	1.185	0.236
Clipping to Air temperature	0.333	1.513	0.756	2.002	0.045
Clipping to Litter biomass	-0.579	-11.553	2.071	-5.579	<0.001
Clipping to Root biomass	0.073	0.071	0.151	0.47	0.638
Clipping to Evenness	0.204	0.16	0.138	1.158	0.247
Clipping to Soil temperature	-0.152	-0.723	0.595	-1.216	0.224
Clipping to Soil moisture	-0.144	-2.914	2.685	-1.085	0.278
Clipping to Nitrogen	0.09	0.163	0.374	0.436	0.663
Precipitation to Shoot biomass	0.436	0.008	0.003	3.143	0.002
Precipitation to Air temperature	-0.315	-0.009	0.005	-2.016	0.044
Precipitation to Litter biomass	-0.264	-0.035	0.018	-1.897	0.058
Precipitation to Root biomass	0.252	0.002	0.001	1.362	0.173
Precipitation to Evenness	-0.082	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.273	-0.009	0.004	-2.041	0.041
Precipitation to Soil moisture	0.335	0.045	0.019	2.377	0.017
Precipitation to Nitrogen	-0.336	-0.004	0.002	-1.638	0.101
Litter biomass to Root biomass	0.021	0.001	0.008	0.13	0.896
Litter biomass to Evenness	0.084	0.003	0.007	0.483	0.629
Litter biomass to Soil temperature	-0.504	-0.12	0.031	-3.868	<0.001
Litter biomass to Soil moisture	-0.176	-0.179	0.143	-1.252	0.21
Litter biomass to Nitrogen	-0.099	-0.009	0.019	-0.469	0.639
Root biomass to Evenness	-0.011	-0.009	0.119	-0.074	0.941
Soil temperature to Shoot biomass	-0.015	-0.009	0.036	-0.262	0.793
Soil temperature to Root biomass	0.165	0.034	0.033	1.014	0.311
Soil temperature to Evenness	0.223	0.037	0.024	1.545	0.122
Soil temperature to Soil moisture	-0.424	-1.804	0.564	-3.2	0.001
Soil temperature to Nitrogen	-0.113	-0.043	0.071	-0.602	0.547
Soil moisture to Shoot biomass	-0.194	-0.028	0.027	-1.038	0.299
Soil moisture to Root biomass	0.332	0.016	0.008	1.931	0.053
Soil moisture to Evenness	0	0	0.005	0	1
Soil moisture to Nitrogen	0.075	0.007	0.015	0.435	0.663
Nitrogen to Shoot biomass	-0.017	-0.027	0.049	-0.564	0.572
Nitrogen to Root biomass	0.08	0.043	0.048	0.888	0.375
Nitrogen to Evenness	0.084	0.037	0.035	1.039	0.299

Table D-8. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model with for richness response variable at AB, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.282	0.173	0.069	2.519	0.012
Air temperature to Shoot biomass	0.127	0.035	0.042	0.836	0.403
Air temperature to Litter biomass	0.123	0.364	0.378	0.964	0.335
Air temperature to Root biomass	-0.094	-0.011	0.025	-0.448	0.654
Air temperature to Richness	-0.502	-0.085	0.023	-3.654	<0.001
Air temperature to Soil temperature	0.445	0.353	0.09	3.905	<0.001
Air temperature to Soil moisture	-0.225	-0.584	0.42	-1.39	0.164
Clipping to Shoot biomass	0.015	0.032	0.235	0.135	0.893
Clipping to Air temperature	0.096	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.56	-12.844	2.035	-6.311	<0.001
Clipping to Root biomass	0.031	0.028	0.152	0.186	0.853
Clipping to Richness	0.338	0.443	0.223	1.986	0.047
Clipping to Soil temperature	-0.109	-0.673	0.618	-1.089	0.276
Clipping to Soil moisture	-0.12	-2.411	2.773	-0.87	0.385
Clipping to Nitrogen	0.101	0.292	0.385	0.759	0.448
Precipitation to Shoot biomass	0.328	0.007	0.002	3.32	<0.001
Precipitation to Air temperature	-0.135	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	0.143	0.034	0.038	0.894	0.371
Precipitation to Root biomass	0.143	0.001	0.001	1.244	0.213
Precipitation to Richness	-0.307	-0.004	0.002	-1.929	0.054
Precipitation to Soil temperature	-0.133	-0.009	0.004	-2.011	0.044
Precipitation to Soil moisture	0.201	0.042	0.019	2.205	0.027
Precipitation to Nitrogen	-0.113	-0.003	0.002	-1.412	0.158
Litter biomass to Root biomass	0.011	0	0.008	0.053	0.958
Litter biomass to Richness	-0.129	-0.007	0.009	-0.842	0.4
Litter biomass to Soil temperature	-0.601	-0.161	0.033	-4.824	<0.001
Litter biomass to Soil moisture	-0.198	-0.174	0.149	-1.165	0.244
Litter biomass to Nitrogen	-0.016	-0.002	0.019	-0.109	0.914
Root biomass to Richness	0.1	0.142	0.097	1.468	0.142
Soil temperature to Shoot biomass	-0.288	-0.1	0.048	-2.07	0.038
Soil temperature to Root biomass	0.093	0.014	0.031	0.455	0.649
Soil temperature to Richness	0.047	0.01	0.03	0.337	0.736
Soil temperature to Soil moisture	-0.415	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.031	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.107	-0.011	0.009	-1.207	0.227
Soil moisture to Root biomass	0.28	0.013	0.008	1.649	0.099
Soil moisture to Richness	0.02	0.001	0.006	0.23	0.818
Soil moisture to Nitrogen	0.046	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	-0.007	-0.005	0.073	-0.068	0.946
Nitrogen to Root biomass	0.056	0.018	0.047	0.381	0.703
Nitrogen to Richness	-0.228	-0.103	0.044	-2.35	0.019

Table D-9. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for richness response variable at SK, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.304	0.173	0.069	2.519	0.012
Air temperature to Shoot biomass	0.075	0.035	0.042	0.836	0.403
Air temperature to Litter biomass	0.078	0.364	0.378	0.964	0.335
Air temperature to Root biomass	-0.03	-0.011	0.025	-0.448	0.654
Air temperature to Richness	-0.32	-0.085	0.023	-3.654	<0.001
Air temperature to Soil temperature	0.285	0.353	0.09	3.905	<0.001
Air temperature to Soil moisture	-0.089	-0.584	0.42	-1.39	0.164
Clipping to Shoot biomass	0.016	0.032	0.235	0.135	0.893
Clipping to Air temperature	0.171	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.625	-12.844	2.035	-6.311	<0.001
Clipping to Root biomass	0.017	0.028	0.152	0.186	0.853
Clipping to Richness	-0.194	-0.224	0.181	-1.241	0.214
Clipping to Soil temperature	-0.124	-0.673	0.618	-1.089	0.276
Clipping to Soil moisture	-0.084	-2.411	2.773	-0.87	0.385
Clipping to Nitrogen	0.507	1.697	0.637	2.664	0.008
Precipitation to Shoot biomass	-0.183	-0.004	0.004	-1.075	0.282
Precipitation to Air temperature	-0.24	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	-0.138	-0.03	0.019	-1.589	0.112
Precipitation to Root biomass	0.082	0.001	0.001	1.244	0.213
Precipitation to Richness	-0.078	-0.001	0.001	-0.74	0.46
Precipitation to Soil temperature	-0.151	-0.009	0.004	-2.011	0.044
Precipitation to Soil moisture	0.141	0.042	0.019	2.205	0.027
Precipitation to Nitrogen	-0.098	-0.003	0.002	-1.412	0.158
Litter biomass to Root biomass	0.005	0	0.008	0.053	0.958
Litter biomass to Richness	-0.131	-0.007	0.009	-0.842	0.4
Litter biomass to Soil temperature	-0.61	-0.161	0.033	-4.824	<0.001
Litter biomass to Soil moisture	-0.124	-0.174	0.149	-1.165	0.244
Litter biomass to Nitrogen	-0.013	-0.002	0.019	-0.109	0.914
Root biomass to Richness	0.199	0.142	0.097	1.468	0.142
Soil temperature to Shoot biomass	-0.265	-0.1	0.048	-2.07	0.038
Soil temperature to Root biomass	0.047	0.014	0.031	0.455	0.649
Soil temperature to Richness	0.047	0.01	0.03	0.337	0.736
Soil temperature to Soil moisture	-0.257	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.024	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.16	-0.011	0.009	-1.207	0.227
Soil moisture to Root biomass	-0.369	-0.021	0.011	-1.888	0.059
Soil moisture to Richness	0.033	0.001	0.006	0.23	0.818
Soil moisture to Nitrogen	0.057	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	-0.008	-0.005	0.073	-0.068	0.946
Nitrogen to Root biomass	0.037	0.018	0.047	0.381	0.703
Nitrogen to Richness	-0.297	-0.103	0.044	-2.35	0.019

Table D-10. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for richness response variable at MB, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Richness	0.345	0.173	0.069	2.519	0.012
Air temperature to Shoot biomass	0.072	0.035	0.042	0.836	0.403
Air temperature to Litter biomass	0.079	0.364	0.378	0.964	0.335
Air temperature to Root biomass	0.371	0.077	0.044	1.761	0.078
Air temperature to Richness	-0.348	-0.085	0.023	-3.654	<0.001
Air temperature to Soil temperature	0.346	0.353	0.09	3.905	<0.001
Air temperature to Soil moisture	-0.132	-0.584	0.42	-1.39	0.164
Clipping to Shoot biomass	0.014	0.032	0.235	0.135	0.893
Clipping to Air temperature	0.164	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.614	-12.844	2.035	-6.311	<0.001
Clipping to Root biomass	0.03	0.028	0.152	0.186	0.853
Clipping to Richness	-0.202	-0.224	0.181	-1.241	0.214
Clipping to Soil temperature	-0.145	-0.673	0.618	-1.089	0.276
Clipping to Soil moisture	-0.119	-2.411	2.773	-0.87	0.385
Clipping to Nitrogen	0.158	0.292	0.385	0.759	0.448
Precipitation to Shoot biomass	0.5	0.007	0.002	3.32	<0.001
Precipitation to Air temperature	-0.365	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	-0.215	-0.03	0.019	-1.589	0.112
Precipitation to Root biomass	0.221	0.001	0.001	1.244	0.213
Precipitation to Richness	-0.13	-0.001	0.001	-0.74	0.46
Precipitation to Soil temperature	-0.279	-0.009	0.004	-2.011	0.044
Precipitation to Soil moisture	0.319	0.042	0.019	2.205	0.027
Precipitation to Nitrogen	-0.284	-0.003	0.002	-1.412	0.158
Litter biomass to Root biomass	0.009	0	0.008	0.053	0.958
Litter biomass to Richness	-0.139	-0.007	0.009	-0.842	0.4
Litter biomass to Soil temperature	-0.552	-0.123	0.042	-2.918	0.004
Litter biomass to Soil moisture	-0.18	-0.174	0.149	-1.165	0.244
Litter biomass to Nitrogen	-0.023	-0.002	0.019	-0.109	0.914
Root biomass to Richness	0.122	0.142	0.097	1.468	0.142
Soil temperature to Shoot biomass	-0.209	-0.1	0.048	-2.07	0.038
Soil temperature to Root biomass	0.068	0.014	0.031	0.455	0.649
Soil temperature to Richness	0.042	0.01	0.03	0.337	0.736
Soil temperature to Soil moisture	-0.313	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.037	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.104	-0.011	0.009	-1.207	0.227
Soil moisture to Root biomass	0.272	0.013	0.008	1.649	0.099
Soil moisture to Richness	0.024	0.001	0.006	0.23	0.818
Soil moisture to Nitrogen	0.073	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	-0.004	-0.005	0.073	-0.068	0.946
Nitrogen to Root biomass	0.035	0.018	0.047	0.381	0.703
Nitrogen to Richness	-0.171	-0.103	0.044	-2.35	0.019

Table D-11. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at AB, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.025	-0.009	0.052	-0.175	0.861
Air temperature to Shoot biomass	0.123	0.034	0.042	0.808	0.419
Air temperature to Litter biomass	0.175	0.495	0.368	1.345	0.179
Air temperature to Root biomass	-0.093	-0.011	0.025	-0.446	0.656
Air temperature to Evenness	-0.102	-0.01	0.019	-0.547	0.584
Air temperature to Soil temperature	0.458	0.353	0.091	3.882	<0.001
Air temperature to Soil moisture	-0.225	-0.584	0.422	-1.384	0.166
Clipping to Shoot biomass	0.011	0.022	0.237	0.095	0.925
Clipping to Air temperature	0.096	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.612	-13.388	2.076	-6.449	<0.001
Clipping to Root biomass	0.028	0.026	0.155	0.168	0.867
Clipping to Evenness	0.168	0.131	0.134	0.978	0.328
Clipping to Soil temperature	-0.113	-0.673	0.625	-1.077	0.282
Clipping to Soil moisture	-0.12	-2.411	2.824	-0.854	0.393
Clipping to Nitrogen	0.101	0.292	0.386	0.758	0.449
Precipitation to Shoot biomass	0.329	0.007	0.002	3.307	<0.001
Precipitation to Air temperature	-0.135	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	0.148	0.034	0.034	0.995	0.32
Precipitation to Root biomass	0.143	0.001	0.001	1.244	0.213
Precipitation to Evenness	-0.05	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.137	-0.009	0.004	-2.017	0.044
Precipitation to Soil moisture	0.201	0.042	0.019	2.206	0.027
Precipitation to Nitrogen	-0.113	-0.003	0.002	-1.415	0.157
Litter biomass to Root biomass	0.005	0	0.008	0.026	0.979
Litter biomass to Evenness	0.013	0	0.007	0.07	0.944
Litter biomass to Soil temperature	-0.591	-0.161	0.034	-4.759	<0.001
Litter biomass to Soil moisture	-0.189	-0.174	0.15	-1.16	0.246
Litter biomass to Nitrogen	-0.016	-0.002	0.018	-0.112	0.911
Root biomass to Evenness	0.177	0.149	0.082	1.825	0.068
Soil temperature to Shoot biomass	-0.27	-0.096	0.048	-1.988	0.047
Soil temperature to Root biomass	0.09	0.014	0.031	0.453	0.651
Soil temperature to Evenness	0.221	0.029	0.022	1.287	0.198
Soil temperature to Soil moisture	-0.403	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.03	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.108	-0.011	0.009	-1.202	0.229
Soil moisture to Root biomass	0.279	0.013	0.008	1.645	0.1
Soil moisture to Evenness	-0.006	0	0.004	-0.053	0.958
Soil moisture to Nitrogen	0.046	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	0.002	0.002	0.072	0.023	0.982
Nitrogen to Root biomass	0.056	0.018	0.047	0.381	0.703
Nitrogen to Evenness	0.184	0.049	0.034	1.468	0.142

Table D-12. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at SK, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.022	-0.009	0.052	-0.175	0.861
Air temperature to Shoot biomass	0.072	0.034	0.042	0.808	0.419
Air temperature to Litter biomass	0.1	0.495	0.368	1.345	0.179
Air temperature to Root biomass	-0.03	-0.011	0.025	-0.446	0.656
Air temperature to Evenness	-0.055	-0.01	0.019	-0.547	0.584
Air temperature to Soil temperature	0.28	0.353	0.091	3.882	<0.001
Air temperature to Soil moisture	-0.089	-0.584	0.422	-1.384	0.166
Clipping to Shoot biomass	0.011	0.022	0.237	0.095	0.925
Clipping to Air temperature	0.171	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.616	-13.388	2.076	-6.449	<0.001
Clipping to Root biomass	0.016	0.026	0.155	0.168	0.867
Clipping to Evenness	-0.476	-0.394	0.178	-2.218	0.027
Clipping to Soil temperature	-0.122	-0.673	0.625	-1.077	0.282
Clipping to Soil moisture	-0.084	-2.411	2.824	-0.854	0.393
Clipping to Nitrogen	0.507	1.697	0.638	2.662	0.008
Precipitation to Shoot biomass	-0.175	-0.004	0.004	-1.023	0.306
Precipitation to Air temperature	-0.24	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	-0.123	-0.028	0.02	-1.397	0.163
Precipitation to Root biomass	0.082	0.001	0.001	1.244	0.213
Precipitation to Evenness	-0.047	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.148	-0.009	0.004	-2.017	0.044
Precipitation to Soil moisture	0.141	0.042	0.019	2.206	0.027
Precipitation to Nitrogen	-0.098	-0.003	0.002	-1.415	0.157
Litter biomass to Root biomass	0.003	0	0.008	0.026	0.979
Litter biomass to Evenness	0.012	0	0.007	0.07	0.944
Litter biomass to Soil temperature	-0.635	-0.161	0.034	-4.759	<0.001
Litter biomass to Soil moisture	-0.132	-0.174	0.15	-1.16	0.246
Litter biomass to Nitrogen	-0.013	-0.002	0.018	-0.112	0.911
Root biomass to Evenness	0.292	0.149	0.082	1.825	0.068
Soil temperature to Shoot biomass	-0.26	-0.096	0.048	-1.988	0.047
Soil temperature to Root biomass	0.047	0.014	0.031	0.453	0.651
Soil temperature to Evenness	0.192	0.029	0.022	1.287	0.198
Soil temperature to Soil moisture	-0.262	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.024	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.16	-0.011	0.009	-1.202	0.229
Soil moisture to Root biomass	-0.368	-0.021	0.011	-1.885	0.059
Soil moisture to Evenness	-0.008	0	0.004	-0.053	0.958
Soil moisture to Nitrogen	0.057	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	0.003	0.002	0.072	0.023	0.982
Nitrogen to Root biomass	0.037	0.018	0.047	0.381	0.703
Nitrogen to Evenness	0.2	0.049	0.034	1.468	0.142

Table D-13. Standardized and unstandardized path coefficients, the standard error of the unstandardized coefficients, t test results, and associated p-values from the climate change and clipping structural equation model for evenness response variable at MB, with missing values estimated. Paths are from exogenous to endogenous variables.

Path	Standardized coefficients	Unstandardized coefficients	Standard error	t value	P-value
Shoot biomass to Evenness	-0.024	-0.009	0.052	-0.175	0.861
Air temperature to Shoot biomass	0.069	0.034	0.042	0.808	0.419
Air temperature to Litter biomass	0.102	0.495	0.368	1.345	0.179
Air temperature to Root biomass	0.372	0.078	0.044	1.761	0.078
Air temperature to Evenness	-0.472	-0.086	0.037	-2.317	0.02
Air temperature to Soil temperature	0.342	0.353	0.091	3.882	<0.001
Air temperature to Soil moisture	-0.132	-0.584	0.422	-1.384	0.166
Clipping to Shoot biomass	0.01	0.022	0.237	0.095	0.925
Clipping to Air temperature	0.164	0.746	0.58	1.285	0.199
Clipping to Litter biomass	-0.607	-13.388	2.076	-6.449	<0.001
Clipping to Root biomass	0.027	0.026	0.155	0.168	0.867
Clipping to Evenness	0.157	0.131	0.134	0.978	0.328
Clipping to Soil temperature	-0.143	-0.673	0.625	-1.077	0.282
Clipping to Soil moisture	-0.119	-2.411	2.824	-0.854	0.393
Clipping to Nitrogen	0.158	0.292	0.386	0.758	0.449
Precipitation to Shoot biomass	0.5	0.007	0.002	3.307	<0.001
Precipitation to Air temperature	-0.365	-0.011	0.005	-2.315	0.021
Precipitation to Litter biomass	-0.192	-0.028	0.02	-1.397	0.163
Precipitation to Root biomass	0.221	0.001	0.001	1.244	0.213
Precipitation to Evenness	-0.075	0	0.001	-0.462	0.644
Precipitation to Soil temperature	-0.275	-0.009	0.004	-2.017	0.044
Precipitation to Soil moisture	0.319	0.042	0.019	2.206	0.027
Precipitation to Nitrogen	-0.284	-0.003	0.002	-1.415	0.157
Litter biomass to Root biomass	0.005	0	0.008	0.026	0.979
Litter biomass to Evenness	0.012	0	0.007	0.07	0.944
Litter biomass to Soil temperature	-0.576	-0.123	0.04	-3.09	0.002
Litter biomass to Soil moisture	-0.19	-0.174	0.15	-1.16	0.246
Litter biomass to Nitrogen	-0.025	-0.002	0.018	-0.112	0.911
Root biomass to Evenness	-0.185	-0.163	0.168	-0.967	0.334
Soil temperature to Shoot biomass	-0.204	-0.096	0.048	-1.988	0.047
Soil temperature to Root biomass	0.069	0.014	0.031	0.453	0.651
Soil temperature to Evenness	0.162	0.029	0.022	1.287	0.198
Soil temperature to Soil moisture	-0.317	-1.36	0.544	-2.501	0.012
Soil temperature to Nitrogen	-0.037	-0.015	0.069	-0.212	0.832
Soil moisture to Shoot biomass	-0.103	-0.011	0.009	-1.202	0.229
Soil moisture to Root biomass	0.271	0.013	0.008	1.645	0.1
Soil moisture to Evenness	-0.006	0	0.004	-0.053	0.958
Soil moisture to Nitrogen	0.073	0.007	0.015	0.456	0.648
Nitrogen to Shoot biomass	0.001	0.002	0.072	0.023	0.982
Nitrogen to Root biomass	0.035	0.018	0.047	0.381	0.703
Nitrogen to Evenness	0.109	0.049	0.034	1.468	0.142

Table D-14. Results from linear mixed models of main effects of air temperature (°C), clipping intensity (%), and precipitation (mm), all fixed factor covariates, and interactions with site (AB, SK, and MB) as fixed factors, on both species richness and evenness response variables.

Source of variation	**Richness**		**Evenness**	
	F_{df}	**p-value**	**F_{df}**	**p-value**
Precipitation	$0.682_{2,54}$	0.413	$1.864_{1,54}$	.178
Air temperature	$5.325_{1,54}$	0.025	$1.322_{1,54}$	.255
Clipping intensity	$0.046_{1,54}$	0.832	$.033_{1,54}$	.856
Site	$0.217_{1,54}$	0.806	$2.326_{2,54}$	.107
Precipitation*Site	$1.179_{2,54}$	0.316	$.443_{2,54}$	.644
Air temperature*Site	$0.378_{2,54}$	0.687	$2.714_{2,54}$	.075
Clipping intensity*Site	$3.474_{2,54}$	0.038	$2.052_{2,54}$	.138

12. APPENDIX E: Supplemental material for Chapter 6

E-1. Presenters at the session "Climate change experiments in temperate grasslands" convened on June 20th and 21st, 2011 in Lyon, France, during the 54th Annual Symposium of the International Association for Vegetation Science

Introduction: Carlyle, C. N., White, S. R., Fraser, L. H., and J. C. Cahill. “Climate change experiments in temperate grasslands: Session introduction.”

1) Fraser, L. H, Carlyle, C. N., and R. Turkington. “Interacting effects of climate change and grazing on grassland plant communities along a natural productivity gradient.”
2) Cahill, J. C., White, S. R., Bork, E., Attaien, B., Chang, S., Wilson, S., Nyanumba, S., Newton, J., and H. Proctor. “Complex responses to altered warming, water, and defoliation: Results from a field experiment in three northern grasslands.”
3) Collins, S. L., Thomey, M. L., Xia, Y., Pockman, W. T., and S. Báez. “Rainfall variability and community dynamics in Chihuahuan desert vegetation.”
4) Knapp, A., Smith, M., Collins, S., and J. Blair. “Grassland ecosystem responses to experimental manipulations of the precipitation regime.”
5) Smith, M., Hoover, D., and A. Knapp. “Impacts of climate extremes on a mesic grassland.”

6) Fridley, J., Grime, J. P., Askew, A., Moser, B., and Ravenscroft, C. “The basis of community resistance to climate change in a limestone grassland, and why it may not last.”
7) E. Cleland. “Community trait composition and ecosystem feedbacks to global change: Inter- vs. intra-specific variability and temporal scale.”
8) Knops, J. M. H., Brassil, C. E., and E. K. Miles. “Biodiversity is not at equilibrium but reflects the ghost of ecological processes past.”
9) Henry, H., Hutchison, J., Kim, M. K., Moise, E., and M. Turner. “Plant and soil responses to four years of warming and nitrogen addition in a grass-dominated, northern temperate old field.”
10) Soussana, J., Picon-Cochard, C., Bloor, J., Cantarel, A., Alessio, G., Roy, J., and S. Lavorel. “Assessing the impacts of climate change, elevated CO_2 and extreme events on mesic and alpine grasslands”.
11) Beierkuhnlein, C., Wellstein, C., Kreyling, J., Astor, T., and A. Jentsch. “Sensitivity of plant species to climatic extremes during grassland succession.”
12) Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzae, H., Nadler, S. E., Nagy, L., Otieno, D., Pritsch K., Rascher, U., Schädler, M., Schloter, M., Singh, B. K., Stadler, J., Walter, J. Wellstein, C., Wöllecke, J., C. Beierkuhnlein. “Effects of recurrent severe drought on multiple ecosystem functions in temperature grasslands.”

13) Maalouf, J., Bagousse-Pinguet, Y. L., Marchand, L., Bâchelier, E., Touzard, B., and R. Michalet. “Adapting ecosystem conservation management to climate change: The case of calcareous grasslands subject to simulated drought.”
14) Mariotte, P., Vandenberghe, and A. Buttler. “Subordinate plants species promote resistance of plant community during summer drought”.
15) Dutoit, T., and H. Frederic. “Discriminating climate changes from land-use changes to explain the absence of shrub colonization in a Mediterranean steppe.”
16) Casper, B., Liancourt, P., Petraitis, P., Ariuntsetseg, L., Boldgiv, B., Helliker, B., and A. Plante. “Flowing in the Mongolian steppe: Effects of topography, year, and climate manipulation.”
17) Doležal, J., Janeček. S., Lanta, V., de Bello, F., Lepš, J., and J. Klimešová. “A comparative analysis of water economy-related traits in species-rich seminatural meadows of Central Europe.” (poster)
18) Mantilla-Contreras, J., Möller, I., Spencer, T., and S. Zerbe. “Influence of climate and land use changes on low lying Baltic coastal habitats.” (poster)