Ecology, 95(11), 2014, pp. 3093–3103

Direct and indirect drivers of plant diversity responses to climate and clipping across northern temperate grassland

SHANNON R. WHITE,[1,3] EDWARD W. BORK,[2] AND JAMES F. CAHILL, JR.[1]

[1]*Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada*
[2]*Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5 Canada*

Abstract. It is well known that climate can influence plant community assembly via a multitude of indirect and direct pathways. However, interpretations of plant diversity responses to simulated climate change experiments, and subsequent predictions of plant communities under future climate scenarios, rarely address the importance of indirect effects. Networks of direct and indirect effects are also critical in understanding linkages between climate and grazing, a common land use of grasslands, and implications for plant diversity. We characterized the roles of indirect vs. direct effects in determining plant diversity responses to climate and grazing using data from three northern temperate grasslands in which we conducted factorial experiments manipulating precipitation, air temperature, and clipping intensity. Utilizing a structural equation modeling framework to address the multivariate networks, we found warming operated directly, causing species loss at all sites. We identified shoot biomass as the key indirect driver of diversity loss in response to both precipitation and clipping, regardless of site. However, site-specific contingencies in the network of interactions were important for understanding varied precipitation effects. At the driest site only, shoot biomass was resistant to reduced precipitation, and diversity was consequently unaltered. Similarly, disconnect between primary drivers and responses explained relatively idiosyncratic responses of evenness compared to richness. Importantly, the finding of widespread, directly controlled plant diversity loss with warming aligns with concerns about declining biodiversity under climate change. However, using a framework of network interactions also allowed us to pinpoint the source of variability in response across systems. Looking forward, we can use the identification of this key indirect pathway to guide an understanding of community assembly under factors likely to control shoot biomass. Viewing a multifactorial, multisite experimental approach through a framework of network interactions allowed us to both identify generalized responses and distill the complexity of contingent responses. This, along with the practical need to identify diversity responses to climate change and grazing, underscores the importance of understanding both indirect and direct drivers of ecosystem responses to global change factors.

Key words: climate change; defoliation; evenness; global warming; grazing; land use; manipulative experiment; multisite; rangeland; richness; structural equation modeling.

INTRODUCTION

There is widespread concern over biodiversity loss and subsequent reduced provision of ecosystem services in response to anthropogenic global changes, particularly rising temperatures and altered precipitation patterns (Bellard et al. 2012, Tilman 2012). Accordingly, the responses of plant diversity to these factors have been widely tested in manipulative experiments in many systems, including temperate grassland (i.e., Sternberg et al. 1999, Price and Waser 2000, Zavaleta et al. 2003, Harmens et al. 2004, Klein et al. 2004, Kardol et al. 2010, Yang et al. 2011, Hoeppner and Dukes 2012), the world's largest terrestrial ecosystem. Despite this extensive investigation, synthesis of results have been limited to responses other than plant diversity (Rustad et al. 2001, Lin et al. 2010, Wu et al. 2011) or to systems other than grassland (Elmendorf et al. 2012). Indeed, variability in diversity outcomes from these studies has impeded the development of general conclusions and predictive frameworks (Gornish and Tylianakis 2013). Developing a synthetic understanding of varied responses of biodiversity to climate change will necessitate approaches distinct from those currently utilized. Specifically, the investigation of indirect vs. direct drivers has been identified as an approach capable of addressing variability in complex systems (Shaver et al. 2000, Rustad 2008, Gornish and Tylianakis 2013).

Many complex interacting processes, both indirect and direct, are known to link climate to plant community assembly (Shaver et al. 2000, Rustad 2008, Gornish and Tylianakis 2013). Shaver et al. (2000) identified numerous processes by which climate impacts ecosystem responses, including modifications in nitrogen

Manuscript received 22 January 2014; revised 22 April 2014; accepted 25 April 2014. Corresponding Editor: A. Classen.

[3] E-mail: shannon.white@ualberta.ca

mineralization rates, litter quality and quantity, and soil moisture. Climate change can be directly or indirectly linked to ecosystem processes through these drivers. For example, it has been suggested that heat stress from warming can directly reduce diversity (Klein et al. 2004). Soil moisture, directly controlled by altered precipitation, is known to increase diversity; this linkage between soil moisture and diversity can also be driven indirectly by warming (Yang et al. 2011).

Land use is an additional factor that has been recognized to interact with environmental changes, thus further contributing to the network of pathways linked to diversity. Grasslands are predominantly managed for grazing, which is known to affect diversity directly through species removal, as well as indirectly (Socher et al. 2012) and in interaction with climate (Klein et al. 2004, Butof et al. 2012). However, because of logistical challenges, grazing is rarely included as a treatment in climate manipulations (Rustad 2008). Further, unlike climate, grazing pressure is largely controlled by land managers and can be adjusted for biodiversity targets following an understanding of how grazing affects diversity under various climate change scenarios.

Interactive effects among and between climate and grazing may be best understood through shared pathways. For example, both temperature and precipitation impact soil moisture (Bell et al. 2010) and might be expected to interact. Indeed, Hoeppner and Dukes (2012) found that drought limited ecosystem resistance to warming, with warming decreasing plant species richness, but only when coinciding with drought. One can use knowledge of the specific pathways acting in a system to predict and interpret nonadditive effects elicited by multiple stressors, sensu Folt et al. (1999).

Because the numerous direct and indirect pathways can operate any given system, the net effect of climate and grazing on plant diversity can be highly nuanced. Response patterns, whether similar or dissimilar, can be driven by varied strengths or direction of the same drivers, or, alternatively, from altogether different underlying processes (Gornish and Tylianakis 2013). For example, Price and Waser (2000) observed no effect of warming on structure of an alpine plant community. Rather than attributing this result as community resistance to the direct effect of warming, they posited that indirect negative effects of warming due to decreased soil moisture were offset by the stimulation of earlier snowmelt by warming. By restricting investigations in responses to net effects, we obtain a potentially superficial understanding of the system responses and may fail to reveal the processes driving variability in general.

Despite the acknowledgement of the inherent complexity driving system responses, there has been little attempt to characterize the complicated networks driving ecosystem responses. Indeed, the relative strengths of indirect vs. direct drivers in climate change studies, and their contribution to variability in response, is unknown. However, using a framework that explicitly accounts for networks of responses can actually help distill complexity into common patterns. For example, identifying key indirect drivers operating across systems can allow us to predict how untested systems might respond to other factors impacting those same drivers.

When might system responses be expected to be associated with either direct vs. indirect drivers? Although little work has addressed this question, knowledge of the ecology of the system can be used to hypothesize whether responses to a driver will be directly and indirectly linked to the drivers of diversity. For species at the northern, coldest limit of their range, diversity might be expected to be directly controlled by temperature. However, control of species range is thought to be controlled by an array of variables (Sexton et al. 2009), and it is possible that these communities are inherently less sensitive to the direct effects of temperature and might instead be indirectly affected by warming. Likewise, grasslands at the grassland–forest ecotone, and at the wettest limits at their range, might be expected to be sensitive to changes in moisture (Schneider et al. 2009), which can be either directly or indirectly controlled. Alternatively, mechanisms might be largely site-specific, with large variation in both direct and indirect drivers due to unique climatic conditions and management history.

We hypothesize that understanding indirect vs. direct drivers at multiple sites will help explain not just variability vs. consistency in response patterns across sites, but the underlying processes contributing to any variability. To do this, we created a hypothetical model of direct and indirect pathways linking climate and grazing to plant diversity in northern temperate grassland, sensu Gibson (2009), and challenged the model with data from a multisite experiment. The sites chosen for the experiment are all superficially similar, but differ in terms of climatic conditions (both those relating to temperature and precipitation), as well as plant productivity and grazing history. Because of these differences, the sites might be expected to vary in terms of their responses to climate and grazing, especially when viewed as a network of indirect and direct drivers. By characterizing the complexity of diversity responses to climate and grazing and by identifying key drivers, we aim to contribute to a predictive understanding of how direct and indirect effects drive variability in grassland diversity.

Materials and Methods

We established a fully randomized, factorial experiment manipulating temperature (control and warming), precipitation (ambient and reduced), and clipping intensity (none, low, high) at three sites across the Canadian prairies for three growing seasons (2007–2009). Consistent experimental methodology was used across sites to ensure any variability in results was due to biologically based, system-specific dynamics (Vicca et al.

2012, White et al. 2014). We present values from the final year, 2009, of experimentation, as these data represent the greatest cumulative responses (data from other years not shown). Clipping was used as an alternative to grazing as experimental infrastructure precluded the presence of large herbivores within plots. Plots were circular, 2 m in diameter, and separated by at least 0.5 m. In each plot, we sampled a central permanent subplot (50 × 50 cm) where all disturbances other than the treatments were minimized. The 12 treatment combinations were replicated five times at each site, for a total of 60 plots at each site. We used these 180 plots in a complementary, univariate, generalized linear mixed model (GLMM) approach. For the multivariate approach used to separate indirect and direct effects, we used data from a subset (two replicates of each combination from each site; 72 plots total) of plots fully monitored for environmental variables. We were thus able to expand our analysis by using continuous predictor variables, rather than the five treatment combinations used as categorical predictor variables in the GLMM, allowing us to integrate the unique environmental conditions expected to result from landscape variability and interactions between treatment variables. As our multivariate modeling approach, structural equation modeling (SEM) is based on regression of continuous variables, sample size in SEM is based on total sample size, rather than replicates (Grace 2006, Clark et al. 2007).

Site description

Sites were chosen to represent a range of climate and vegetation conditions across North American northern temperate grassland. The Alberta (AB) site was at the University of Alberta Kinsella Research Station, about 170 km southeast of Edmonton (53°0′59.5404″ N, 111°32′23.6322″ W), within the Aspen Parkland ecoregion, in a landscape of rough fescue (*Festuca hallii* [Vasey] Piper) dominated grassland and intermittent aspen (*Populus tremuloides*) stands. The Saskatchewan (SK) site was at the Gap Community Pasture, 130 km south of Regina (49°18′1.404″ N, 104°38′2.259″ W), in the mixed grassland ecoregion, dominated by *Hesperostipa curtiseta*, *Pascopyrum smithii* (Rydb.) Á. Löve, and dryland *Carex* spp. Also in Aspen Parkland, the Manitoba (MB) site was located in Riding Mountain National Park, about 200 km northwest of Winnipeg (50°46′52.4964″ N, 100°35′36.222″ W) in rough fescue grassland amidst primarily boreal forest. Dominant plant species at MB included *Poa secunda* J. Presl., dryland *Carex* spp., and *Monarda fistulosa* L. Based on control plots, the biomass at AB and SK were predominantly (approximately 75%) graminoids, with the remainder composed of forbs, and minimal (<1%) shrubs at AB. Site MB was 50% forbs, with 40% graminoids and 10% shrub biomass. All grasslands were dominated by native, C_3, perennial plant species, and thought to be free of previous tillage.

All sites have a history of grazing by endemic ungulates (deer, elk, antelope, moose, and bison), which continues today with the exception of bison, which have been replaced by cattle in most areas. Grazing history at AB and SK is similar and consistent with local cattle grazing practices, while grazing at MB has been restricted to native ungulates since 1970 due to its national park status (Brook 2009). At all sites, fencing was used to prevent grazing by large mammals during the study. Long-term mean annual precipitation (MAP) is highest at MB, followed by AB and SK (White et al. 2014). SK has the highest long-term mean annual temperature (MAT), followed by AB and MB. For more site details see White et al. (2014).

Treatments

Half the plots received a passive warming treatment using fiberglass open-top chambers (OTCs; Sunlite-HP, Solar Components/Kalwall, Manchester, New Hampshire, USA), which increased mean daytime air temperatures by 1–3°C (White et al. 2014). 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.

Precipitation was reduced in half the plots, while the other half received ambient (control) precipitation. Precipitation was manipulated using rain-out shelters composed of 6.25 m^2, 60–120 cm tall wood frames with plastic tops (Dura-Film Super 4 6-mil polyethylene film; AT Plastics, Edmonton, Alberta, Canada); see details in White et al. (2012). 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 control for any effects of the structure itself. To maintain consistency of other environmental conditions between treatments, shelters were installed over all plots.

Clipping treatments were applied annually in late June, with plots either not clipped (i.e., controls) or clipped to a height of 7.5 cm (low intensity) or 3.5 cm (high intensity) above ground level. These clipping intensities are consistent with recommended local grazing management (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.

Model overview

To identify and quantify direct and indirect responses of diversity to the treatments, we used SEM, a technique well-suited to assessing relationships among networks of variables (Didham et al. 2005, Grace 2006, Gornish and Tylianakis 2013). Unlike traditional regression tech-

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

Variables	Source	Units	Sampling period
Treatment variables			
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 (7.5 cm) and high (25 cm) data loggers	°C	mid-May until late July
Clipping intensity	clipped biomass per plot divided by average nonclipped shoot biomass for that temperature and precipitation treatment	%	clipped biomass (June); shoot biomass (late July)
Intermediary environmental variables			
Soil moisture	average of shallow (0–5 cm) and deep (7.5–17.5 cm) soil probes (04:00–06:00 only)	% VWC	mid-May until late July
Soil temperature	average of shallow and deep soil probes	°C	mid-May until late July
Soil nitrogen	plant root simulator (PRS) probes	(mg total nitrogen)/(10 cm^2 ion-exchange membrane surface area)	late June through early September
Intermediary vegetation variables			
Litter biomass	10 × 100 cm vegetation strip	g/m^2	late July
Shoot biomass	10 × 100 cm vegetation strip (nonclipped plots), and 50 × 50 cm sampling plot (clipped plots)	g/m^2	late July (non-clipped plots); late July and June (clipped plots)
Root biomass	5 cm diameter, 20 cm deep core	g/m^2	late July
Response variables			
Richness	percent cover in 50 × 50 cm sampling plot	number of species/0.25m^2	late July
Evenness	percent cover in 50 × 50 cm sampling plot	no unit (index)	late July

Note: MB means Manitoba and VWC means volumetric water content.

niques, variables in SEM are able to act simultaneously as both predictor and response variables, termed exogenous and endogenous variables, respectively, in SEM (Grace 2006). Further, to understand how variation in this network of interactions contributes to divergence in responses among sites, we used a multigroup SEM approach (Grace and Jutila 1999, Grace 2006), which differentiates pathways operating consistently across sites from those acting idiosyncratically. Using this multivariate statistical modeling framework, we assessed the direct effects of the environmental drivers associated with the treatments, as well as effects that acted indirectly via intermediary variables.

Environmental variables directly controlled by the treatments were air temperature (°C), precipitation (mm), and clipping intensity (%). Intermediary environmental variables were soil moisture (percentage of soil volumetric water content [VWC]), soil temperature (°C), and soil nitrogen (mg total inorganic nitrogen/10-cm^{-2} ion-exchange membrane surface area). Litter biomass (g/m^2), shoot biomass (g/m^2), and root biomass (g/m^2) were intermediary vegetation variables. Species richness (number of species/0.25 m^2) and evenness (per 0.25 m^2), representing plant diversity, were the ultimate response variables. With the exception of precipitation, which was coded as a binary variable, all environmental variables and response variables were monitored in each of the 72 plots. See Table 1 for summary information on variables and sampling and Table 2 for control plot average values of sampled variables at each site. Full sampling methodology can be found in Appendix A.

We created an initial model linking these variables based on our expectation of biological processes likely to be important for diversity in northern temperate grassland systems (see Appendix A: Fig. A1). Paths with single-headed arrows represent hypothesized causal relationships, while paths with double-headed curved arrows represent variables that covary. The model was tested separately for richness and evenness, using Amos 18.0 (Amos Development, Crawfordville, Florida, USA). See Appendix A for further details on model specification.

Univariate analyses

To evaluate net treatment effects on both richness and evenness, and to perform explicit testing for interactions between treatments, we analyzed the data using generalized linear mixed models (GLMMs). We tested for the main and interactive effects of warming, precipitation, and clipping (fixed factors), and site (random factor) on

TABLE 2. Values of variables in structural equation models of precipitation, air temperature, and clipping intensity on richness and evenness.

Variable	AB	SK	MB
Precipitation (mm)	66.7	68.5	107.4
Air temperature (°C)	20.7 ± 0.2	22.6 ± 0.1	21.8 ± 0.1
Clipping intensity: low (%)	0.33 ± 0.02	0.36 ± 0.17	0.36 ± 0.09
Clipping intensity: high (%)	0.45 ± 0.25	0.41 ± 0.16	0.34 ± 0.04
Soil moisture (% VWC)	17.0 ± 0.1	17.2 ± 3.2	21.4 ± 2.6
Soil temperature (°C)	13.9 ± 1.1	17.7 ± 0.2	12.9 ± 0.4
Soil nitrogen (mg total N)†	48.7 ± 15.1	17.0 ± 9.4	8.8 ± 4.0
Litter biomass (g/m^2)	21.8 ± 2.9	190.4 ± 23.0	445.5 ± 6.5
Shoot biomass (g/m^2)	248.5 ± 53.4	119.5 ± 43.4	355.4 ± 160.0
Root biomass (g/m^2)	637.6 ± 28.0	441.2 ± 46.2	1106.0 ± 341.0
Richness (number of species/0.25m^2)	7.5 ± 0.5	7.0 ± 0.0	10 ± 1
Evenness (no unit, index)	0.88 ± 0.04	0.88 ± 0.01	0.918 ± 0.02

Notes: Values (mean ± SE) are from control plots, with the exception of precipitation, which was not measured at the plot level, and clipping intensity. Clipping intensity was calculated as percent biomass removed by dividing clipped biomass per plot by average nonclipped shoot biomass of plots with the same corresponding climate treatment. Abbreviations are: AB, Alberta; SK, Saskatchewan; MB, Manitoba; VWC, volumetric water content.

† See Table 3.

richness and evenness. A Poisson distribution and log link was chosen to model richness as a count variable. Evenness was modelled with a gamma distribution and log link, as it has only nonnegative values. We used variance components as the covariance structure for the random effect. Both GLMMs were estimated using SPSS Version 22.0.0 (SPSS IBM, Armonk, New York, USA).

RESULTS

Final SEM models provided satisfactory fit to the data (richness, $\chi^2_{91} = 83.6$, $P = 0.697$, Tucker-Lewis Index (TLI) = 1.073, root mean square error of approximation (RMSEA) = <0.001; evenness $\chi^2_{92} = 85.1$, $P = 0.683$, TLI = 1.070, RMSEA = <0.001). Standardized coefficients, unstandardized coefficients, and t test results are presented in Appendix B. We first present those statistically significant ($P \leq 0.05$) pathways involving the response variables, then pathways that did not affect the response variables but were nonetheless statistically significant, and finally results from the GLMMs.

Richness

At all sites, richness decreased with increases in air temperature and clipping intensity, but increased with more precipitation (Fig. 1 and Table 3). Air temperature decreased richness through direct rather than indirect effects at all sites. The opposite was true of clipping intensity, with clipping only indirectly decreasing richness. The indirect effect of clipping on richness was initiated through a negative effect of clipping on litter; litter was associated with increased shoot biomass, and shoot biomass increases richness. At AB (Fig. 1a) and MB (Fig. 1c), richness also decreased with clipping intensity via an increase in air temperature, which negatively affects richness. At SK (Fig. 1b), clipping intensity did not affect air temperature, with no associated indirect pathway to richness. However, clipping did increase soil nitrogen at this location, which in turn reduced richness (Fig. 1b). Notably, the influence of soil nitrogen in reducing richness was apparent at all three sites, but, with the exception of SK, was not mediated by the main treatments.

Precipitation increased richness through several indirect pathways. At all sites, increased precipitation decreased air temperature, which in turn led to greater richness (Fig. 1). At SK, no further linkages between precipitation and richness were apparent. At AB and MB, however, precipitation increased shoot biomass, and ultimately richness. The total effect of precipitation on richness was strongest in MB and weakest in SK.

Evenness

Evenness was less responsive to both treatment variables and intermediary variables than richness, and responses to evenness varied widely among sites, with no consistent effects among sites (Table 4 and Fig. 2). At MB (Fig. 2c), changes in evenness were related directly and indirectly to air temperature. Increased air temperature directly decreased evenness, but greater precipitation also indirectly increased evenness via reductions in air temperature. Clipping intensity decreased evenness at SK and MB, albeit through different pathways. At SK, this effect was direct (Fig. 2b), while in MB it was mediated by increases in air temperature (Fig. 2c). There were no other effects of the environmental treatments on evenness at SK, although root biomass, which remained decoupled from the treatments, was associated with increased evenness (Fig. 2b). In AB, there were no significant relationships with evenness (Fig. 2a).

Other

In both the richness (Fig. 1) and evenness (Fig. 2) models at all sites, greater precipitation increased soil moisture and decreased both air and soil temperatures. At AB and MB, but not SK, reduced precipitation decreased shoot biomass and clipping intensity increased

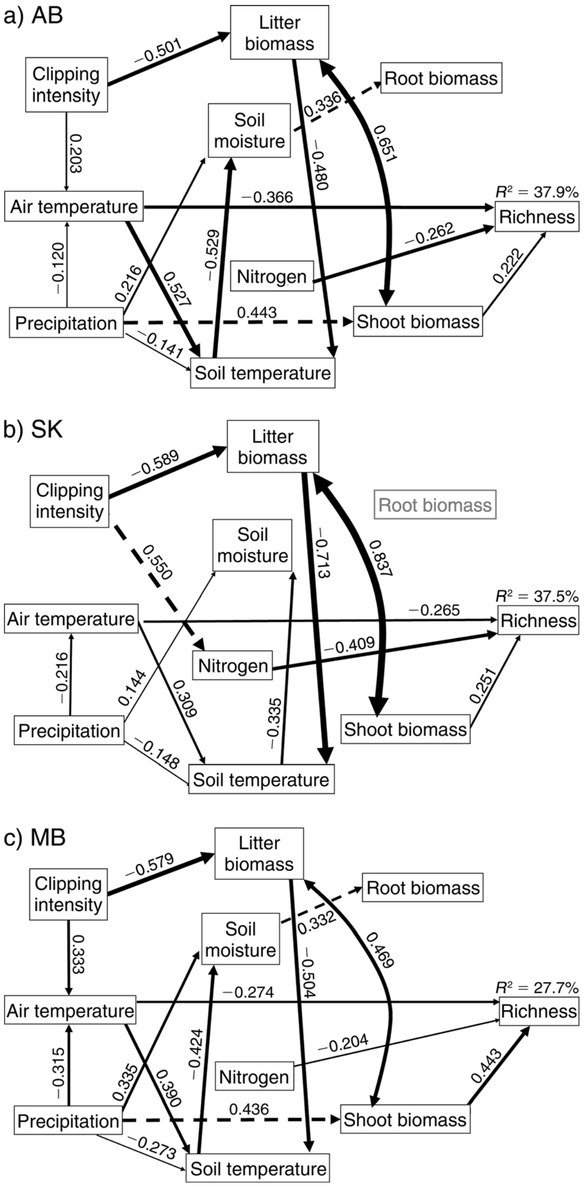

FIG. 1. Arrows represent significant ($P < 0.05$) paths in a fitted multigroup structural equation model depicting the effects of treatment variables on intermediary environmental and biological variables, and ultimately species richness, for (a) Alberta (AB), (b) Saskatchewan (SK), and (c) Manitoba (MB). Thick 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 covarying variables. Numbers on arrows and width of arrows correspond to standardized path strength. The variable lacking a significant relationship with other variables in the model is presented in gray.

air temperature, in both models of richness and evenness. Increased air temperature also increased soil temperature at all sites, which decreased soil moisture. Clipping intensity had a consistent negative effect on litter, while litter had a positive relationship with shoot biomass and decreased soil temperature.

GLMMs

Generalized linear mixed models revealed a main effect of precipitation on richness ($P = 0.026$); richness decreased in the reduced precipitation treatment (Fig. 3 and Appendix C). There were no significant ($P < 0.05$) treatment effects on evenness (Fig. 4 and Appendix C).

DISCUSSION

Plant species richness was sensitive to variables associated with all three experimental treatments (warming, precipitation, and clipping) across the sites. Effects of air temperature on richness were direct rather than indirect, while clipping intensity and precipitation generally acted indirectly. Key drivers controlling richness were consistent across sites, mainly involving changes in shoot biomass or air temperature. Conversely, evenness showed more resistance to change, but also more site-specific variation in responses when they did occur, suggesting that generality of results cannot be extended across response variables. Univariate statistical results were consistent with those of the SEM models but led to greatly oversimplified conclusions. In contrast, the SEM approach revealed additional insight on the underlying ecological relationships (Grace 2006).

At all three sites, we found increased air temperature decreased richness. The negative effect of air temperature was consistently a result of direct effects despite the expectation that responses to warming involve indirect pathways (Shaver et al. 2000, Yang et al. 2011). Meta-analysis of tundra responses (Walker et al. 2006) and a number of individual grassland warming studies (Klein et al. 2004, Yang et al. 2011) have revealed negative responses of richness to warming; however, resistance of grassland diversity to the direct effect of warming has been displayed as well (Grime et al. 2008, Hoeppner and Dukes 2012). It remains unknown whether the resistance to diversity exhibited in these studies stems from a lack of direct linkages from warming to community assembly or from a positive indirect pathway counteracting the negative direct effect of warming. Our results reveal warming itself as a key determinant of species diversity in northern temperate grassland. Warming can directly affect diversity through species-specific physiological responses, such as heat stress (Klein et al. 2004), or through ecological factors such as altered species interactions (Farrer et al. 2014). Further work is required to determine if diversity in systems at the northern, and thus coldest, edge of their range are more likely to be directly reduced by warming.

As observed at the AB and MB sites, precipitation generally has a positive relationship with grassland richness (Zavaleta et al. 2003, Yang et al. 2011, Hoeppner and Dukes 2012). We were able to further identify the indirect driver, shoot biomass, linking reduced precipitation to decreased richness. However, while this pathway was not shared by SK, shoot biomass did influence richness at this site, with a disconnect between precipitation and shoot biomass accounting for

TABLE 3. 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 ($P < 0.05$) SEM paths.

Treatments and effects	AB	MB	SK
Precipitation			
Direct	···	···	···
Indirect	precipitation, shoot biomass, richness (0.10) precipitation, air temperature, richness (0.04)	precipitation, shoot biomass, richness (0.19) precipitation, air temperature, richness (0.09)	precipitation, air temperature, richness (0.06)
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, air temperature, richmess (−0.07)	clipping intensity, litter, shoot biomass, richness (−0.12) clipping intensity, air temperature, richness (−0.09)	clipping intensity, litter, shoot biomass, richness (−0.12) clipping intensity, nitrogen, richness (−0.22)
Total	−0.15	−0.21	(−0.32)

Notes: Only paths originating with the treatment variables and terminating with richness included here. SEM means structural equation modeling. Cells with ellipses signify no significant effect observed. The numbers in parentheses correspond to the standardized values of pathway strength. See Table 2 for site abbreviations.

the anomaly. Thus, although the effects of these direct and indirect drivers might shift over prolonged manipulation, SK, the driest site, appeared largely resistant to soil moisture effects over the three-year period of our study. Others studies (Knapp and Smith 2001, Gerten et al. 2008) have suggested that arid and semiarid plant communities are adapted to drought compared to their mesic counterparts, and thus, biomass is resistant to reduced precipitation. We also observed a weak indirect effect of precipitation on air temperature, and subsequently, richness, at all sites. However, the increase in air temperature with precipitation is most likely an artefact of the experimental infrastructure used (Beier et al. 2012).

Clipping intensity consistently decreased diversity at all sites. Many studies have shown that clipping increases plant diversity (Collins et al. 1998, Harmens et al. 2004, Klein et al. 2004), while others, including those in Canadian prairies, have shown very high grazing intensities, represented here by the 3.5 clipping height decrease diversity, consistent with the intermediate disturbance hypothesis (Bai et al. 2001, Vujnovic et al. 2002). Through reductions in litter, clipping treatments were sufficiently intense to reduce shoot biomass, a major control of richness at our sites, consistently resulting in loss of richness. Litter has previously been shown to increase shoot growth in these systems (Willms et al. 1986). There was also a weak negative effect of clipping intensity on richness through air temperature, likely driven by changes in vegetation structure and the loss of insulating litter (Facelli and Pickett 1991). At SK only, clipping also decreased richness by an increase in

TABLE 4. 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 ($P < 0.05$) SEM paths.

Treatments and effects	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

Notes: Only paths originating with the treatment variables and terminating with evenness included here. SEM means structural equation modeling. Cells with ellipses signify no significant effect observed. The numbers in parentheses correspond to the standardized values of pathway strength. See Table 2 for site abbreviations.

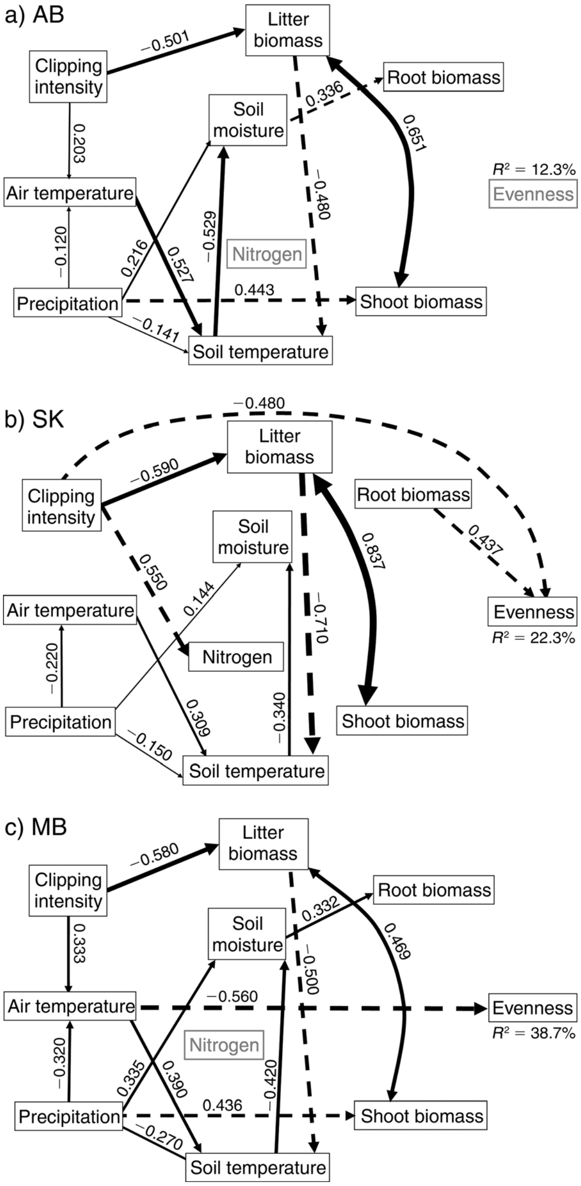

FIG. 2. Arrows represent significant ($P < 0.05$) paths in a fitted multigroup structural equation model depicting the effects of treatment variables on intermediary environmental and biological variables, and ultimately species evenness, for (a) Alberta (AB), (b) Saskatchewan (SK), and (c) Manitoba (MB). Thick arrows indicate paths consistent across all sites; dashed arrows indicate paths that varied by site. Single-headed arrows represent causal relationships, while double-headed arrows represent covarying variables. Numbers on arrows and width of arrows correspond to standardized path strength. Variables lacking significant relationships with variables in the model are presented in gray.

soil nitrogen. Nitrogen enrichment is known to cause species loss, although the magnitude of loss is highly variable across sites (Clark et al. 2007).

Shoot biomass consistently acted as a key driver of diversity, mediating effects of precipitation and clipping on richness. Although highly debated, a positive quadratic relationship between biomass or productivity and species richness has often been described, shifting from positive to negative as productivity increases (Waide et al. 1999, Mittelbach et al. 2001). As our precipitation treatment reduced rather than added resources, and because our grasslands were initially of low to moderate productivity (Gibson 2009), we would expect a positive relationship between shoot biomass and richness, consistent with expectations under lower productivity. Compared to other factors, biomass has been shown to be a strong determinant of richness (Grace 1999). Our results suggest that by using our knowledge of which factors are expected to influence shoot biomass, we can predict which factors will indirectly affect grassland diversity.

The relatively consistent responses and sensitivity of richness across the three sites contrasted with the inconsistent responses and relative stability of evenness. Indeed, the link between evenness and shoot biomass, the main intermediary variable controlling richness, is less established than the link between richness and shoot biomass (Wilsey and Potvin 2000, Mulder et al. 2004, Wilsey et al. 2005). Decreases in evenness are often the first step towards species loss (Chapin et al. 2000, Hoeppner and Dukes 2012). However, the only examples of parallel decreases in both richness and evenness were at MB and involved air temperature. Species loss

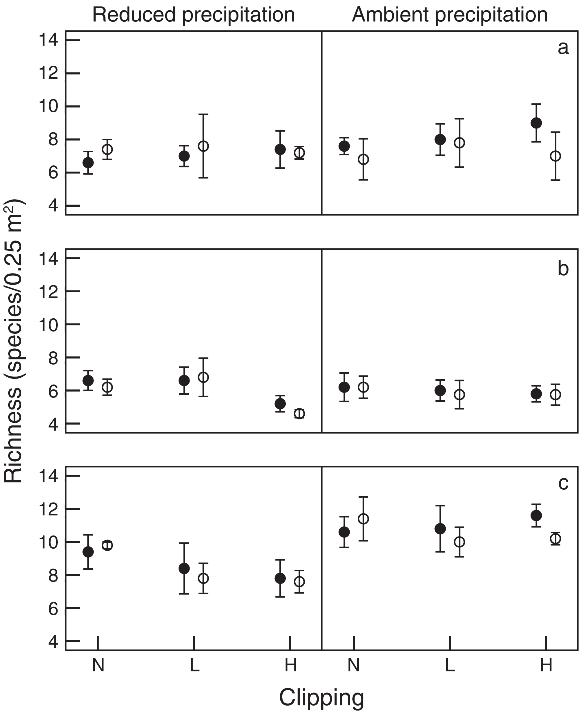

FIG. 3. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, N; low intensity, L; high intensity, H) on species richness for (a) Alberta (AB), (b) Saskatchewan (SK), and (c) Manitoba (MB). Error bars represent ±SE.

was likely linked to dominance of select species, perhaps facilitated at only the MB site due to its high productivity and species richness and lack of recent grazing history.

Site differences can help interpret the variation observed in evenness responses across the sites. In SK, evenness was directly controlled by clipping and root biomass. As previously discussed, SK is the driest site, and thus plant controls may be predominantly belowground rather than aboveground. It is also the site with the lowest net primary productivity (NPP), and thus clipping may have an exaggerated ability to remove species. Our model explained very little variation at the AB site, suggesting that evenness is controlled by additional factors not considered in this study. Lamb (2008) found that litter, which was not directly manipulated in our study, was the main driver of evenness at a nearby rough fescue grassland.

By identifying the indirect pathways by which these environmental drivers act, we can identify potential interacting mechanisms regulating diversity. In our grasslands, richness decreased with warming, both as a direct result of the warming, and indirectly, by clipping leading to higher temperatures. In MB, these effects were further extended to include evenness. This suggests that clipping and warming together may have a stronger effect on richness than their single-factor responses would indicate, although this interaction was not evident in the GLMM, possibly because the clipping effect was insignificant as well or because the identities of sensitive species were redundant between the two treatments. Klein et al. (2004) found that clipping and warming had interactive effects on richness, with clipping dampening the effect of warming on richness, although they did not assess the indirect contributions to this interaction. In AB and MB, both precipitation and clipping treatments decreased richness via the same mediating factor (shoot biomass), and thus have the potential to interact to exacerbate plant species loss. We did not observe this interaction in the GLMM; this may be due to a threshold to the decrease in shoot biomass or because these relationships were observed in only two of the three sites.

We demonstrate negative effects of the environmental drivers associated with warming, reduced precipitation, and clipping treatments on species richness at three grassland sites representing gradients of biogeoclimatic conditions. Not only was the effect of air temperature on richness consistent in direction and mechanism among sites, but this finding is consistent with concerns over declining diversity under climate change (Bellard et al. 2012). The implications of diversity 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 other factors, such as grazing, that are known to influence diversity, air temperature cannot be directly modified by resource managers. However, grazing can be further utilized to moderate air temperatures and conserve litter, both which help maintain species diversity.

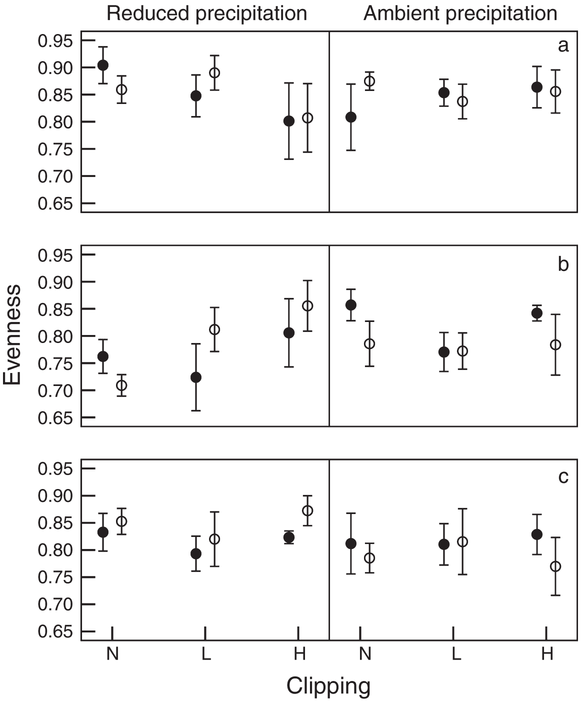

FIG. 4. Effects of precipitation (ambient and reduced), warming (solid circles, control; open circles, warmed), and clipping (no clipping, N; low intensity, L; high intensity, H) on species evenness for (a) Alberta (AB), (b) Saskatchewan (SK), and (c) Manitoba (MB). Error bars represent ±SE.

By using a framework able to identify both direct and indirect responses, we were able to identify the primary drivers of richness in these grasslands: air temperature and shoot biomass. Other intermediaries affected by the treatments, but not related to diversity (e.g., soil moisture, soil temperature) did not contribute to responses. Additional global change factors that affect air temperature and shoot biomass might thus be expected to further impact diversity at these sites, and similar systems might be expected to respond in the same way to warming and factors that influence shoot biomass. Using this framework, we were also able to attribute the lack of response in evenness to resistance to these factors, rather than to a net response of positive and negative effects. In terms of both direct and indirect pathways, evenness responded idiosyncratically across sites, while richness responses were relatively consistent across sites, suggesting that certain aspects of diversity may have predictable responses to factors associated with climate change and grazing. We were also able to attribute the stability of richness despite reduced precipitation at the driest site to the ecosystem's overall resistance in shoot biomass, rather than from the resistance of richness itself; this was not evident using the GLMM approach.

We also illustrate the complexity of ecosystem responses that can unfold following seemingly simple modifications of single factors. Without appropriate experimental designs (De Valpine and Harte 2001) or statistical frameworks, it is difficult to identify these complex effects and differentiate the nature of pathways involved in ecosystem change (Didham et al. 2005). However, by understanding the key drivers of system responses, we can identify the factors underlying variability between systems and move toward predicting how systems will respond. We suggest that large-scale multisite experimental approaches, such as the coordinated distributed experiments described in Fraser et al. (2013), coupled with statistical or experimental techniques able to parse out indirect pathways, be used to further investigate ecosystem responses to factors of interest, including climate change.

ACKNOWLEDGMENTS

This research was supported by the University of Alberta, a Natural Sciences and Engineering Research Council (NSERC) Strategic grant, BIOCAP Canada, Alberta Environment and Sustainable Resource Development, the Agricultural Research Extension Council of Alberta, Ducks Unlimited Canada, Agriculture and Agri-Food Canada, the Alberta Cooperative Conservation Research Unit, and the Alberta Prairie Conservation Forum. S. R. White was supported by a NSERC Postgraduate Scholarship—Masters and by the Prairie Adaptation Research Collaborative. Heather C. Proctor, Scott Chang, Scott D. Wilson, Samson M. Nyanumba, Yang Lin, Behnaz Attaeian, and Jeffrey Newton contributed extensively as project collaborators. Rolf Vinebrooke, M. Derek Mackenzie, and Greg H. R. Henry provided feedback in the final stages. We would like to thank numerous technical assistants and Bryon H. Shore, the project manager.

LITERATURE CITED

Bai, Y. G., Z. Abouguendia, and R. E. Redmann. 2001. Relationship between plant species diversity and grassland condition. Journal of Range Management 54:177–183.

Beier, C., et al. 2012. Precipitation manipulation experiments—challenges and recommendations for the future. Ecology Letters 15:899–911.

Bell, J. E., R. Sherry, and Y. Q. Luo. 2010. Changes in soil water dynamics due to variation in precipitation and temperature: an ecohydrological analysis in a tallgrass prairie. Water Resources Research 46. http://dx.doi.org/10.1029/2009WR007908

Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity. Ecology Letters 15:365–377.

Brook, R. K. 2009. Historical review of elk–agriculture conflicts in and around Riding Mountain National Park, Manitoba, Canada. Human–Wildlife Interactions 3:72–87.

Burkinshaw, A., M. Willoughby, K. France, H. Loonen, and R. McNeill. 2009. Range plant communities and range health assessment guidelines for the central parkland subregion of Alberta. Alberta Sustainable Resource Development. Government of Alberta, Red Deer, Alberta.

Butof, A., L. R. von Riedmatten, C. F. Dormann, M. Scherer-Lorenzen, E. Welk, and H. Bruelheide. 2012. The responses of grassland plants to experimentally simulated climate change depend on land use and region. Global Change Biology 18:127–137.

Chapin, F. S., III, et al. 2000. Consequences of changing biodiversity. Nature 405:234–242.

Clark, C. M., E. E. Cleland, S. L. Collins, J. E. Fargione, L. Gough, K. L. Gross, S. C. Pennings, K. N. Suding, and J. B. Grace. 2007. Environmental and plant community determinants of species loss following nitrogen enrichment. Ecology Letters 10:596–607.

Collins, S. L., A. K. Knapp, J. M. Briggs, J. M. Blair, and E. M. Steinauer. 1998. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 280:745–747.

De Valpine, P., and J. Harte. 2001. Plant responses to experimental warming in a montane meadow. Ecology 82: 637–648.

Didham, R. K., J. M. Tylianakis, M. A. Hutchison, R. M. Ewers, and N. J. Gemmell. 2005. Are invasive species the drivers of ecological change? Trends in Ecology and Evolution 20:470–474.

Elmendorf, S. C., et al. 2012. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecology Letters 15:164–175.

Facelli, J. M., and S. T. A. Pickett. 1991. Plant litter: its dynamics and effects on plant community structure. Botanical Review 57:1–32.

Farrer, E. C., I. W. Ashton, J. Knape, and K. N. Suding. 2014. Separating direct and indirect effects of global change: a population dynamic modeling approach using readily available field data. Global Change Biology 20:1238–1250.

Folt, C. L., C. Y. Chen, M. V. Moore, and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. Limnology and Oceanography 44:864–877.

Fraser, L. H., et al. 2013. Coordinated distributed experiments: an emerging tool for testing global hypotheses in ecology and environmental science. Frontiers in Ecology and the Environment 11:147–155.

Gerten, D., et al. 2008. Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. Global Change Biology 14:2365–2379.

Gibson, D. J. 2009. Grasses and grassland ecology. Illustrated edition. Oxford University Press, New York, New York, USA.

Gornish, E. S., and J. M. Tylianakis. 2013. Community shifts under climate change: mechanisms at multiple scales. American Journal of Botany 100:1422–1434.

Grace, J. B. 1999. The factors controlling species density in herbaceous plant communities: an assessment. Perspectives in Plant Ecology, Evolution and Systematics 2:1–28.

Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge, Massachusetts, USA.

Grace, J. B., and H. Jutila. 1999. The relationship between species density and community biomass in grazed and ungrazed coastal meadows. Oikos 85:398–408.

Grime, J. P., J. D. Fridley, A. P. Askew, K. Thompson, J. G. Hodgson, and C. R. Bennett. 2008. Long-term resistance to simulated climate change in an infertile grassland. Proceedings of the National Academy of Sciences USA 105:10028–10032.

Harmens, H., P. D. Williams, S. L. Peters, M. T. Bambrick, A. Hopkins, and T. W. Ashenden. 2004. Impacts of elevated atmospheric CO_2 and temperature on plant community structure of a temperate grassland are modulated by cutting frequency. Grass and Forage Science 59:144–156.

Hoeppner, S. S., and J. S. Dukes. 2012. Interactive responses of old-field plant growth and composition to warming and precipitation. Global Change Biology 18:1754–1768.

IPCC. 2007. Summary for policymakers. Pages 1–18 *in* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Climate change 2007: the physical science basis. Cambridge University Press, New York, New York, USA.

Kardol, P., C. E. Campany, L. Souza, R. J. Norby, J. F. Weltzin, and A. T. Classen. 2010. Climate change effects on plant biomass alter dominance patterns and community evenness in an experimental old-field ecosystem. Global Change Biology 16:2676–2687.

Klein, J. A., J. Harte, and X. Q. Zhao. 2004. Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. Ecology Letters 7: 1170–1179.

Knapp, A. K., and M. D. Smith. 2001. Variation among biomes in temporal dynamics of aboveground primary production. Science 291:481–484.

Lamb, E. G. 2008. Direct and indirect control of grassland community structure by litter, resources, and biomass. Ecology 89:216–225.

Lin, D. L., J. Y. Xia, and S. Q. Wan. 2010. Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. New Phytologist 188:187–198.

Marion, G. M., et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3:20–32.

Mittelbach, G. G., C. F. Steiner, S. M. Scheiner, K. L. Gross, H. L. Reynolds, R. B. Waide, M. R. Willig, S. I. Dodson, and L. Gough. 2001. What is the observed relationship between species richness and productivity? Ecology 82:2381–2396.

Mulder, C. P. H., E. Bazeley-White, P. G. Dimitrakopoulos, A. Hector, M. Scherer-Lorenzen, and B. Schmid. 2004. Species evenness and productivity in experimental plant communities. Oikos 107:50–63.

Price, M. V., and N. M. Waser. 2000. Responses of subalpine meadow vegetation to four years of experimental warming. Ecological Applications 10:811–823.

Rustad, L. E. 2008. The response of terrestrial ecosystems to global climate change: towards an integrated approach. Science of the Total Environment 404:222–235.

Rustad, L. E., J. L. Campbell, G. M. Marion, R. J. Norby, M. J. Mitchell, A. E. Hartley, J. H. C. Cornelissen, and J. Gurevitch. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126:543–562.

Schneider, R. R., A. Hamann, D. Farr, X. L. Wang, and S. Boutin. 2009. Potential effects of climate change on ecosystem distribution in Alberta. Canadian Journal of Forest Research 39:1001–1010.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annual Review of Ecology, Evolution, and Systematics 40:415–436.

Shaver, G. R., et al. 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. Bioscience 50:871–882.

Socher, S., D. Prati, J. Müller, V. H. Klaus, N. Hölzel, and M. Fischer. 2012. Direct and productivity-mediated indirect effects of fertilization, mowing and grazing intensities on grassland species richness. Journal of Ecology 100:1391–1399.

Sternberg, M., V. K. Brown, G. J. Masters, and I. P. Clarke. 1999. Plant community dynamics in a calcareous grassland under climate change manipulations. Plant Ecology 143:29–37.

Tilman, D. 2012. Biodiversity and environmental sustainability amid human domination of global ecosystems. Daedalus 141: 108–120.

Vicca, S., et al. 2012. Urgent need for a common metric to make precipitation manipulation experiments comparable. New Phytologist 195:518–522.

Vujnovic, K., R. W. Wein, and M. R. T. Dale. 2002. Predicting plant species diversity in response to disturbance magnitude in grassland remnants of central Alberta. Canadian Journal of Botany 80:504–511.

Waide, R. B., M. R. Willig, C. F. Steiner, G. Mittelbach, L. Gough, S. I. Dodson, G. P. Juday, and R. Parmenter. 1999. The relationship between productivity and species richness. Annual Review of Ecology and Systematics 30:257–300.

Walker, M. D., et al. 2006. Plant community responses to experimental warming across the tundra biome. Proceedings of the National Academy of Sciences USA 103:1342–1346.

White, S. R., E. W. Bork, J. Karst, and J. F. Cahill. 2012. Similarity between grassland vegetation and seed bank shifts with altered precipitation and clipping, but not warming. Community Ecology 13:129–136.

White, S. R., J. F. Cahill, and E. W. Bork. 2014. Implications of precipitation, warming, and clipping for grazing resources in Canadian prairies. Agronomy Journal 106:33–42.

Willms, W. D., S. Smoliak, and A. W. Bailey. 1986. Herbage production following litter removal on Alberta native grasslands. Journal of Range Management 39:536–540.

Wilsey, B. J., D. R. Chalcraft, C. M. Bowles, and M. R. Willig. 2005. Relationships among indices suggest that richness is an incomplete surrogate for grassland biodiversity. Ecology 86: 1178–1184.

Wilsey, B. J., and C. Potvin. 2000. Biodiversity and ecosystem functioning: importance of species evenness in an old field. Ecology 81:887–892.

Wu, Z., P. Dijkstra, G. W. Koch, J. Penuelas, and B. A. Hungate. 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. Global Change Biology 17:927–942.

Yang, H. J., M. Y. Wu, W. X. Liu, Z. Zhang, N. L. Zhang, and S. Q. Wan. 2011. Community structure and composition in response to climate change in a temperate steppe. Global Change Biology 17:452–465.

Zavaleta, E. S., M. R. Shaw, N. R. Chiariello, H. A. Mooney, and C. B. Field. 2003. Additive effects of simulated climate changes, elevated CO_2, and nitrogen deposition on grassland diversity. Proceedings of the National Academy of Sciences USA 100:7650–7654.

SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–C are available online: http://dx.doi.org/10.1890/14.0144.1.sm