Ecosystem sensitivity to variation in precipitation and defoliation across grasslands of Alberta

by

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Abstract

Climate models project a greater likelihood of increases in precipitation variability and droughts over the Great Plains of North America. Drought's interactions with other co-occurring factors, such as grazing, can lead to substantial and long-lasting changes to ecosystem goods and services. In this thesis, I examine variations in precipitation and defoliation to better understand how their interactive effects shape ecosystem structure and function. First, I test the effects of grazing on ecosystem sensitivity to precipitation using spatially extensive long-term records of aboveground net plant productivity (ANPP) and precipitation. I found that grazing increased ANPP sensitivity to interannual variability in precipitation, especially at arid grasslands, suggesting that the explanatory power of the precipitation-ANPP relationship may not hold for ecosystems subjected to two or more global change drivers. Second, I examine the commonly assumed but little tested hypothesis – that drought impacts are progressive through time. I do so using a factorial experiment crossing drought and defoliation that I conducted at seven northern temperate grasslands over four years. I found that multi-year drought led to greater changes to community composition than productivity, and effects did not compound through time. Shifts in species composition were driven by variation in the abundance of dominant species, which also likely resulted in stability in ANPP under drought. Third, I examine the sensitivity of both shoot and root biomass to the combined effects of drought and defoliation. This topic is important because root responses are often overlooked in ecosystem models. I found that ANPP increased under drought relative to the ambient treatment, and the combined effects of drought and defoliation reduced ANPP but had no impacts on root biomass. Moreover, using minirhizotrons, I further examine root length dynamics in response to drought and defoliation at two of my seven field sites. I found root length dynamics were affected by every factor manipulated or measured in this thesis. In general, I found decreases in length, production, and lifespan of roots with drought in the shallow soil depth in the absence of defoliation. Notably, I found that the two sites exhibited general convergence in many aspects of their root length dynamics when exposed to both drought and defoliation, but when differences existed, they occurred under the ambient condition or at the deep soil depth. Further, the mortality and lifespan of roots were the aspects of root length dynamics that caused the observed shifts in total root length resulting from drought and variation in defoliation. In sum, this thesis demonstrates that northern temperate grasslands are highly resistant to a reduction in water availability via stability among dominant species or root responses, particularly root length dynamics, but defoliation, regardless of what regime, makes these systems sensitive to drought. Further, my results emphasize that the combined effects of global change drivers on plant root and shoot responses must be included in ecosystem models and will more accurately project ecosystem sensitivity to future variable weather.

Preface

This thesis is an original work by Batbaatar Amgaa

Chapter 2 of this thesis has been published in *Journal of Vegetation Science* in 2021:

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I was responsible for the development of the ideas, all data collation, analysis, and manuscript writing. Data were collected as part of the Rangeland Reference Area program operated by Alberta Environment and Parks. EWB, JFC, and CNC provided significant input on the conception of the ideas and feedback on the manuscript. BT and AMJ collected and archived the data and contributed to writing and editing the manuscript. Data deposited in the University of Alberta Dataverse:

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To my family and friends,
and especially for mom,
Damdinpurev Gankhlkhagva
(1957 - 2001)

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Chapter 1: Introduction

The assembly of plant communities is often determined by multiple interacting abiotic and biotic ecological filters (Hillerislambers et al. 2012), including precipitation, fire, and grazing in grassland ecosystems (Milchunas et al. 1988, Koerner and Collins 2014, Kohli et al. 2020). However, anthropogenic climate change is causing unprecedented changes to the nature and pace of these factors (Smith et al. 2009, IPCC 2021). For example, a warmer temperature due to increased emissions of greenhouse gases (e.g., carbon dioxide and methane) associated with anthropogenic activities increases the water-holding capacity of the atmosphere, leading to an overall intensification of the global water cycle (Trenberth 2011, IPCC 2021). As a result, many parts of the world are predicted to experience substantial increases in precipitation variability and, by extension, the frequency and magnitude of precipitation extremes, such as droughts (Sloat et al. 2018, Zhao et al. 2020, Zhang et al. 2021). Such predictions have prompted ecologists to increasingly study their ecological impacts over the past two decades (Hoover et al. 2018, Wang et al. 2021), partly due to the substantial socio-economic loss often associated with changing precipitation variability (Weaver 1968, Knapp et al. 2020). Rising interest in this field of research also reflects a growing concern that traditional approaches may not be predictive of responses to a condition imposed by the increased precipitation variability and extremes (Hsu et al. 2012, Estiarte et al. 2016, Knapp et al. 2017b), which is also likely to result in broad-scale shifts in land use (Foley et al. 2005, Knapp et al. 2008).

Grasslands are expected to be especially sensitive to changes in rainfall patterns because plant growth in grasslands is primarily controlled by water compared to other ecosystems (Huxman et al. 2004, Smith 2011, Knapp et al. 2015a, Maurer et al. 2020). Yet, observed responses to altered precipitation variability within grasslands are highly variable, ranging from negligible to major changes in ecosystem structure and function (Wilcox et al. 2017, Hoover et al. 2018, Wang et al. 2021). This is likely due to several factors, but I seek to address three of those through my thesis project. First, most systems are almost always simultaneously subject to multiple stressors (Vinebrooke et al. 2004, Crain et al. 2008, Ma et al. 2020b). However, much of our knowledge about the ecological impacts of altered precipitation variability is derived from responses to precipitation and assessed independently of other global change drivers such as grazing in grasslands. This is a critical knowledge gap, especially when grazing is an important disturbance in grasslands that maintain ecosystem services, such as biodiversity and carbon storage, either through modification of root systems (Ingrisch et al. 2020, Ma et al. 2021) or by altering microclimatic and edaphic conditions (Willms et al. 1986, Deutsch et al. 2010). Further, evidence suggests the interaction of variable precipitation with other global change drivers can result in greater ecological impacts than when either factor is considered alone (Komatsu et al. 2019, Ma et al. 2020b, Avolio et al. 2021). Therefore, it will be critical to understanding how variation in individual elements of management practices that are within the control of land managers (e.g., the timing or intensity of grazing) would influence responses of ecological communities to changes in precipitation patterns (Di Virgilio et al. 2019, Godde et al. 2019). This is a key question, with significant implications for nutrient cycling, carbon sequestration, and storage, as well as suggestions of more sustainable grazing practices in the face of changing climate (Briske et al. 2020, Godde et al. 2020).

Second, although new methods are continually being introduced into the literature and put to use by empirical studies (Fraser et al. 2013, Felton et al. 2019), our ability to make broad generalizations and develop a predictive framework remains a challenge for ecologists today

(Filazzola and Cahill 2021). Especially, short-term responses to precipitation manipulation experiments may not reflect ecosystem feedbacks to climate change over time, likely because of the experiments' limited duration and geographical extent (Hoover et al. 2018, Wang et al. 2021). Thus, long-term experiments with consistent methodology are required, given that the occurrence of multi-year drought is predicted to increase globally (Zhao et al. 2020, Zhang et al. 2021). Finally, much of the past experimental and observational research on the sensitivity of ecosystems to altered precipitation have focused on aboveground plant responses (Knapp et al. 2017b, Wilcox et al. 2017), while root responses, particularly root length dynamics, are often overlooked, despite their importance to current and future ecosystem function and services (e.g., carbon sequestration and drought resistance) (Bardgett et al. 2014, Wilson 2014, Ma et al. 2020a).

The aforementioned knowledge gaps inspired my dissertation, and I addressed them by utilizing both spatially and temporally extensive long-term observational data and conducting a multi-year and multi-site factorial experiment. There are practical limitations of coupling rainfall manipulation experiment and live cattle; instead, I used artificial defoliation rather than actual cattle in my field experiment. Consequently, relative to cattle grazing, the mechanical simulation I applied is more uniformly applied across species and lacks cattle selectivity or saliva-induced plant responses, thereby allowing the standardization of treatments across sites (Waterman et al. 2019, Filazzola and Cahill 2021). Moreover, in combination with defoliation treatments, I examine ecological consequences of changes in water availability across northern temperate grasslands, with a specific focus on rainfall reduction (e.g., growing season drought) and between-year precipitation variability (e.g., amount) in my research. Therefore, I used both variability and reduction in precipitation throughout my dissertation. In tandem, I can elucidate how ecosystems respond to the variation in co-occurring factors of precipitation and defoliation.

Thesis overviews

Changes to water availability, whether through increased year-to-year variability or altered amounts, can have cascading consequences for numerous ecosystem goods and services such as forage availability and habitat quality (Sloat et al. 2018, Godde et al. 2020, Klemm et al. 2020). This is due to the well-established positive linear relationship between precipitation and ecosystem function, measured as aboveground net primary productivity (ANPP), across most grassland ecosystems (Smoliak 1986, Sala et al. 1988, Knapp and Smith 2001). This tight association between precipitation and ANPP serves as a benchmark for predicting the vulnerability of ecosystems to changes in precipitation variability in ecosystem models (Estiarte et al. 2016, Knapp et al. 2017b). The slope of this relationship can be thought of as a measure of ecosystem sensitivity because it describes the magnitude of the response that is likely to occur with per mm change in precipitation (Huxman et al. 2004, Sala et al. 2012, Maurer et al. 2020). However, the relationship between precipitation and ANPP under normal precipitation variability may not hold as the magnitude of precipitation variability increases (Hsu et al. 2012, Knapp et al. 2017b, Felton et al. 2019, Felton et al. 2021). For example, multiple lines of inferences have reported a non-linear precipitation-ANPP relationship that portrayed asymmetric ANPP response to dry and wet years (Wilcox et al. 2017, Zhang et al. 2017, Wu et al. 2018). However, the test of this hypothesis has not fully been applied in concert with co-occurring factors like grazing, primary land use in grasslands (but see, Irisarri et al. 2016). In Chapter 2, I examine whether grazing alters the sensitivity of ANPP to interannual variability in precipitation using long-term (14-28 years) data from 31 grazed grasslands, each with a paired non-grazed livestock exclosure.

In the next three chapters of my thesis, I use data from a factorial experiment I conducted at seven northern temperate grasslands over four years, crossing extreme growing season drought with five levels of aboveground plant biomass removal. I collected data on a range of biotic response variables, including species composition, shoot and root biomass, and root length dynamics, to gain a comprehensive understanding of ecological responses to the variation of co-occurring factors of precipitation and defoliation. In Chapter 3, I examine the response of northern temperate grasslands to four consecutive years of extreme drought. To date, few studies have examined the effects of drought duration on ecosystems, even though the likelihood of multi-year extreme drought is predicted to increase globally (Zhao et al. 2020, Zhang et al. 2021). Further, accumulating evidence suggests that drought effects on ecosystems are likely to compound through time (Evans et al. 2011, Zhang et al. 2019, Orth et al. 2020). However, while this hypothesis is often assumed, it has not been tested, likely because the duration of drought experiments is often limited to 1-2 years (Hoover et al. 2018, Wang et al. 2021). Thus, it is important to test for a treatment × year interaction within multi-year experimental studies to ascertain the ecological effects of prolonged droughts.

The effects of drought and grazing on plant growth have been relatively well studied (McNaughton 1979, 1985, Milchunas et al. 1988, Milchunas and Lauenroth 1993), but their effects are often viewed as independent, neglecting potential interaction effects. Previous studies have shown that responses of grasslands to the combined effects of reduced rainfall and simulated grazing across the Canadian prairies are varied (Carlyle et al. 2014, White et al. 2014a, White et al. 2014b), suggesting an incomplete understanding of ecosystem responses to drought under different land-use practices. Further, existing studies are limited in spatial and temporal extent, making it difficult to generalize patterns across ecosystems or determine what type might be better if grazing occurs in drought years. Such information is a key, yet rarely addressed, question with major implications for the global carbon cycle (Di Virgilio et al. 2019, Godde et al. 2019, Godde et al. 2020, Nielsen et al. 2020). It has been suggested that manipulation of individual grazing impacts can be a promising set of adaptive management options to sustain plant growth in the face of climate change (Döbert et al. 2021, Hulvey et al. 2021). In Chapters 4 and 5 of this thesis, I focus on the combined effects of drought either with timing (early vs late) or intensity (none, light, heavy) of defoliation early in the growing season. Specifically, in Chapter 4, I use the fourth-year data to compare the effects of defoliation timing or intensity on plant shoot and root biomasses under drought through a priori hypothesis testing.

Predicting grassland responses to both precipitation reduction and grazing requires an understanding of root responses (Frank 2007, Wilcox et al. 2017, Ma et al. 2020a, Slette et al. 2021), given that a substantial portion of total photosynthates are allocated to roots (Gherardi and Sala 2020), making up to 85% of plant biomass in semi-arid temperate grasslands (Coupland and Johnson 1965). The response of plants to water stress and grazing is characterized by their ability to acquire and efficiently use limited resources (Comas et al. 2013, Bardgett et al. 2014, Erktan et al. 2018b, Freschet et al. 2021b). Root system efficiency is better understood when root length dynamics rather than standing root biomass are considered because attributes of individual roots, such as appearance (e.g., birth), disappearance (e.g., death), and lifespan, can change meaningfully without resulting changes to the root biomass (Wilson 2014, Mueller et al. 2018, Ma et al. 2020a, Weigelt et al. 2021). Thus, it will be critical to determine how variation in defoliation would influence the responses of root length dynamics to drought. Yet, this remains one of the least understood questions, with significant implications for predicting the trajectory of these ecosystems in response to the combined effects of co-occurring global change drivers. In Chapter 5, I examine the responses of root length dynamics to four consecutive years of extreme drought and defoliation at two northern temperate grasslands that differed in climatic conditions.

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Chapter 2: Grazing alters the sensitivity of plant productivity to precipitation in northern temperate grasslands

Abstract

Interannual variability in precipitation is expected to increase in grasslands, potentially causing additional stress to systems already impacted by anthropogenic activities such as livestock grazing, which can induce changes to grassland vegetation. Yet, the sensitivity of key ecosystem functions to these co-occurring factors is often overlooked. Here, we determine: (1) the effects of grazing on the sensitivity of aboveground net primary productivity (ANPP sensitivity) to interannual variation in water-year precipitation (the sum of precipitation from September through to the following August); (2) whether ANPP sensitivity to precipitation is associated with shifts induced by grazing in functional group biomass (grass vs. forb) contribution to total ANPP, litter, and species richness, and mean annual water-year precipitation; and (3) whether the impacts of grazing on ANPP vary between dry and wet years. We used long-term (14-28 years) ANPP and precipitation data from 31 grazed grasslands, each with a paired non-grazed livestock exclosure. ANPP was sampled annually within exclosures and adjacent grazed locations at each site. We found that grazing increased ANPP sensitivity to interannual changes in precipitation. Increased ANPP sensitivity to precipitation in grazed, relative to non-grazed, locations was associated with both an increase in the contribution of forbs to total ANPP and a decrease in the contribution of grasses to total ANPP; reduced litter also increased ANPP sensitivity to precipitation. Species richness was not associated with ANPP sensitivity in both grazed and non-grazed locations. Arid grasslands were more sensitive to interannual variation in precipitation when grazed than were mesic grasslands. Similarly, grazing reduced ANPP during dry years but had no effect during wet years. Overall, these findings suggest that grazed grasslands are more

vulnerable to reductions in primary productivity in dry years, which may present a challenge for maintaining ecosystem services in an era of increasing precipitation variability.

Introduction

Climate change is likely to increase interannual variability in precipitation (Diffenbaugh et al. 2015, Sloat et al. 2018), and interactions with other global change drivers (e.g., grazing, fire, drought, and nitrogen deposition) could have significant and long-lasting impacts to the carbon cycle and the delivery of production-derived services such as carbon uptake, forage, and livestock productivity (Smith et al. 2009, Sloat et al. 2018, Godde et al. 2019, Maurer et al. 2020). This is particularly relevant to water-limited ecosystems, such as grasslands that have naturally high precipitation variability (Knapp and Smith 2001) and widespread grazing by large herbivores (Frank et al. 1998). However, much of current understanding of ecosystem sensitivity to precipitation variability is derived from responses of aboveground net primary productivity (ANPP) to precipitation (Knapp et al. 2017b) and assessed independently of grazing effects (but see Irisarri et al. 2016), despite grazing being the predominant land-use of grasslands (Asner et al. 2004). As such, understanding ecosystem responses to the interactive effects of co-occurring factors is critical, and a reliable benchmark is needed for predicting ecosystem sensitivity robustly from ecosystem models (Dangal et al. 2017, Chen et al. 2018, Ma et al. 2020b).

Precipitation is a key driver of variation in aboveground net primary productivity (ANPP) in grasslands (Knapp and Smith 2001); however, ecosystems can vary substantially in their sensitivity to changes in precipitation variability (Huxman et al. 2004, Knapp et al. 2015a). Particularly, ANPP in arid ecosystems is expected to be more sensitive to change in precipitation than mesic systems (Maurer et al. 2020). Multiple mechanisms have been proposed for the reported differences in ANPP sensitivity defined as the change in ANPP per mm change in annual precipitation between ecosystems. Among them are variation in the magnitude of precipitation events, such as dry and wet years, experienced by any particular system (Knapp et al. 2017b, Petrie et al. 2018, Wu et al. 2018, Felton et al. 2019), inherent differences in the assembly of the local plant community (Byrne et al. 2017, Mulhouse et al. 2017, Sternberg et al. 2017), and disturbance-induced alterations to abiotic properties (e.g., soil moisture and nutrients Willms et al. 1986, Willms et al. 1993). However, evidence indicates that the sensitivity of ecosystem functions (e.g., ANPP) to climate change may not represent simple additive responses to multiple environmental factors (Felton et al. 2020, Ma et al. 2020b). This suggests that our ability to project ecosystem sensitivity to predicted changes in precipitation variability is hindered by a limited understanding of combined factors, which in grasslands will be the by-product of interannual variability in precipitation coupled with grazing.

Grazing is an important disturbance in grasslands that can change vegetation structure and composition directly through impacts on plants (Milchunas and Lauenroth 1993, Filazzola et al. 2020) and indirectly through alteration of edaphic conditions as a result of trampling and litter removal (Willms et al. 1986, Deutsch et al. 2010). In temperate grasslands, grazing increases the contribution of forb biomass to total ANPP at the expense of grasses (Bork et al. 2012, Bork et al. 2019). Such shifts in the relative contribution of functional group biomass to total ANPP can influence ANPP sensitivity to precipitation (Verón and Paruelo 2010, Gaitán et al. 2014, Irisarri et al. 2016) as forbs and grasses exhibit differential sensitivity to water availability (Felton et al. 2019). Likewise, water-use efficiency varies greatly among plant species (Jiang et al. 2017), and grazing-induced changes in vegetation structure (e.g., species richness Lyseng et al. 2018, Souther et al. 2020) may influence ANPP sensitivity to precipitation (Fischer et al. 2018).

Finally, grazing could affect ANPP sensitivity through changes in soil structure and litter mass, which are tightly linked to available soil water for plant growth (Willms et al. 1986, Naeth et al. 1991, Vandandorj et al. 2017).

Here, we use long-term ANPP data collected from a network of 31 northern temperate grasslands with and without exposure to long-term livestock grazing to examine the effects of grazing on ANPP and ANPP sensitivity to changes in both precipitation amount and the occurrence of dry and wet years. Long-term data is imperative, not only to better understand grazing effects on ANPP sensitivity to precipitation, but also to devise grazing management recommendations in light of future changes in precipitation amount, variability, and extremes. Site-level data that have been collected over extensive temporal and spatial scales and sampled simultaneously from grazed and adjacent non-grazed locations within the same ecosites enabled us to address three questions: 1) does grazing alter ANPP sensitivity to precipitation? 2) is ANPP sensitivity to precipitation associated with local climatic and grazing-induced vegetation changes? and 3) do grazing effects on ANPP vary between dry and wet years?

Methods

Study sites

This study took place in the northern extent of the North American Great Plains, with study sites distributed throughout southern Alberta, Canada. A range of climatic conditions characterize the region; mean annual temperature ranges from 2.3 °C to 4.4 °C, and mean annual precipitation from 333 to 470 mm (Downing and Pettapiece 2006). All sites were part of the Rangeland Reference Area (RRA) program currently operated by Alberta Environment and Parks. The RRA is a network of long-term livestock exclosures that serve as non-grazed reference plant communities for monitoring of provincial grazing lands (RRA 2017). Exclosures typically consisted of four-strand barbed-wire fences and varied in size from 18 × 40 m to 30 × 50 m, and range in age from 9 to 62 years. Exclosures are located on public lands that are subject to light to moderate grazing by beef cattle from early summer to mid-autumn each year. Though no site-specific stocking rate data are available, the stocking rates used on these public lands are considered to be low to moderate intensity and range from a low of 0.6 animal-unit-months (AUM) ha^{-1} in arid grasslands to 1.4 AUM ha^{-1} in mesic grasslands (Adams et al. 2013). All grasslands are susceptible to wildlife grazing, but the latter is considered very light for the grasslands assessed (Gross and Knight 2000). There were 56 sites with paired grazed and non-grazed locations in the RRA dataset. The average duration of monitoring across sites was 19 years, ranging from 9 to 28 years (Appendix 2-S1).

Vegetation sampling

To estimate ANPP from grazed and non-grazed locations, we harvested aboveground plant biomass annually to ground level during the period of peak biomass. In the non-grazed exclosures, ANPP was collected from ten 50 x 50 cm (0.25 m^2) subplots. In the adjacent grazed locations, samples were collected from another ten subplots under portable ungulate exclusion cages (1.5 × 1.5 m in size). Cages were randomly placed in new locations in the spring of every year, which enabled an estimate of ANPP from grazed locations without the effects of cattle grazing in the current year and eliminating the need to estimate the biomass removed by cattle. All harvested samples were separated into grass, forb, and litter (standing dead and detached material) in the field. Samples were dried to constant mass and weighed. Biomass values from the ten subplots were averaged and converted to g m^{-2} for analyses.

We compiled species richness data from grazed and non-grazed locations at each site from two published studies that examined changes in species composition as a function of grazing. We matched the data to the sites included in this study (see Lyseng et al. 2018 for a description of data collection, Bork et al. 2019). We used average species richness to capture the long-term cumulative effects of grazing on species richness for each plant community.

Precipitation data

The remote location of many of the field sites precluded the use of local weather stations. Thus, we used the ClimateNA v5.5 software package, available at http://tinyurl.com/ClimateNA, to obtain annual and monthly climate variables, including mean annual temperature, mean annual precipitation, and monthly precipitation, for the last 116 years (1901-2016) for each study site. ClimateNA uses data from weather stations to generate high-resolution (4 × 4 km) interpolated climate variables for specific locations based on latitude, longitude, and elevation (Wang et al. 2016).

We assessed the relationship between ANPP and precipitation over two precipitation time periods: calendar-year (January 1 – December 31) and water-year (September of the previous year – August of the calendar year). Water-year precipitation was compared to calendar year precipitation due to its potentially greater utility in predicting ANPP, particularly in more arid grasslands of the region where precipitation during fall and winter contributes to water recharge and plant growth the following growing season (Smoliak 1986). Monthly precipitation was used to calculate water-year precipitation for every year for each study site using the *water_year* function from the ‘lfstat’ package (Koffler et al. 2016). To evaluate whether water-year or calendar-year precipitation explained the relationship best, we used Akaike’s Information Criteria model weights (AIC) to compare our model fits. Consistent with Smoliak (1986), water-year precipitation (AIC = 8985.9) was a better predictor of ANPP than calendar year precipitation (AIC = 9002.1). Thus, we used water-year precipitation in all further analyses.

Data analysis

Some sites did not experience substantial variation in water-year precipitation during the period of observation, which could decrease the ability to detect relationships between ANPP and water-year precipitation. Thus, we used a subset of sites for which there was substantial water-year precipitation variability during the study period. To subset the dataset, we used the long-term precipitation data (1901-2016) to calculate the expected range of water-year precipitation for each site based on its mean annual water-year precipitation and coefficient of variation of water-year precipitation (mean ± mean*CVs). Our inclusion of sites for all further analyses was determined by the observed range of precipitation within the sites during the study period, which determined the strength of the relationship between ANPP and water-year precipitation in our study, and thus our estimate for ANPP sensitivity to precipitation. We only retained sites if the observed range of water-year precipitation was greater than the expected range during the observational period. This process reduced the number of sites from 56 in the original dataset to 31 in the subset (Appendix 2-S1). ANPP values were square-root transformed to meet assumptions of normality; all figures show non-transformed values to aid visual interpretation. All statistical analyses were performed using R studio (RStudio Team 2019) based on R version 3.6.1 (R Core Team 2019). Statistical significance was set at $\alpha = 0.05$ for all analyses.

To determine overall changes in ANPP response to precipitation due to grazing, we performed a linear mixed-effect model including data from every year and every site using the 'lme4' package (Bates et al. 2015). In this model, precipitation, exposure to grazing, and their interaction were fixed effects, and site and year were included as random effects. A significant grazing term ($p < 0.05$) indicated that exposure to grazing affected ANPP (different y-intercepts), and a significant grazing × precipitation interaction indicated that grazing altered ANPP sensitivity to precipitation (different slopes).

To examine how changes in functional group biomass (grass vs. forb) contribution to total ANPP, litter, and species richness induced by grazing, and mean annual water-year precipitation were associated with ANPP sensitivity to precipitation, data were processed by following three steps. First, we calculated each variable's average values for grazed and non-grazed locations for each site over the sampling period. Second, we estimated ANPP sensitivity for every grazed and non-grazed location for each site by computing the slope of the relationship between ANPP (response variable) and water-year precipitation (predictor variable) using linear regression (Huxman et al. 2004, Byrne et al. 2017). Third, slope values of the relationships were used as a response variable (a measure of response in ANPP per mm change in interannual water-year precipitation) in mixed-effect models, where site was a random effect. Fixed effects were grazing, mean annual water-year precipitation, the relative contribution of grass and forb biomass to total ANPP, species richness, litter, and two-way interactions between grazing and the predictors aforementioned. We used grazing by predictor variable interactions to determine whether ANPP sensitivity to precipitation was associated with functional group biomass (grass vs. forb) contribution to total ANPP, litter, species richness, and mean annual water-year precipitation. Significant interactions indicated grazing effects on ANPP sensitivity to precipitation were mediated by those variables.

We used two subsets of data to examine whether the response of ANPP to grazing varied between dry and wet years. First, we used only the five driest and five wettest years recorded for each site (Appendix 2-S2). Second, we selected extreme dry and wet years based on the 5th and 95th percentiles of long-term water-year precipitation (1901-2016) of each site (Knapp et al. 2015b, Knapp et al. 2017a). Both sets of data provided the same interpretation of results (i.e., Figure 2-4 versus Appendix 2-S3), but there were fewer sites that experienced extremely dry conditions (Appendix 2-S4), and typically infrequently (i.e., only in 1 year). We therefore chose to report and discuss findings from the analysis using five years of data in order to use a more representative dataset. We used a mixed-effect model with grazing, precipitation level (dry and wet years), and their interaction as fixed effects, and site and year as random effects. A significant grazing × precipitation level indicated that the grazing effects on ANPP varied between dry and wet years. To identify whether the effects of grazing on ANPP were more pronounced in dry or wet years, we conducted post-hoc Tukey pairwise comparisons using the *emmeans* function from 'emmeans' package (Lenth 2019).

Results

Across the 31 study sites, mean annual temperatures ranged from 2.4 to 5.7 °C (4.1 ± 1.3 °C; mean ± SD), and mean annual water-year precipitation ranged from 308 to 540 mm (398 ± 82 mm) during the period of observation (Appendix 2-S1). Although ANPP was higher in non-grazed (153 ± 84 g m^{-2}; mean ± SD) than grazed (143 ± 87 g m^{-2}) locations on average, the range of ANPP was greater in grazed (3.36 to 579 g m^{-2}) relative to non-grazed (5.27 to 486 g m^{-2}) sampling locations.

Across all years, total ANPP increased with water-year precipitation within both grazed and non-grazed locations ($F_{1,746.53} = 52.46$; $p < 0.0001$), and was further affected by grazing ($F_{1,1368.48} = 20.72$; $p < 0.0001$), and the interaction of grazing × water-year precipitation ($F_{1,1368.48}$

= 13.81; p = 0.0002). Grazing altered ANPP sensitivity to precipitation as the slope of the relationship between ANPP and water-year precipitation was steeper for grazed than non-grazed locations (Figure 2-1). In other words, across all locations, temporal changes in precipitation caused greater changes in ANPP when grazed, relative to non-grazed locations. The same pattern of increased sensitivity of ANPP to precipitation due to grazing was found when the full dataset of 56 sites was examined (Appendix 2-S5).

Mean annual water-year precipitation and grazing-induced changes in vegetation significantly interacted with grazing to influence ANPP sensitivity (Figure 2-2 & 2-3). More specifically, grazing increased ANPP sensitivity to inter-annual changes in precipitation for arid sites, but ANPP sensitivity decreased with grazing for mesic sites (Figure 2-2) as indicated by a significant main effect of grazing ($F_{1,29} = 16.12$; p = 0.0004) and an interaction between grazing × water-year precipitation ($F_{1,29} = 11.21$; p = 0.002). The relative contribution of forb to total ANPP was greater in grazed relative to non-grazed locations and was positively associated with ANPP sensitivity specifically for grazed locations (Figure 2-3b). The opposite pattern existed for grasses whereby the relative contribution of grass biomass to total ANPP was reduced with grazing, and this in turn led to a negative association with ANPP sensitivity under grazed conditions (Figure 2-3a; $F_{1,29.74} = 4.45$; p = 0.0435). Consistent with the relative contribution of grass to total ANPP, reduced litter due to grazing increased ANPP sensitivity to precipitation (Figure 2-3d) as evidenced by a significant effect of grazing ($F_{1,29.09} = 18.47$, p = 0.0001), litter ($F_{1,57.5} = 9.57$, p = 0.003), and their interaction ($F_{1,40.64} = 10.2$, p = 0.0027). By contrast, we found no evidence of association between grazing-induced changes in species richness and ANPP sensitivity to precipitation (Figure 2-3c) as neither the main effect of richness ($F_{1,56.74}$ =

1.36, p = 0.25) nor an interaction between grazing × richness was significant ($F_{1,31.03} = 0.017$, p = 0.89).

During the wettest and driest years documented for each site, the effects of grazing ($F_{1,562.33} = 15.37$, $p < 0.0001$), precipitation level ($F_{1,306.79} = 17.06$, $p < 0.0001$) and an interaction between grazing × precipitation level ($F_{1,562.33} = 10.19$, p = 0.0015) remained apparent on ANPP. As expected, ANPP was lower in dry years compared to wet years. However, the effect of grazing on ANPP was only apparent during dry years, which resulted in a 12% reduction in ANPP within grazed locations relative to their non-grazed counterparts (Figure 2-4).

Discussion

We found ANPP in grazed grasslands was more sensitive to interannual changes in precipitation than non-grazed grasslands, with the greatest differences between grazed and non-grazed grasslands in terms of ANPP sensitivity to precipitation apparent in more arid grasslands. This pattern was highly consistent within and across sites and, hence, can have significant implications for predictions from ecosystem models (Dangal et al. 2017) as well as the provisioning of ecosystem services (Sloat et al. 2018, Maurer et al. 2020). Thus, there is a need to understand the mechanisms behind grazing-induced increases in ANPP sensitivity to precipitation.

We found that an increased contribution of forb biomass to total ANPP at the expense of grasses led to an increased ANPP sensitivity to precipitation, and this occurred specifically in grazed rather than non-grazed grasslands (Figure 2-3). Previous long-term grazing studies report that grazing has a deterministic impact on plant functional group composition (Verón and Paruelo 2010, Gaitán et al. 2014, Irisarri et al. 2016) and their contribution to total ANPP (Bork et al. 2012, Bork et al. 2019), which includes a decline in grasses under grazing. Together these results strongly suggest that any compositional shifts induced by grazing, even among coarse plant functional groups, are likely to account for differences in ANPP sensitivity among ecosystems with comparable mean annual precipitation (Verón and Paruelo 2010, Irisarri et al. 2016). Moreover, it highlights the need for more in-depth studies into the specific role of plant compositional changes under grazing for their ability to regulate ecosystem stability.

Findings from two studies conducted at the same sites as ours showed that species richness increased with ongoing grazing (Lyseng et al. 2018, Bork et al. 2019), but we found no evidence of association between species richness and ANPP sensitivity to precipitation. The result is unexpected given the fact that species richness is considered an important determinant of changes in ecosystem productivity and stability (Isbell et al. 2009, Hallett et al. 2014, Tilman et al. 2014). This could be attributed to the diversity of species traits within the existing plant communities examined, which determine the capacity of existing vegetation to respond rapidly to both variable precipitation and grazing (Milchunas et al. 1988, Díaz et al. 2007). For example, previous studies show that grazing-induced increases in the abundance and diversity of non-native, or annual, species at the expense of perennial native species, led to increased ecosystem sensitivity to fluctuations in annual precipitation, particularly during drought years (Liang et al. 2018, Souther et al. 2020). On the other hand, an increased contribution of non-natives species in the grazed communities examined here (Lyseng et al. 2018) could reduce ANPP sensitivity to precipitation due to an increased utilization of available resources, particularly in mesic systems. Notably, non-native species representation was generally lower in more arid grasslands of the current study area (Lyseng et al. 2018), including those exposed to grazing, which in turn, were those with the greatest overall susceptibility to grazing-induced increases in variability in ANPP.

This suggests that factors other than grazing-induced changes to composition likely play at least some role in regulating ANPP sensitivity to precipitation.

We found that litter was another underlying mechanism explaining the differential sensitivity in ANPP between grazed and non-grazed grasslands. Compared with non-grazed grasslands, ANPP sensitivity to precipitation increased when grazing resulted in a greater reduction in litter. Litter is known to regulate the availability of resources in grasslands, chiefly soil moisture, and the removal of litter can markedly reduce plant productivity in the prairies of western Canada (Willms et al. 1986, Naeth et al. 1991, Deutsch et al. 2010, Hilger and Lamb 2017). Further, litter-induced declines in productivity are generally larger (ca. 60%) in arid than in mesic grasslands (Willms et al. 1986), with the current findings therefore highlighting the critical role of litter in not only increasing ANPP, but also stabilizing ANPP during climatic variation. Thus, grazing can alter ecosystem sensitivity to changes in precipitation indirectly through feedback effects of grazing-induced changes in litter on structural and functional conditions of the environment (Naeth et al. 1991, Deutsch et al. 2010, Hilger and Lamb 2017, Vandandorj et al. 2017).

There was a reduction in ANPP associated with grazing during dry years that did not occur in wet years. This finding is in agreement with studies conducted in grasslands of Asia, Europe, and North America (Liang et al. 2018, Stampfli et al. 2018, Souther et al. 2020). The absence of grazing effects on ANPP during wet years could be due to compensatory plant growth induced by either improved light conditions (Borer et al. 2014) or increased nitrogen availability induced by grazing (Frank and Evans 1997), which becomes the limiting factor for plant growth when water is more available (Huxman et al. 2004). By contrast, reductions in ANPP are likely if grazing-induced soil moisture deficits occur due to litter removal (Deutsch et al. 2010) and a shift from deep to shallow rooted plant species (Stampfli et al. 2018), as is known to occur under moderate to heavy grazing in the region (Smoliak et al. 1972, Willms et al. 1985).

In summary, our findings from this multi-site study show that the primary productivity of grasslands is most sensitive to precipitation when grazed, particularly within arid grasslands during periods of below-normal precipitation. Although changes in species richness responses did not contribute to the influence of grazing on ANPP sensitivity to precipitation, the observed difference in ANPP sensitivity between grazed and non-grazed grasslands was associated with changes induced by grazing in the contribution of functional group biomass to total ANPP and litter. Thus, we recommend that the interactions between precipitation and historical land-uses, such as grazing, need to be incorporated into process-based models when evaluating ecosystem responses. This incorporation may reduce the previously identified inconsistencies among studies (Wilcox et al. 2017, Zhang et al. 2017) as well as the discrepancy between predictions of experimental and simulation modeling (Wu et al. 2018). Finally, these findings suggest that primary productivity, and therefore land use potential, of grazed grasslands may remain relatively similar to non-grazed grasslands during wet years. In contrast, during dry years (i.e., below normal precipitation) the urgency for adaptive grazing practices such as reduced stocking may be heightened. Perhaps most important, conservative stocking rates in general are likely to minimize overgrazing and maintain stability in ANPP during dry years.

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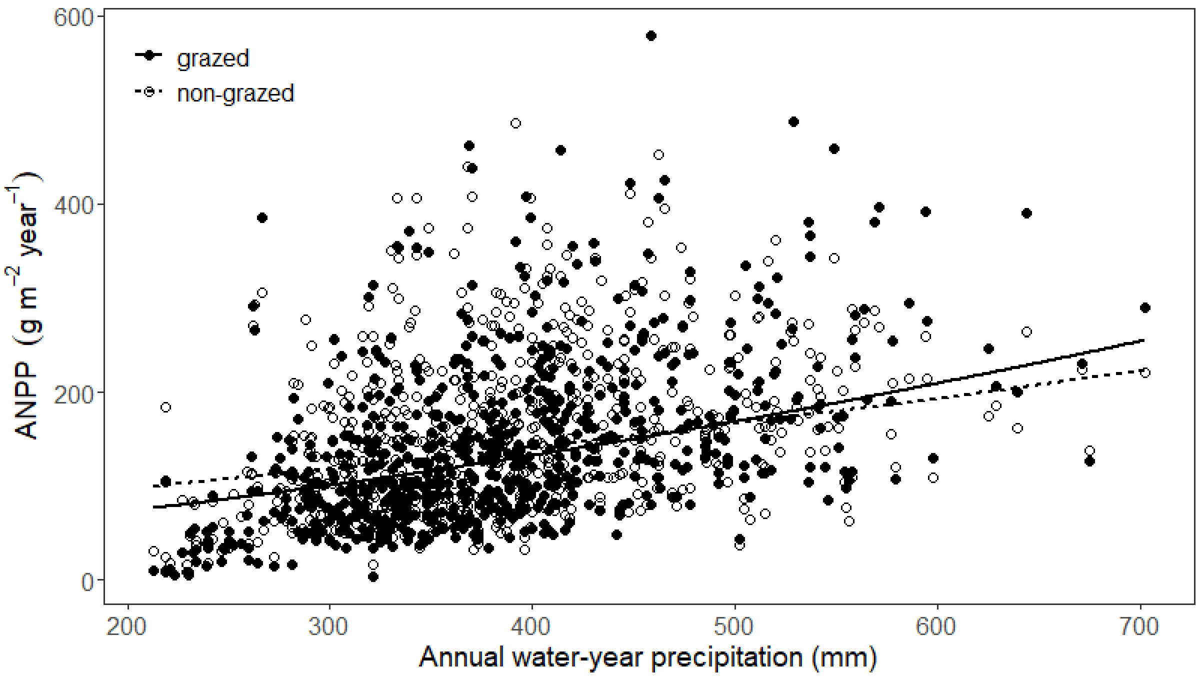

Figure 2-1. The relationship between aboveground net primary production (ANPP) and annual water-year precipitation (September 1-August 31) across 31 sites with pairs of long-term grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation (Appendix 2-S1). Lines are linear mixed-effect model fits for grazed and non-grazed locations.

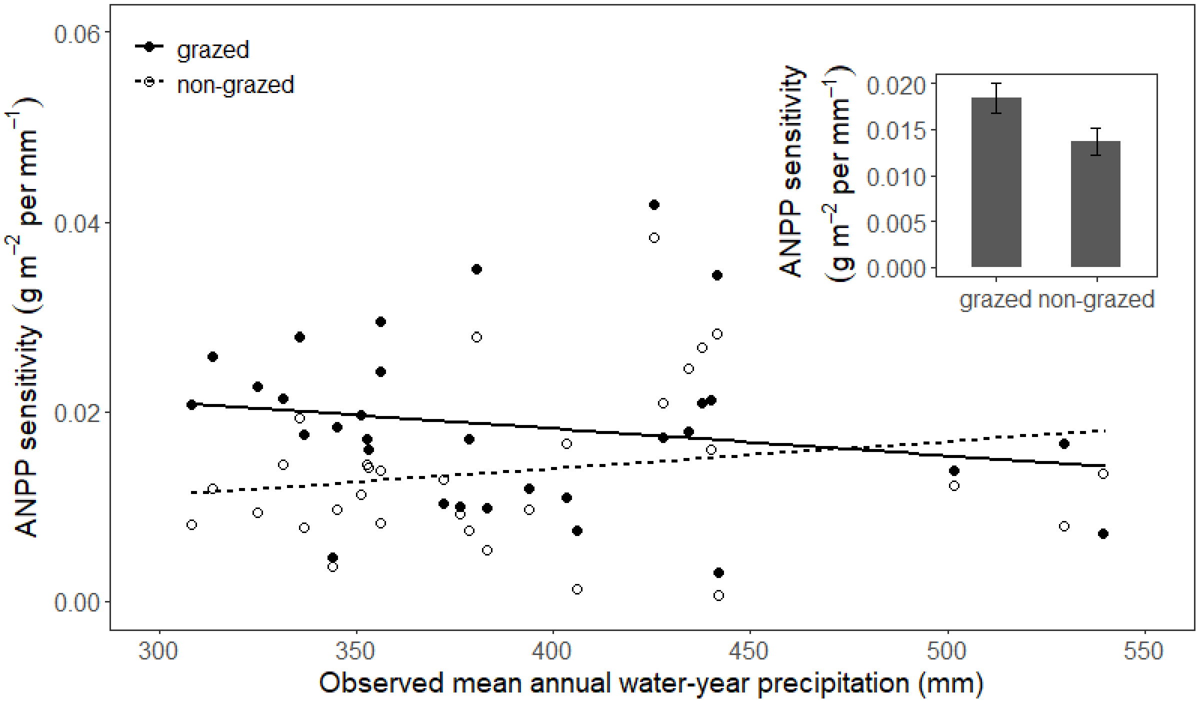

Figure 2-2. The relationship between ANPP sensitivity and observed mean annual water-year precipitation (September 1-August 31). Data points represent 31 sites with pairs of grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation. Lines are linear mixed-effect model fits for grazed and non-grazed locations. The inset compares mean ANPP sensitivity (± SE) of grazed and non-grazed locations across the same set of sites.

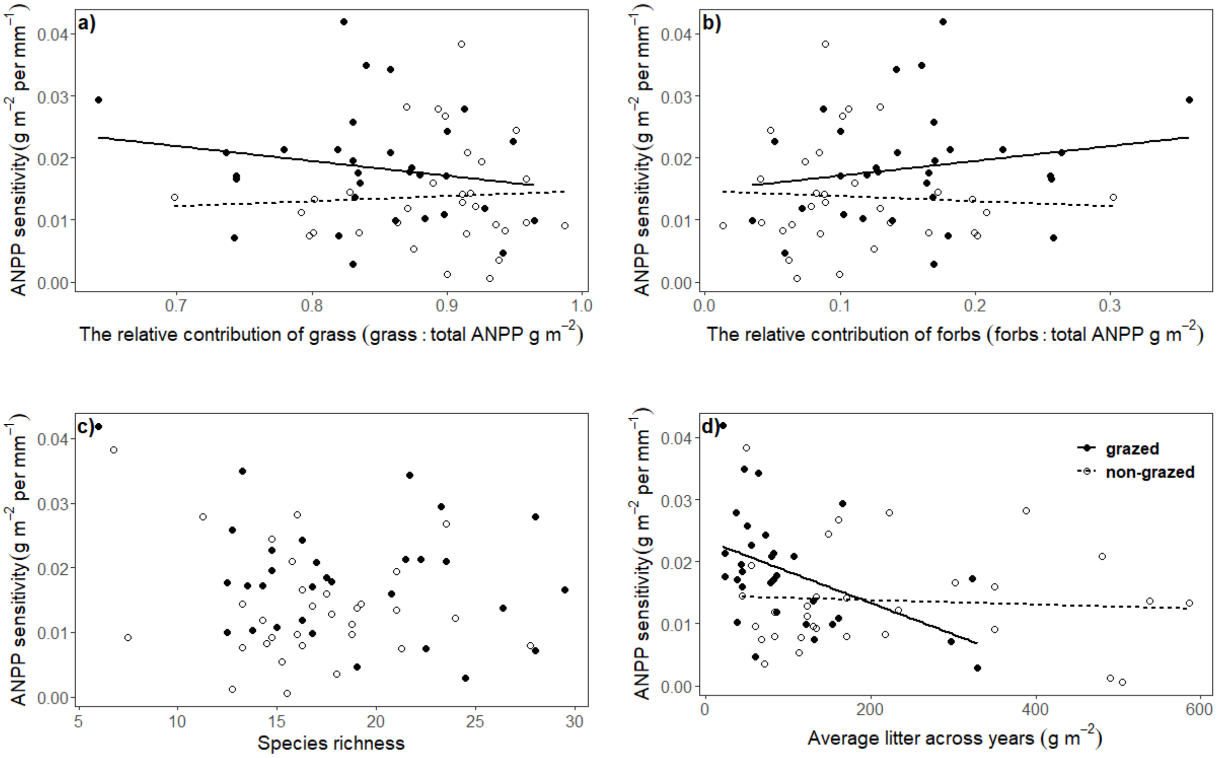

Figure 2-3. The relationships of ANPP sensitivity with the relative contribution of grass (a) and forbs (b) biomass to total ANPP, species richness (c), and litter (d), respectively. Data points represent 31 sites with pairs of grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation.

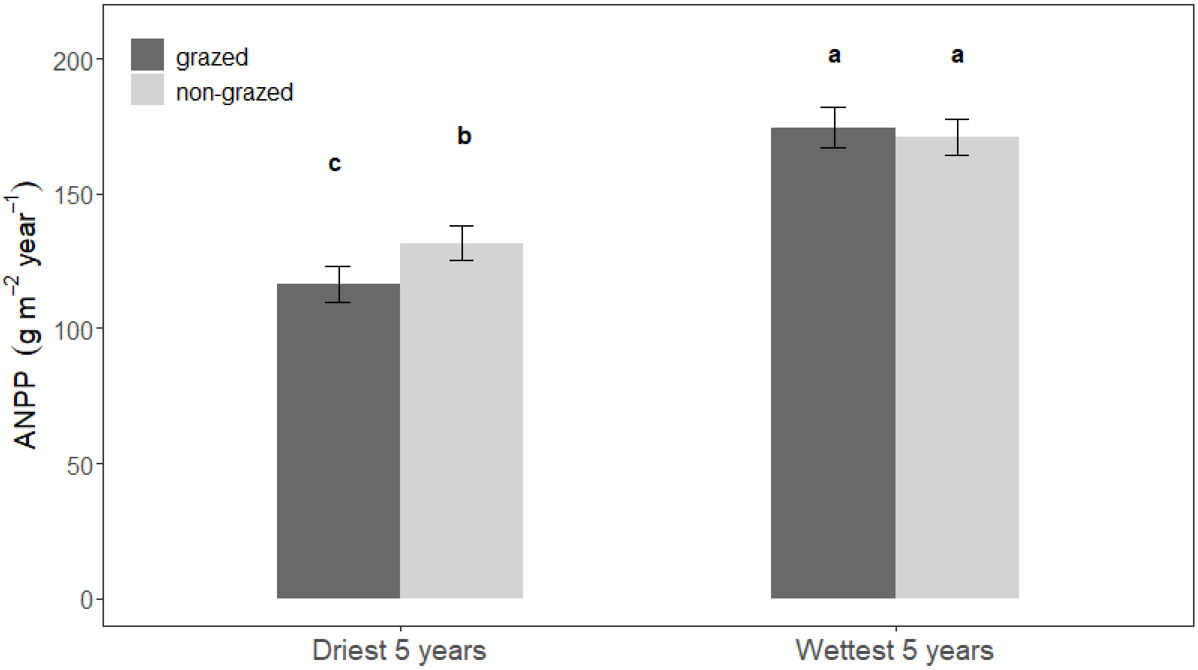

Figure 2-4. The effects of grazing on ANPP (means ± SE) during the five driest and five wettest years on record for 31 sites that experienced substantial variability of precipitation during the period of observation (see Appendix 2-S2). Bars sharing a letter are not statistically different ($p > 0.05$).

Chapter 3: Multi-year drought alters plant species composition more than productivity across northern temperate grasslands

Abstract

The occurrence of multi-year drought is predicted to increase globally with climate change. However, it is unclear whether drought effects on ecosystems are progressive through time. Here, we experimentally reduced growing season precipitation (GSP) by 45% at seven North American temperate grasslands for four consecutive years to determine: 1) whether the effects of reduced precipitation on plant community structure and biomass components (shoot, root, litter) are compounding over time, 2) whether prior year climatic and soil conditions influence subsequent drought impacts on plant community structure and biomass components, and 3) whether the effects of reduced precipitation on individual ecosystem components are related to one another. Across the seven field sites, we observed neither consistent nor progressive effects of reduced precipitation on any biomass component during the experiment, despite having extreme drought conditions imposed for four consecutive years. Relative to the ambient treatment, aboveground net primary productivity (ANPP) declined in response to drought during the early years of the experiment but increased above the ambient treatment in the fourth year, while root and litter biomass were stable across the sites throughout the study. Similarly, graminoid cover decreased initially but recovered by the final year of the experiment, contributing to observed differences in species composition between treatments across sites. Compositional changes were not associated with any declines in species richness or evenness. Divergent responses among years were not driven by lag effects based on prior year climatic and soil conditions. Further, precipitation effects on ecosystem components were largely independent as we found only two positive links: between ANPP and plant species richness, and between

species evenness and composition. Overall, our results suggest that these northern grasslands are relatively resistant to short-term multi-year drought in the context of supporting plant diversity and biomass production.

Introduction

Climate change models predict that grasslands in North America will experience more frequent, severe, and prolonged droughts in the future (Dai 2011, 2013). Understanding how changes in drought characteristics influence ecosystem structure and function has gained interest over the past two decades (Hoover et al. 2018, Zhang et al. 2019), in part due to the substantial socio-economic loss often associated with drought (Weaver 1968, Knapp et al. 2020). Despite this extensive effort, our ability to make a broad generalization and develop a predictive framework remains a challenge for ecologists today because of differences in methodology (Wilcox et al. 2017, Hoover et al. 2018, Song et al. 2019) or lack of standardized assessments of the magnitude of droughts tested in the studies, particularly relative to site-specific normal precipitation variability (Slette et al. 2019). Another overlooked attribute of drought is the effect of duration, and those that have assessed drought duration report conflicting patterns (Estiarte et al. 2016, Gao et al. 2019, Komatsu et al. 2019). Evidence exists that, over time, drought effects can either increase (Orth et al. 2020, Felton et al. 2021), decrease (Wang et al. 2021) or remain unchanged through time (Jentsch et al. 2011, Carroll et al. 2021). The occurrence of a multi-year drought is expected to increase dramatically over this century across North America (Zhao et al. 2020); thus, it is important to ascertain the ecological consequences of prolonged droughts.

Drought as a result of an extended period of precipitation deficiency usually results in reductions in ecosystem function and alterations in grassland structure (Weaver 1968, Knapp et al. 2020). Notably, evidence is accumulating that drought effects on vegetation are likely to intensify with the duration of drought (Evans et al. 2011, Zhang et al. 2019, Orth et al. 2020) as changes in the variability and pattern of precipitation further limit the functioning of water-limited systems (Smoliak 1986, Knapp and Smith 2001). For example, a two-year drought experiment in the Great Plains of North America showed that the reduction in plant productivity was greater in the second year than in the first year (Hoover et al. 2014). Several lines of evidence suggest that climatic, vegetation and soil conditions experienced in prior years may have been an underlying factor regulating progressive drought impacts through time (Sala et al. 2012, Reichmann and Sala 2014, Gherardi and Sala 2015, Petrie et al. 2018). Thus, an ecosystem may respond differentially to multi-year drought than a single (non-consecutive) drought year. Despite this, the effects of multi-year drought on ecosystems have received less attention than the magnitude of drought, likely because the duration of drought experiments is often limited to 1-2 years (Hoover et al. 2018, Zhang et al. 2019).

There are seemingly conflicting patterns of ecosystem responses to drought across existing studies and meta-analyses (White et al. 2012, Carlyle et al. 2014, White et al. 2014a, Wilcox et al. 2015, Wilcox et al. 2017, Song et al. 2019). These varying impacts of drought have been attributed to differences in site characteristics such as precipitation (Carlyle et al. 2014, White et al. 2014a, Wilcox et al. 2015, Hoover et al. 2018). For instance, reduced precipitation is expected to have minimal impacts on ecosystems if the ambient precipitation is low compared to long-term norms during the investigation (Heitschmidt et al. 2005, Hoover et al. 2018) or the condition imposed by rainfall reduction is within natural precipitation variability of given a site (Slette et al. 2019). Additionally, drought can alter the availability of soil nutrients through impacts on plant growth; such changes in the availability of nutrients, particularly nitrogen, may lead to divergent ecosystem responses to drought (He and Dijkstra 2014). It is also possible that ecosystems falling within transition zones, or the edge of their range, might not respond similarly to climate change compared to ecosystems that have been relatively stable in the past (Schneider et al. 2009). For example, for grasslands with their northern range limit in Alberta, Canada, the coldest limit of their range might be expected to be less sensitive to the direct effects of reduced precipitation (Carlyle et al. 2014, White et al. 2014a). However, few precipitation manipulation experiments have been conducted at the northern edge of the Great Plains (Hoover et al. 2018). Thus, standardized and multi-year drought experiments are needed to identify general patterns about different ecological responses within and across ecosystems (Fraser et al. 2013).

In addition to site characteristics, hierarchical responses in ecosystem components (e.g., plant physiological to community change) to drought may lead to contrasting responses across different ecosystems, or alternatively, varied responses among individual ecosystem components, e.g., richness and biomass (Smith et al. 2009, Walter et al. 2013, White et al. 2014a). For example, across grasslands globally, drought reduced aboveground net primary productivity (ANPP) but had no impact on root biomass (Wilcox et al. 2017). This may be attributed to the preferential allocation of carbohydrates to roots in order to maximize the capture of water (Friedlingstein et al. 1999, Sanaullah et al. 2012, Hasibeder et al. 2015). Moreover, shifts in the abundance of functional groups induced by drought are known to either reduce ANPP (Hoover et al. 2014) or maintain ANPP (Grime et al. 2008, Liu et al. 2018), potentially due to compensation of growth responses by different plant species under drought. Since drought can simultaneously affect multiple ecosystem properties, an understanding of the interrelationship of the response of different ecosystem components to drought is needed to better understand whether there are general patterns across these different components of the ecosystem (Jentsch et al. 2011, Smith 2011).

To assess the effects of drought on community structure and biomass components, we experimentally imposed drought by reducing growing season precipitation (GSP) by 45% at each of seven grasslands in the Northern Great Plains over four consecutive years. Here we report responses in plant species richness, evenness, and composition as community structure, as well as responses in ANPP, and standing root and litter biomass as biomass components. Four years of experimentally imposed drought allowed us to address the following questions: 1) do the effects of reduced precipitation on plant community structure and biomass components compound over time, 2) do prior year climatic and soil conditions influence the effects of reduced precipitation on plant community structure and biomass components in the current year, and 3) are the responses of different ecosystem components to reduced precipitation related?

Methods:

Site description

This study was conducted at seven native grasslands in the province of Alberta, Canada. These sites represented the range of variation in climate, vegetation, and soil properties of the northern extent of the North American Great Plains (Appendix 3; S1 & S2). Mean (115-yr) annual precipitation ranged from 312 to 533 mm, with mean annual temperatures varying from 1.9 to 4.9 °C (Appendix 3-S1). Across all sites, 60-70% of annual precipitation falls as rainfall during the growing season, while the dormant season (October-April) inputs make up the remaining precipitation. All sites had a prior history of grazing by large mammals (deer, antelope, elk, moose, and bison), with a recent history of moderate cattle grazing based on local rainfall and productivity. Cattle were excluded during the four-year experiment through the use of fencing. Additional information is given in Appendix 3-S1.

Precipitation treatment

We implemented a standardized and fully randomized experimental design at all seven sites. Treatments (ambient or reduced precipitation) were applied to 2.5 × 2.5 m plots, each separated from neighbouring plots by a 1 m buffer zone. Non-destructive measures were recorded in a central 50 × 50 subplot, with destructive sampling conducted at least 20 cm from the subplot edge (Appendix 3-S2b). At two of the seven sites (Mattheis, Kinsella) treatments were replicated five times, while at the remaining five sites there were four replicates of each treatment for a total of 60 study plots. Plots at each site were located within a topographically uniform area (similar slope, aspect, drainage class).

We reduced precipitation using rainout shelters designed to intercept 45% of ambient rainfall (Gherardi and Sala 2013) from May to September (the growing season), except in the first year, 2016, when shelters were erected in July. Given that the likelihood of multi-year extreme drought over North America is predicted to increase with climate change (Zhao et al. 2020), the percentage of rainfall reduction for each site was determined by the 1st percentile of long-term annual precipitation (1901-2015), corresponding to a threshold level below the normal variability (Knapp et al. 2015b). Although the percentage varied slightly among sites (Appendix 3-S1), we standardized the reduction across sites by evenly placing ten V-shaped transparent acrylic slats on tall wooden frames to cover 45% of the area occupied by the shelter roofs. Shelters were sloped from 120 cm height at the top to 90 cm on the low end, where gutters collected the water and drained it away from plots. Because these open-sided shelters permit free airflow over plots and transmit more than 90% of photosynthetically active radiation to the top of the plant canopy (Appendix 3-S3), these shelters had minimal effects on plot microclimatic and plant photosynthetic processes (Loik et al. 2019) in the current study. Each October we removed the acrylic slats for winter and reinstalled them the following May.

Abiotic and biotic data collection

To answer our research questions, we required data characterizing site-specific differences in climate, soil, and vegetation conditions over multiple years. Although all sites were situated within the northern Great Plains, they are separated from each other by 200 to 780 km. Thus, each site experienced different weather conditions, which could impact the potential severity of rainfall interception from our passive shelters. To quantify the extent of rainfall reduction experienced at each site, and its interaction with year of drought imposed, we used monthly precipitation, minimum, and maximum temperature to calculate two drought metrics: precipitation percentiles (Knapp et al. 2015b) and the Standardized Precipitation-Evapotranspiration Index (SPEI) (Beguería et al. 2014). We used monthly precipitation obtained from the ClimateNA v6.3 software package (Wang et al. 2016) to determine the 5th percentile of long-term growing season precipitation (1901-2015) as an indicator of a threshold for extreme drought conditions (Knapp et al. 2015b). Growing season precipitation (GSP) for each ambient and reduced precipitation treatment (GSP × 55%) during each year (2016-2019) was calculated from a nearby weather station (typically < 10 km distance) to each study site. GSP is the total rainfall from May to September. We used average data of three surrounding weather stations for three sites (Oyen, Twin River, and Sangudo) where there was no weather station within a 10 km radius. Although the percentile-based index contextualizes the magnitude of imposed drought relative to historical precipitation, it does not consider potential confounding effects of temperature. Thus, we calculated a second metric, SPEI, which uses the difference between precipitation and reference evapotranspiration for a given location, based on monthly precipitation and both minimum and maximum temperature (Beguería et al. 2014).

The SPEI was calculated for a time scale of 1-month using the spei R package (Beguería and Vicente-Serrano 2017). To do so, we calculated monthly SPEI values of each experimental year (2016-2019) and for each site, from nearby weather station data and normally distributed monthly reference SPEI values from ClimateNA (1901-2015). To estimate each site's drought severity during every growing season, we averaged monthly SPEI values from May to September. Positive SPEI values indicated a water surplus, and negative values indicated a water shortage relative to the reference value (Beguería and Vicente-Serrano 2017).

To determine whether rainout shelters altered soil resources, we measured soil water content (VWC), soil organic carbon (SOC), and total nitrogen (N) within each plot (Appendix 3-S2c). Soil water content was measured every two hours throughout each growing season using Decagon 5TM sensors, attached to Em50 ECH20 loggers. Sensors were placed near the central subplot of each plot (5 cm deep; Appendix 3-S2c), and multi-temporal readings were averaged to produce monthly mean soil water content. SOC and N were measured from composite soil samples collected from each plot in August each year by taking five random soil cores (3.25 cm diameter) to a depth of 0-15 cm (Appendix 3-S2c). Samples (~10 g) from each plot were dried at 60 °C for 48 h and ground to 0.1 mm in a ball mill (Retsch MM400 Mixer Mill, Retsch, Haan, Germany), and then analyzed with a LECO TruSpec CN elemental analyzer (LECO Corporation, St. Joseph, MI, USA).

We measured plant community composition, aboveground net primary productivity (ANPP), root biomass, and litter mass at each plot between 10 and 30 July, each year, coincident with peak biomass across the study sites. We estimated the percent cover (to the nearest 1%) for each plant species rooted in the 50 × 50 cm subplots (Appendix 3-S2c). Species richness and evenness were calculated from species abundance data for each plot using the *community_structure* function from the codyn R package (Hallett et al. 2020). We used Evar as a measure of species evenness, which is based on the variance in abundance values among all species present in a plot, and is independent of species richness (Smith and Wilson 1996). We sampled ANPP and litter (standing dead and detached material) from within a 0.1 × 1 m strip quadrat in each plot, with a different location sampled each year. All harvested living plants were sorted into species. To quantify standing root biomass, two root cores (Appendix 3-S2c), 5 cm in diameter and 15 cm deep, were taken from within the clipped quadrat of each plot. Root samples were sieved over a 2 mm sieve and then washed over a 1 mm sieve in the lab. All vegetation samples were dried at 65 ºC for at least 48 h and weighed. Values from the two root cores were averaged to estimate standing root biomass, while biomass values across species were summed to estimate ANPP. The ANPP estimate did not include shrub biomass, which was found only at one site, Stavely, and made up less than 9% of the total ANPP at the site. All biomass values were converted to g m^{-2}.

Calculation of treatment effects

To measure the effects of reduced precipitation on species richness, evenness, ANPP, standing root biomass, and litter mass, we used the log response ratio of reduced precipitation relative to ambient precipitation (LRR = ln[reduced/ambient]). Departures in species composition relative to the ambient treatment were assessed with a Bray-Curtis dissimilarity index. To calculate the Bray-Curtis dissimilarity index (pairwise distance between centroids) for each year and site, we used the *multivariate_difference* function from the codyn R package

(Hallett et al. 2020). Because ambient and reduced precipitation treatments were not paired within each site, each variable's LRR was calculated by pairing every possible combination of replicates of treatments within each year by site combination and then averaged (n = 28) (Lamb and Cahill 2006). Negative values of LRR indicate that reduced precipitation decreased the variable relative to the ambient treatment, while positive values indicate that rainfall reduction increased the variable relative to the ambient treatment. Bray-Curtis dissimilarity values range from 0 to 1: where 0 indicates an identical community, while 1 indicates a completely different community.

Statistical analyses

All statistical analyses were conducted using R studio (RStudio Team 2019) based on R version 3.6.1 (R Core Team 2019). Data from the first year (2016) were excluded from all analyses due to the incomplete nature of treatments in that year, and instead commenced using the fully imposed drought over the entire growing season starting in 2017. To better generalize drought impacts across the northern edge of the Great Plains, we sacrificed within site replicates (4-5 replicates) to allow more sites to be studied in our research and treated sites as a form of replication in our analyses (Filazzola and Cahill 2021). Further, our objective was to minimize type 2 errors relative to the effects involving a drought treatment or the combination of treatment and year; thus, we used an alpha of 0.1 to determine significance.

To determine whether rainout shelters altered soil water content (VWC), soil organic carbon (SOC), or total soil nitrogen (N), we used linear mixed-effects models. Fixed effects were the primary rainfall treatment (ambient and reduced), year, and treatment by year interactions. Plots, nested within site and month, were random effects for VWC, SOC, and N models. All linear mixed-effect models were fitted with the lme4 package (Bates et al. 2015).

To determine whether the effects of reduced precipitation on ANPP, root biomass, litter mass, species richness, evenness, and composition were compounding over time, we ran repeated-measures mixed-effect models on each variable. Treatment (ambient and reduced), year (treatment duration), and their interaction were fixed effects, while site was a random effect in all models. Raw values of ANPP, root, litter, and species evenness were log-transformed to meet normality assumptions. The effect of precipitation reduction on species richness was tested using a generalized linear mixed-effect model with a Poisson distribution (log link). To test whether reduced precipitation altered species composition, we used repeated-measures permutational multivariate analysis of variance (perMANOVA) using the *adonis* function in the vegan package, and used the option strata (plots nested within site) to account for differences in vegetation across sites (Oksanen et al. 2017). We expected to find a significant treatment by year interaction if the effects of reduced precipitation on community structure and biomass components increases progressively over time due to compounded effects, or alternatively, decreased over time due to ecosystem adjustments.

In addition, we conducted three complementary analyses to gain insights into what aspects of community composition resulted in differences in species composition between the ambient and reduced precipitation treatments. First, we took a general approach to examine whether the reduced precipitation treatment altered community composition through changes in the cover of dominant growth forms (graminoids vs forbs). To do so, we summed the cover of all graminoid and forb species separately within each replicate of ambient and reduced precipitation treatments, for each year by site combination. Reduced precipitation effects on the cover of graminoid and forb were assessed with repeated-measures mixed-effect models, with treatment, year, and treatment by year interaction as fixed effects, and plots nested within site as a random factor. Secondly, we carried out a more in-depth examination of community differences between treatments for each year using measures derived from rank abundance curves (Avolio et al. 2019). Four measures of community difference were calculated with *RAC_difference()* function based on species relative abundance data in the codyn R package (Hallett et al. 2020). To estimate the magnitude of each measure contributing to differences in species composition between treatments without confounding impacts of directionality, we used the absolute values of differences in species richness, evenness, rank, and species substitution (e.g., a species replacement component of beta diversity, Carvalho et al. 2012) between ambient and reduced precipitation treatments from *RAC_difference()* output. Lastly, we conducted a similarity percentage analysis (SIMPER, Clarke 1993) to identify the relative contribution of each species to the divergence of community composition between the ambient and reduced precipitation treatments using *simper()* function in the vegan R package (Oksanen et al. 2017). Since three sets of analyses provided the same interpretation of results (i.e., Figure 3-1 vs. Appendix 3-S8; Appendix 3-S11), we report and discuss the findings from the growth form analysis in the main text and place outputs from rank abundance curves and SIMPER analysis in the supplementary information (Appendix 3; S8-11).

We used linear regression to investigate whether site-specific prior year climatic (GSP, SPEI) or soil conditions (VWC, SOC, and N) influenced the effects of reduced precipitation on community structure and biomass components the following year. Data from 2019 were used for this analysis as they represented the cumulative effects of the treatments over time. Site-specific prior year climatic or soil conditions for each site were calculated by averaging GSP, SPEI, VWC, SOC, and soil N of the ambient treatment from 2017 to 2019. The VWC, SOC, and soil N were averaged first across all replicates of the ambient treatment for each site and year. In the models, LRRs of ANPP, root biomass, litter mass, species richness, species evenness, and Bray-Curtis dissimilarity index of species composition, were the response variables. Predictors were averages of five site-specific variables, including GSP, SPEI, VWC, SOC, and N, during the experiment.

To test whether the effects of reduced precipitation on individual ecosystem components were associated with impacts on other components, we carried out Spearmen correlation analyses between LRRs of ANPP, root biomass, litter mass, species richness, evenness, and the Bray-Curtis dissimilarity index of species composition. A significant correlation between variables indicates that the variable's response to reduced precipitation is proportional to that variable.

Results

The efficacy of rainout shelters

During the experiment, growing season precipitation (GSP) varied among sites and years (Appendix 3-S1). Overall, GSP within the ambient treatment plots was lower than the long-term GSP in 17 of the 21 site × year combinations. The three exceptions all occurred in 2019, wherein GSP was above the long-term average at the three wettest sites (Kinsella, Sangudo, and Stavely). Consequently, due to the lower than average precipitation throughout this region for most of this study, the rainout shelters reduced GSP to extreme levels, effectively below the 5th percentile of long-term GSP in 18 of the 21 site × year combinations (Appendix 3-S4). We also found a similar pattern in the drought severity index, with SPEI indicating the rainout shelters created conditions comparable to severe drought across all site × year combinations, except as noted, for the three wettest sites during 2019 (Appendix 3-S5).

Rainout shelters were effective in decreasing soil moisture throughout all three consecutive growing seasons ($F_{1,\,210} = 12.67$, $P = 0.0005$), including reducing soil moisture in 18 of 21 site × year combinations (Appendix 3-S6). Soil moisture further varied among growing seasons ($F_{2,\,760} = 13.68$, $P < 0.0001$), however, the differences in soil moisture between treatments were similar in magnitude as there was no interaction between treatment and year ($F_{2,\,764} = 0.69$, $P = 0.5$; Appendix 3-S6). Further, rainout shelters did not alter total soil nitrogen ($F_{1,\,146} = 0.06$, $P = 0.81$) or soil organic carbon ($F_{1,\,139} = 0.20$, $P = 0.66$) in the top 15 cm of soil (Appendix 3-S7), and the interaction between treatment and year was not significant for soil nitrogen ($F_{2,\,115} = 1.12$, $P = 0.33$) or for carbon ($F_{2,\,114} = 1.95$, $P = 0.15$).

Are the effects of drought on community structure and productivity compounding?

Four years of reduction in precipitation led to greater changes to community composition (Table 3-1) than the biomass components (Table 3-2) across the seven sites. However, the difference induced by multi-year drought in species composition between the ambient and reduced precipitation treatments was not directional over the course of the experiment (Figure 3-1a), as indicated by the non-significant treatment by year interaction term (Table 3-1). While there were no overall effects of reduced precipitation on plant species richness (Figure 3-1b, Table 3-1) or evenness (Figure 3-1c, Table 3-1), we found that the reduced precipitation treatment decreased graminoid cover during 2017 and 2018 but not in 2019 (Table 3-1; Figure 3-1d). The cover of forbs did not differ significantly between treatments throughout the study duration (Table 3-1; Figure 3-1e). Rank abundance curves indicated that differences in species richness and evenness between treatments were relatively small, whereas rank (e.g., reordering in species abundance) and species substitution were aspects of community composition that differed between the treatments across sites during the experiment (Appendix 3-S8). Shifts in species' ranks within a community were modest and occurred without the loss or changes in the identity of dominant species under extreme drought (Appendix 3-S9). Further, SIMPER analysis revealed that changes in the abundance of graminoids contributed primarily to compositional shifts with drought (Appendix 3-S11). Thus, this combination of results suggests that the difference in species composition between the ambient and reduced precipitation treatments was driven by changes in the abundance of the dominant growth form (graminoids), rather than changes in plant species gains or losses (Appendix 3-S9).

Rainout shelters reduced ANPP to different degrees among years, as indicated by the significant treatment by year interaction (Table 3-2). However, there was no evidence of cumulative effects of the multi-year drought, as ANPP with rainfall reduction in 2019 was found to be higher than under ambient conditions (Figure 3-2a). We found no effects of rainfall reduction on root biomass or litter mass (Table 3-2; Figure 3-2), suggesting that the shifts observed in ANPP were more complicated than simple biomass reallocation or alteration of edaphic conditions.

Do prior year climatic and soil conditions influence subsequent drought impacts on community structure and productivity?

Overall, across the seven sites, the effects of reduced precipitation treatment on community structure or biomass components in 2019 were largely independent of site-specific climatic and soil conditions experienced in the prior year (Figures 3-3 and 3-4), with two exceptions. First, we found an association between the effects of reduced precipitation on species evenness and prior year GSP ($F_{1,5} = 4.1$, $p = 0.097$). However, this relationship appears to be driven by a single data point, and thus we urge caution in assuming biological realism (Figure 3-3b). Potentially a more biologically plausible finding was the greater magnitude of drought effects on ANPP occurred in a given year when soil moisture content in prior years was high ($F_{1,5} = 4.7$, $p = 0.082$; Figure 3-4a). In other words, across all seven sites, drought may cause greater reductions in ANPP when previous years were relatively wet.

Are the effects of drought on ecosystem components related to one another?

Of the 15 possible pairwise correlations, there were only two positive associations between individual ecosystem components in response to the reduced precipitation (Figure 3-5). Despite no observable effects of reduced precipitation on species richness and evenness, impacts of reduced precipitation on ANPP were positively correlated with richness. Similarly, reduced rainfall effects on composition were positively associated with rainfall impacts on evenness. This small number of coupled responses of ecosystem components suggests that the effects of reduced precipitation are primarily limited to very few components of the system.

Discussion

Reducing water availability at extreme levels for four consecutive years caused changes to these grassland ecosystems, but not in a way consistent with expectations that the effects of prolonged drought would intensify over time (Zhang et al. 2019, Orth et al. 2020, Felton et al. 2021) or other precipitation manipulation experiments conducted in the Great Plains of North America (Wilcox et al. 2017, Hoover et al. 2018). The primary impacts of reduced growing season rainfall were limited to differences in plant species composition, which occurred without substantive changes in overall community richness and evenness. While aboveground net primary productivity changed, the effect did not compound through time. Root biomass and litter mass remained stable over the course of the experiment. Together these findings show that many ecosystem properties within these grasslands appear to be relatively resistant to consecutive years of water deficit, consistent with several other multi-year drought experiments within grasslands that found a high degree of resistance to multi-year drought (Heitschmidt et al. 2005, Evans et al. 2011, Jentsch et al. 2011, Estiarte et al. 2016).

Since the prairies of western Canada have experienced both short and extended periods of drought in the past (Bonsal et al. 2011), the strong resistance to extreme multi-year drought found here may be due to the adaptations of plant communities incurred through previous exposure to episodic deficits in water availability. It is worth noting that the amount of precipitation falling on ambient plots during the experiment was well below the long-term site averages. Thus, plants within the ambient treatment may have been constrained by natural drought, thereby muting comparative treatment impacts (i.e., drought-induced effects vs the controls) on community structure and productivity (Heitschmidt et al. 2005, Hoover et al. 2018). Related to this, the timing and seasonality of precipitation can also confound the effects of rainfall reduction treatment on ecosystems (Heisler-White et al. 2009, Cherwin and Knapp 2012, Post and Knapp 2020). For example, grasslands in our study were dominated by C_3 species that may mitigate the adverse effects of drought on plant growth by taking advantage of conditions such as high soil water availability and low air temperature early in the season in association with winter precipitation (Smoliak 1986, Knapp et al. 2020). Specifically, root system efficiency, e.g., shoot biomass produced per unit root length, is another mechanism that can maintain or stimulate ANPP under drought conditions (Frank 2007, Ma et al. 2020a). Plants under drought can shift the allocation of recently assimilated carbohydrates to roots to maximize water capture (Hasibeder et al. 2015). Such preferential allocation of assimilated carbohydrates to belowground enables plants to invest in root length growth, which can occur without substantive changes in root biomass (Ma et al. 2020a), thereby improving overall root system efficiency (Frank 2007, Ma et al. 2020a). Finally, as predicted by the mass ratio hypothesis (Grime 1998), both the loss of plant species (Zhang et al. 2019) or changes in the identity of dominant species (Hoover et al. 2014) typically results in significant changes in ecosystem structure and function. Despite finding changes in the abundance of graminoids in response to drought, we found no evidence of plant species loss or changes in dominance under four consecutive years of drought across the seven grasslands. This indicates that stability among the dominant plant species may be a likely explanation for the high resistance to drought, as the abundance of those species often determines the overall community response to altered precipitation (Grime 1998, Evans et al. 2011, Carlyle et al. 2014).

We found that the greater magnitude of drought effects on ANPP occurred in the current year when the soil moisture content in previous years was relatively higher. This suggests that legacy effects of precipitation may develop through soil moisture availability, and a higher maintenance cost associated with enhanced plant growth in a prior wet year can result in ANPP reductions in the drought year (Sala et al. 2012). However, the effects of reduced precipitation on community structure or biomass components in the current year were not affected by any other prior year climatic or soil conditions considered in this study. This overall lack of prior year climatic and soil condition effects could be due to changes in the allocation of carbohydrates to plant organs in response to drought (Friedlingstein et al. 1999, Sanaullah et al. 2012, Hasibeder et al. 2015). Although we found no changes in root biomass within the upper soil profile, the disproportionate allocation of carbohydrates to roots during low soil moisture may shift the distribution of roots deeper into the soil profile (Canadell et al. 1996, Schulze et al. 1996), thereby muting the influence of conditions from prior years. However, further studies are warranted, including on deep root responses, to test this physiological mechanism as the root biomass quantified in this study only represented the upper soil profile (0-15 cm).

We found that none of the individual ecosystem components was related to each other in response to reduced precipitation, with two exceptions (see below). The overall lack of associations between the effects of drought on community properties, including vegetation structure or biomass components, may be attributed to the differences in the nature and pace of responses of ecosystem components to drought (Smith et al. 2009). For example, we observed the following patterns of response to drought in this experiment: immediate changes in soil moisture content, followed by shifts in species composition (though not richness or evenness), but inconsistent effects on ANPP. This may chiefly be through variation in the abundance of graminoids, which contribute most to the stability of productivity to precipitation in the region (Bork et al. 2019, Batbaatar et al. 2021a). The positive link between the effects of reduced precipitation on species composition and evenness further suggests that changes in the abundance of graminoids may have led to differences in species composition between the ambient and reduced precipitation treatments. Moreover, the positive association between the effects of reduced precipitation on ANPP and plant species richness supports findings from a previous experiment conducted in the same study region in which productivity was the key indirect driver of plant species richness responses to precipitation (White et al. 2014a).

Understanding the effects of drought on community structure and productivity through extended periods of time is important for maintaining ecosystem services and developing predictive frameworks across ecosystems. In summary, our findings from this standardized and multi-site experiment show that northern temperate grasslands are highly resistant to four consecutive years of experimental drought. We found that species composition was affected by drought more often than richness, evenness or community productivity. However, we also found little evidence that drought impacts were compounding over time. These non-directional drought effects were largely independent of site-specific climatic and soil conditions experienced during the experiment.

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Table 3-1. Results from repeated measures perMANOVA (composition) and mixed-effect models (all other variables), testing the main and interactive effects of the reduced precipitation treatment (Trt) and year (Yr) on community structure. Degrees of freedom for F-values are given as subscripts in parentheses. Bold values denote statistically significance (p < 0.1) effects.

Fixed effects	Composition		Grass cover		Forb cover		Richness		Evenness	
	F-ps$_{(df)}$	P	F$_{(df)}$	P	F$_{(df)}$	P	$\chi2_{df}$	P	F$_{df}$	P
Trt	$1.69_{(1,171)}$	**0.001**	$0.04_{(1,145)}$	**0.094**	$0.\ 03_{(1,155)}$	0.844	$0.01_{(1)}$	0.906	$0.2_{(1,165)}$	0.653
Yr	$0.52_{(2,171)}$	**0.001**	$8.04_{(1,114)}$	**<0.001**	$1.37_{(2,155)}$	0.257	$4.79_{(2)}$	**0.091**	$3.91_{(2,165)}$	**0.022**
Trt × Yr	$0.09_{(2,171)}$	0.841	$2.76_{(1,114)}$	**0.067**	$0.75_{(2,155)}$	0.474	$1.46_{(2)}$	0.929	$1.06_{(2,165}$	0.348

Table 3-2. Results from repeated measures mixed-effect models, testing the main and interactive effects of reduced precipitation treatment (Trt) and year (Yr) on biomass components. Degrees of freedom for F-values are given as subscripts in parentheses. Bold values denote statistically significance ($p < 0.1$) effects.

Fixed effects	ANPP		Root biomass		Litter mass	
	F_{df}	P	F_{df}	P	F_{df}	P
Trt	$0.2_{(1,167)}$	0.667	$0.1_{(1,165)}1$	0.742	$0.\ 03_{(1,165)}$	0.869
Yr	$5.83_{(2,165)}$	**0.004**	$3.76_{(2,165)}$	**0.025**	$22.25_{(2,165)}$	**<0.0001**
Trt × Yr	$2.34_{(2,165)}$	**0.099**	$0.88_{(2,165)}$	0.416	$0.002_{(2,165)}$	0.998

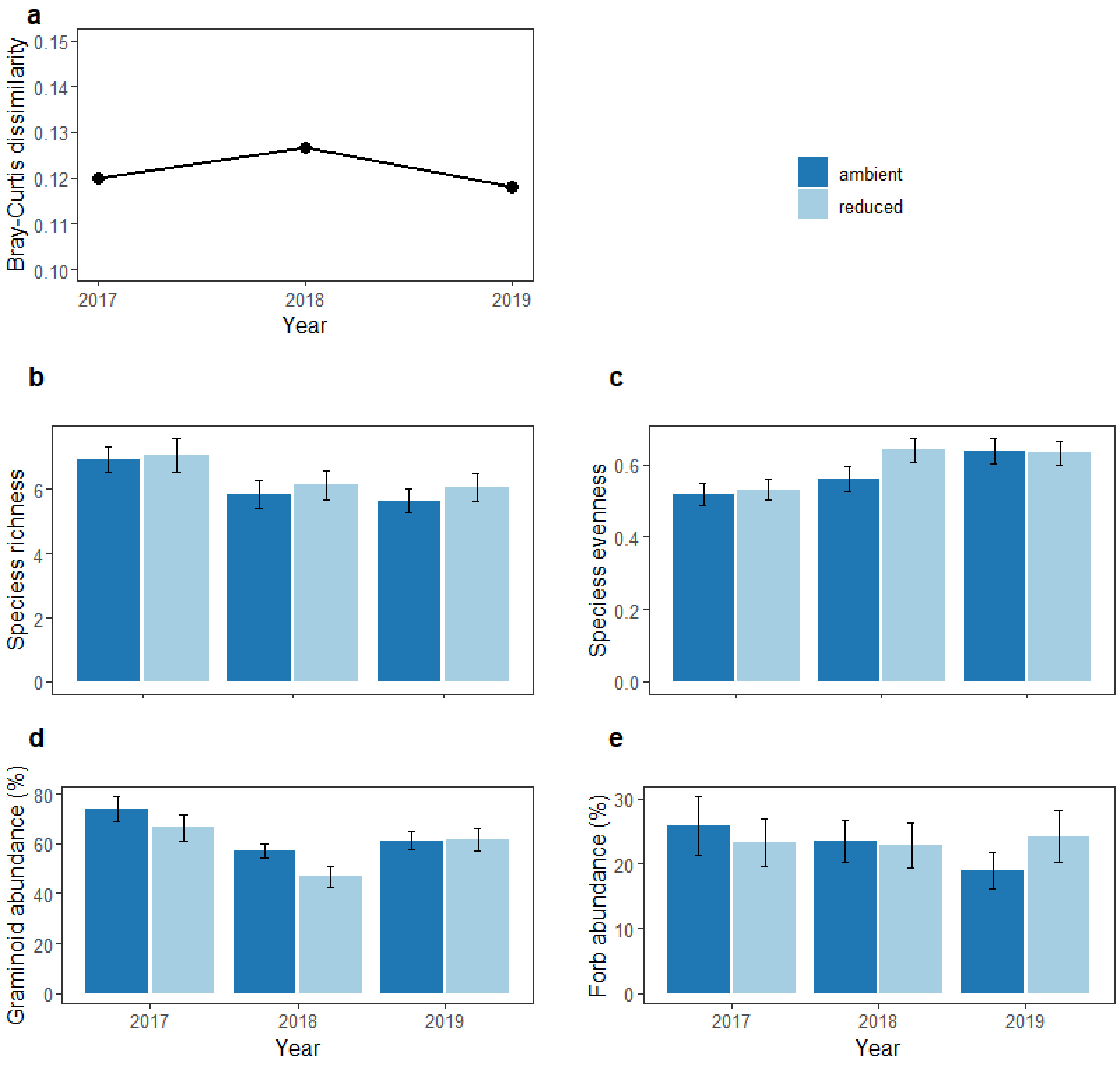

Figure 3-1. The effects of reduced precipitation on (a) species composition as measured by Bray-Curtis dissimilarity, (b) species richness, (c) species evenness, and the abundance of (d) graminoid and (e) forb across the seven sites over the course of the experiment. Bray-Curtis dissimilarity index bounds between 0 (identical) and 1 (completely different).

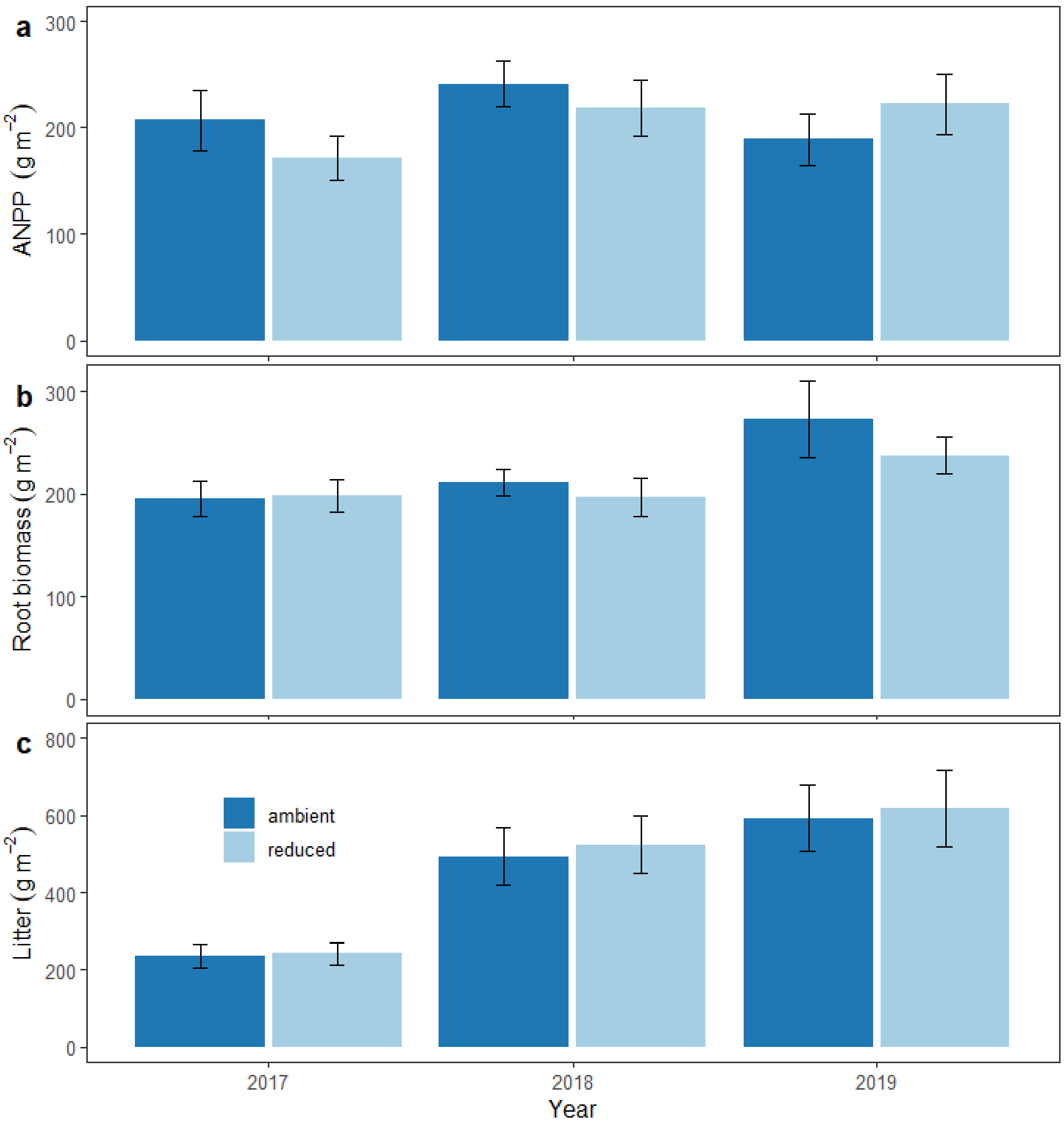

Figure 3-2. The effects of reduced precipitation on (a) aboveground net primary productivity (ANPP, mean ± SE), (b) standing root biomass in the top 15 cm soil, and (c) litter mass across the seven sites.

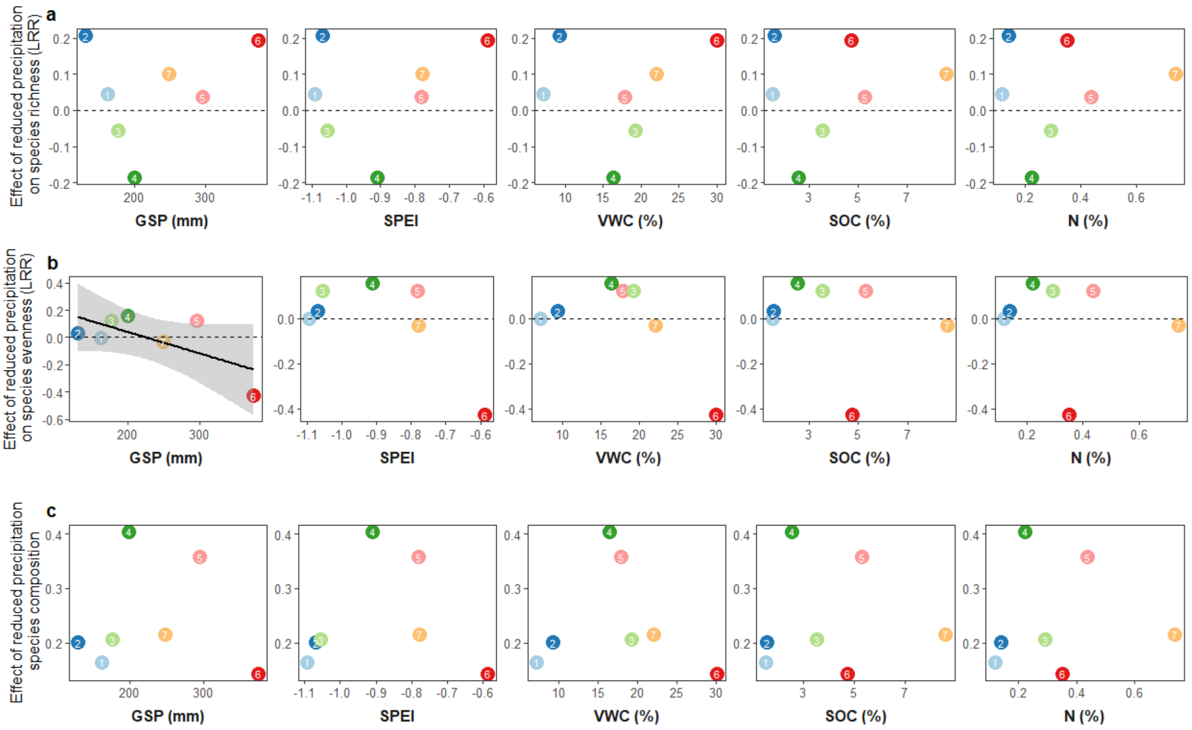

Figure 3-3. The influence of prior year climatic and soil conditions (2017-2019) on the effects of reduced precipitation on (a) species richness, (b) evenness, and (c) composition in 2019. GSP-the average growing season precipitation from 2017 to 2019; SPEI- the average Standardized Precipitation-Evapotranspiration Index; VWC, SOC, and N are the average soil moisture, soil organic carbon, and nitrogen across all replicates of ambient treatment. The effect of reduced precipitation was estimated as the log response ratio (LRR = ln[reduced/ambient]). Bray-Curtis dissimilarity index was used to quantify the difference in species composition between ambient and reduced precipitation treatments (0 = identical; 1 = different). The numbers and colours of points represent each site from most arid to mesic (1-7). The full name and characteristics of sites can be found in Appendix 3-S1.

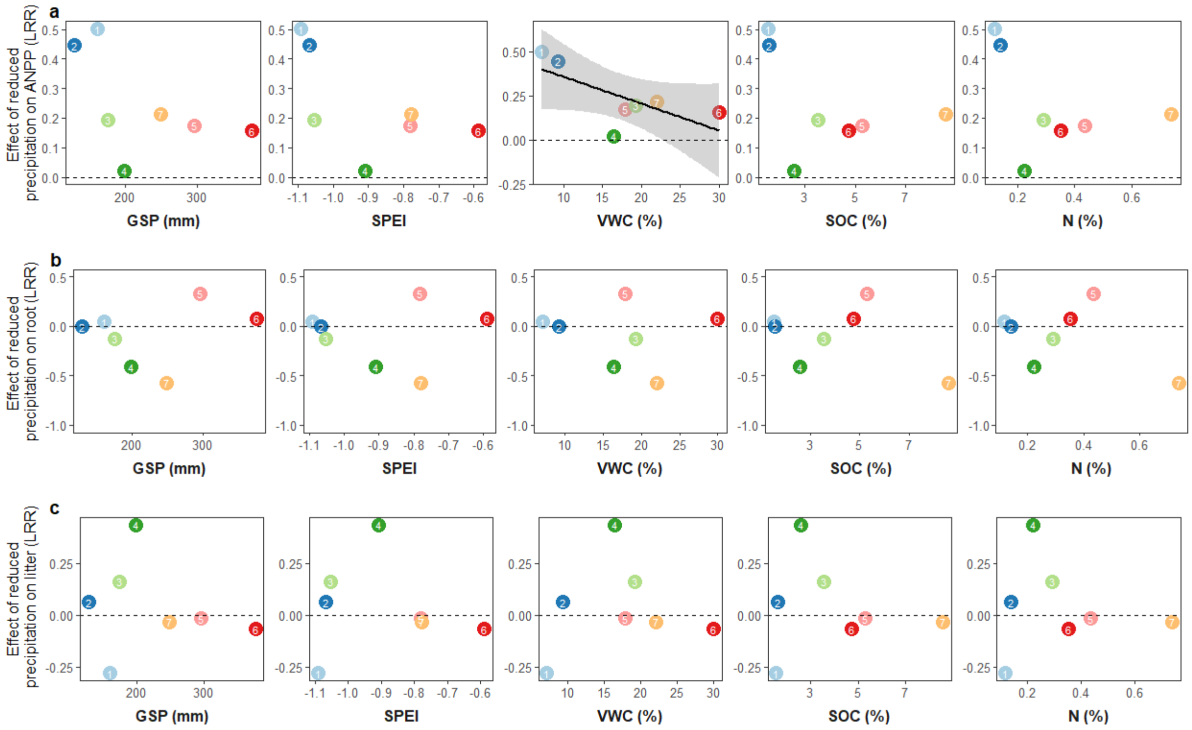

Figure 3-4. The influence of prior year climatic and soil conditions (2017-2019) on the effects of reduced precipitation on (a) ANPP, (b) root and (c) litter biomass in 2019. GSP-the average growing season precipitation from 2017 to 2019; SPEI- the average Standardized Precipitation-Evapotranspiration Index; VWC, SOC, and N are the average soil moisture, soil organic carbon, and nitrogen across all replicates of ambient treatment. The effect of reduced precipitation was estimated as the log response ratio (LRR = ln[reduced/ambient]). The numbers and colours of points represent each site from most arid to mesic (1-7). The full name and characteristics of sites can be found in Appendix 3-S1.

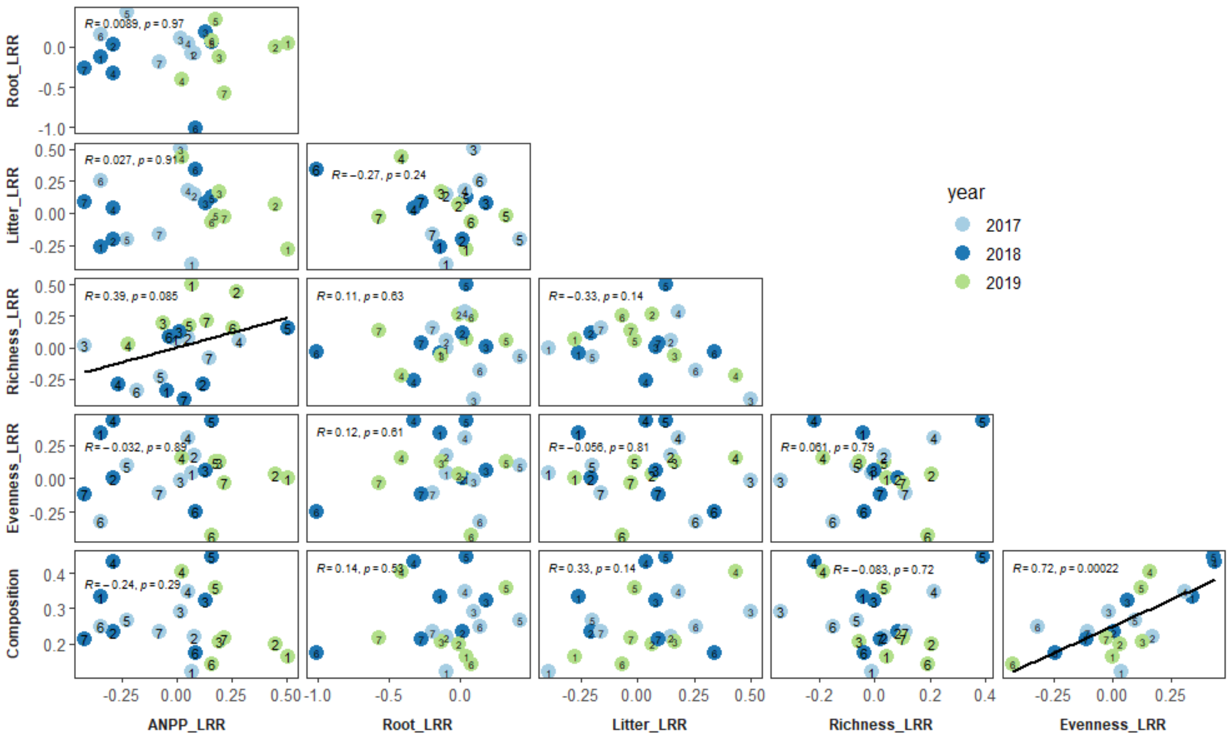

Figure 3-5. Pairwise correlations between the impacts of reduced precipitation on components of the ecosystem. ANPP, root, litter, species richness, and evenness are the log response ratio (LRR = ln[reduced/ambient]). Bray-Curtis dissimilarity index was used to quantify the difference in species composition between ambient and reduced precipitation treatments (composition). The correlation coefficient, R, and significance, p-value, are shown at the top, and lines are included when correlations are significant at $p < 0.1$. Numbers on the points represent each site from most arid to mesic (1-7). The full name and characteristics of sites can be found in Appendix 3-S1.

Chapter 4: Differential sensitivity of above and belowground plant biomass to drought and defoliation in temperate grasslands

Abstract

Co-occurring stressors such as drought and grazing influence plant growth in grasslands; however, little is known how management practices alter plant growth stability under drought. We examined shoot and root biomass responses to extreme drought (ambient vs reduced precipitation) and defoliation (five levels of treatment altering the timing and intensity of plant biomass removal) treatments using the fourth-year data from a factorial experiment conducted at seven temperate grasslands. Relative to ambient treatment, drought increased aboveground net primary productivity (ANPP) in non-defoliated control treatment but generally decreased standing root biomass across the seven grasslands. Defoliation treatments altered ANPP and root biomass differently under ambient and drought conditions. The combined effects of drought and defoliation treatments reduced ANPP but had no impacts on root biomass across sites. However, there was no difference either between early- and late-season defoliation or varying early-season defoliation intensity in their effects on ANPP and root biomass. Further, we found greater ANPP sensitivity to drought in mesic than semi-arid grasslands, whereas the sensitivity of root biomass to drought was not associated with mean annual precipitation. We conclude that these systems are relatively resistant to multi-year droughts, but defoliation is likely to suppress shoot growth under drought.

Introduction

Co-occurring factors such as drought, grazing, and fire drive the structure and function of grasslands (Koerner and Collins 2014, Kohli et al. 2020). Of particular concern in the Northern

Great Plains of North America are potential increases in the intensity and frequency of drought (Zhao et al. 2020, Zhang et al. 2021). Ecosystems affected by drought may experience greater susceptibility to other stressors, such as grazing (Di Virgilio et al. 2019, Godde et al. 2020, Hoover et al. 2020), leading to reduced ecosystem goods and services, including livestock production and provision of wildlife habitat. Indeed, fluctuations in aboveground net primary productivity (ANPP) associated with precipitation variability are projected to adversely impact the ability of beef producers to adapt to environmental risk and maximize livestock production (Klemm et al. 2020). However, understanding of the extent to which grazing management practices influence community-level biomass responses to reduced precipitation is a key, yet rarely addressed, question with major implications for the global carbon cycle (Di Virgilio et al. 2019, Godde et al. 2019). A more nuanced understanding of how drought and aspects of grazing regimes interact to affect above and belowground productivity is needed to identify more sustainable grazing practices (Briske et al. 2020, Godde et al. 2020).

Individual grazing impacts on vegetation are both within (e.g., the timing, frequency or intensity of grazing, and animal type) and outside (e.g., animal behavior) the control of grazing managers, each of which can impact land use outcomes (e.g., forage production) through their influence on plant growth (Briske et al. 2008, Teague et al. 2013, Briske et al. 2020, Derner et al. 2021). Here we focus on two key elements: the timing (early vs late) and intensity (no, light, heavy) of defoliation early in the growing season, as these practices are a promising set of adaptive management options to sustain ecosystem services in the face of climate change (Ingrisch et al. 2017, Döbert et al. 2021, Hulvey et al. 2021). Further, to isolate specific defoliation effects on plant growth, we use controlled clipping rather than herbivores. Mechanical simulation allows examination of plant responses to herbivory in isolation of herbivore feeding-induced (e.g., saliva) plant responses, and eliminates behavioral selection of plants within the community, thereby standardizing treatments to all vegetation present across sites (Waterman et al. 2019).

Experimental studies show that drought reduces the sequestration of carbon by plants (Hoover et al. 2018, Wang et al. 2021) and increases the preferential allocation of recently assimilated carbohydrates to roots to ensure adequate water uptake for maintaining shoot growth (Hasibeder et al. 2015, Ingrisch et al. 2020). However, minimal impacts of drought on ecosystems have been observed across the northern extend of North American Great Plains (Byrne et al. 2013, Carlyle et al. 2014, White et al. 2014a) and temperate grasslands of Europe (Kreyling et al. 2008, Jentsch et al. 2011). Aside from site characteristics (Hoover et al. 2018), root length dynamics have considerable potential to explain differences in ecosystem sensitivity to drought (Frank et al. 2002, Frank 2007, Ma et al. 2020a). Management practices such as a high defoliation intensity can shift the allocation of freshly assimilated carbohydrates to roots due to increased demands for carbon by roots (Ingrisch et al. 2020, Ma et al. 2021). This suggests that changes in belowground carbon allocation may enable plants to grow longer roots, thereby maintaining or even stimulating shoot growth under drought conditions (Frank et al. 2002, Ma et al. 2020a).

We experimentally manipulated growing season precipitation and defoliation regimes (timing/frequency and intensity of defoliation in the growing season) over four years at seven northern temperate grassland sites with a long history of herbivory. Previous research has shown that arid grasslands in the region are more sensitive to drought when grazed, especially in years with below-average precipitation (Irisarri et al. 2016, Batbaatar et al. 2021a). One of the benefits of multi-site experiments is that we can use site-based covariates such as mean annual precipitation to evaluate the consistency in ANPP and root biomass responses to these treatments (Fraser et al. 2013). We more specifically addressed the following questions in this study: 1) does the timing of defoliation alter the effect of drought on plant productivity? 2) how does varying early-season defoliation intensity influence the responses of plant productivity to drought? and 3) do defoliation treatment effects on production sensitivity to drought depend on water availability?

Methods

Study sites

We conducted our study at seven temperate grasslands in Alberta, Canada, with mean annual precipitation ranging from 312 to 533 mm and mean annual temperature from 1.9 to 4.9 °C. Sites encompass a broad geographic area such that there are 3-fold differences in plant productivity across sites (Appendix 4-S1). All sites have a history of moderate cattle grazing, though livestock (but not wildlife) were excluded during the study.

Experimental design and treatments

Two precipitation (ambient and reduced) treatments were crossed with five defoliation treatments in a fully randomized factorial experiment. At each site, treatment combinations were applied to 2.5 × 2.5 m plots, separated by at least a one meter buffer zone. Each treatment combination was applied for four consecutive growing seasons (2016-2019) and replicated five times at two sites (Kinsella and Mattheis) and four times at the remaining five sites. In total there were 300 experimental plots.

We reduced growing season precipitation using rainout shelters (Gherardi and Sala 2013) designed to impose drought conditions greater than site-specific precipitation variability from

May through September each year, except for the first year, 2016, when shelters were set up in early July. Rainout shelters consisted of ten V-shaped transparent acrylic slats spaced evenly on sloped wooden frames (90 cm to 120 cm tall) that excluded 45% of rainfall, imposing drought near or below the 5th percentile of long-term growing season precipitation (Appendix 4-S2). The efficacy of rainout shelter is described more in detail in a previous publication (Batbaatar et al. 2021b) . The ambient treatment at each site received natural precipitation.

We manipulated the timing of defoliation (June or September) in combination with intensity (no, light, and heavy) to simulate five different defoliation treatments. The five treatments represented two common elements of management: variation in the timing and intensity of defoliation relative to the non-defoliated control (No-No). To test whether the timing of defoliation and drought interacted to affect plant growth, we examined two treatments wherein vegetation was clipped once a year at the intensity of 3 cm stubble height (heavy defoliation) at different times of the year: either heavy defoliation in June with no defoliation in September (Heavy-No) or no defoliation in June with heavy defoliation in September (No-Heavy). To test how defoliation intensity early in the season affected the response of plant growth to drought, we examined three treatments that clipped vegetation at the same intensity of 3 cm stubble height in September but were preceded by a different defoliation intensity (either no defoliation (none), 7 cm (light), or 3 cm stubble height (heavy)) in June, resulting in No-Heavy, Light-Heavy, and Heavy-Heavy treatments, respectively.

All defoliation treatments were applied annually starting from 2017, the year after drought shelter installation. Vegetation was defoliated using a mower set at the appropriate height to remove vegetation from most of the 2.5 × 2.5 m plots, except for Stavely, where a string trimmer was used because of uneven ground. For all defoliated plots, the central 50 × 50 cm subplot was hand clipped to minimize disturbance associated with mowing. Vegetation from this central plot was dried to constant mass and weighed (see below).

Data collection

Monthly precipitation data obtained from the ClimateNA v6.3 software package (Wang et al. 2016) was used to quantify the extent of rainfall reduction at each site relative to long-term growing season precipitation (1901-2015) and test whether site-based conditions interacted with defoliation to alter ecosystem sensitivity to drought.

We estimated ANPP annually in all plots using a 0.1 × 1 m strip quadrat between 10 and 30 July, coinciding with the occurrence of peak biomass across the study sites during 2017-19. Standing biomass in the plots receiving the June defoliation treatment represented regrowth biomass, while biomass in the other plots was constituted as peak current annual growth. To estimate ANPP within plots defoliated in June, we added the biomass removed from central subplots during June defoliation to the standing biomass harvested in July. To quantify standing root biomass, two root cores, 5 cm wide x 15 cm deep, were taken from the strip quadrat clipped in July. Root samples were sieved over a 2 mm sieve and then washed over a 1 mm sieve. All vegetation samples were dried at 65 ºC for 48 h and weighed. Values from the two root cores were averaged to estimate standing root biomass, while the ANPP estimate did not include shrub biomass.

Calculation of drought treatment effects

To measure the effects of reduced precipitation on ANPP and root biomass, we used the log response ratio of reduced precipitation treatment relative to ambient (LRR = Ln[reduced precipitation / ambient precipitation]). Because ambient and reduced precipitation treatments were not paired within each site, LRRs of ANPP and root biomass were calculated by pairing all possible combination of replicates of treatments within each site and then averaged (n = 35) (Lamb and Cahill 2006). Negative values indicate that the drought treatment decreased biomass variables relative to ambient, while positive values indicate drought enhanced the variables.

Statistical analyses

We present data from the final year (2019) of the experiment, as these values represent the greatest cumulative treatment effects. We ran all analyses in R version 3.6.1 (R Core Team 2019). To answer our questions, we constructed custom contrast matrices using the emmeans package (Lenth 2019) in linear mixed-effects models fitted with the lme4 package (Bates et al. 2015). In the models, LRRs of ANPP and root biomass were the response variables. Defoliation treatments were a fixed effect, while site was a random factor for all models.

To determine whether the timing of defoliation altered drought effects on ANPP and root biomass, we created a contrast matrix using No-No, Heavy-No, and No-Heavy treatments. This allowed us to test the effects of drought on ANPP and root biomass in the absence of defoliation (No-No) and compare these effects to those varying the timing of heavy defoliation (Heavy-No vs. No-Heavy) under drought. Next, we created a contrast matrix involving No-Heavy, Light-Heavy, and Heavy-Heavy treatments. These treatments differed by the intensity of clipping early in the season (no, light, vs heavy) and enabled us to determine how early-season defoliation intensity influenced the responses of ANPP and root biomass to drought.

Finally, we used analysis of variance to test whether the effects of defoliation treatments on the sensitivity of ANPP and root biomass to drought were associated with water availability (i.e., rainfall). In these models, LRRs of ANPP and root biomass were response variables, while mean annual precipitation, defoliation, and their interaction were predictor variables.

Results

In the absence of defoliation, aboveground net primary productivity (ANPP) in the final year of the experiment increased under reduced precipitation, while root biomass to 15 cm depth tended to decrease relative to the ambient treatment (Figure 1) across the seven sites. By contrast, defoliation treatments reduced ANPP by 10% ($F_{1,24} = 5.93$, $p = 0.002$). While there was an increase in root biomass with defoliation relative to the non-defoliated control treatment across sites (Figures 4-1, 4-2), the trend was not statistically significant ($F_{1,24} = 1.42$, $p = 0.275$).

Across all seven sites, early- and late-season defoliation treatments had comparable effects on ANPP ($t = 0.54$, $p = 0.59$) and root biomass ($t = -1.04$, $p = 0.31$) under drought conditions, though defoliation late in the season tended to elevate root biomass more than early season defoliation (Figure 4-1b). With increased defoliation intensity early in the growing season, there was an increase in ANPP and a decline in root biomass under drought, respectively (Figure 4-2). Despite this, all observed trends in ANPP (none vs light, $t = 0.54$, $p = 0.59$; light vs heavy, $t = -1.04$, $p = 0.31$) and root biomass (none vs light, $t = 0.69$, $p = 0.49$; light vs heavy, $t = 0.5$, $p = 0.62$) were not statistically significant between treatments with varied intensity of defoliation early in the season.

Effects of defoliation on ANPP and root biomass remained independent of water availability (Figure 4-3) as evidenced by non-significant interactions between precipitation and defoliation (ANPP, $F_{4,25} = 0.13$, $p = 0.972$; root biomass, $F_{4,25} = 1.48$, $p = 0.238$). However, drought effects on ANPP were associated with mean annual precipitation ($F_{1,25} = 8.37$, p =

0.008), with more severe reductions in ANPP from drought at mesic rather than xeric grassland sites (Figure 4-3a); this relationship was not observed for root biomass ($F_{1,25} = 1.41$, $p = 0.245$; Figure 4-3b).

Discussion

We found that the sensitivity to drought differed between grassland ANPP and standing root biomass in this multi-site experiment. Surprisingly, there was no drought-induced reduction in ANPP across the seven grasslands in the final year in the absence of defoliation, contradicting findings from previous meta-analyses (Wilcox et al. 2017, Wang et al. 2021). An increase in ANPP under reduced precipitation relative to the ambient treatment occurred at all seven sites, except for one site wherein ANPP remained unchanged (Appendix 4-S3). This further shows that the high resistance to drought we found has not been driven by an unexpected response that occurred at one or two sites. Though not statistically significant, the trend for decreased root biomass in the top 15 cm of soil from drought is consistent with previous findings (Carroll et al. 2021). Differences in water use among plant species and study sites may explain the lack of responses in ANPP and root biomass to drought (Knapp et al. 2020, Ma et al. 2020a). For example, our grasslands were dominated by C_3 species that may take advantage of higher water availability and have lower transpiration rates early in the season due to winter water input (Smoliak 1986, Knapp et al. 2020), thereby mitigating growing season drought impacts. Alternatively, the demand for water needed for shoot growth under drought may be met by improved root system efficiency (e.g., aboveground biomass produced per unit root length) at the community level (Ma et al. 2020a). Notably, the relationship between root and shoot biomass from non-defoliated controls as a proxy measure of root system efficiency (Frank 2007, Ma et al.

2020a) exhibited evidence of increased root system efficiency under reduced precipitation (Appendix 4-S4).

Compared to drought alone, the combined effects of drought and defoliation further altered ANPP and root biomass responses. Under drought conditions, ANPP was greatly reduced by defoliation, while root biomass remained unchanged (or slightly increased). This may reflect a preferential allocation of photosynthates to roots, even under high stress conditions, in order to maintain the potential for future growth (Frank et al. 2002). Contrary to our expectation and results from past studies (Döbert et al. 2021, Hulvey et al. 2021), we found no evidence that the timing or intensity of defoliation affected these patterns, and instead conclude that these grasslands were highly resistant to drought. Furthermore, given that the primary pathway by which carbon enters the soil is through plant biomass production and return to the soil (Piñeiro et al. 2010), our findings of markedly reduced ANPP suggest that defoliation during the growing season, regardless of when or how, is likely to reduce carbon sequestration under drought.

We used the relationship between vegetation productivity and mean annual precipitation as a sensitivity metric (Irisarri et al. 2016, Wilcox et al. 2017, Batbaatar et al. 2021a) to assess whether and how defoliation altered the sensitivity of ANPP and root biomass to drought. Similar to White et al. (2014a), we found that ANPP was more sensitive to reduced precipitation in mesic than arid ecosystems, while root biomass sensitivity to drought was not related to mean annual precipitation across sites. Greater sensitivity of ANPP to reduced precipitation in mesic grasslands could be attributed to vegetation traits promoting drought tolerance (Griffin-Nolan et al. 2019). For example, non-native species representation is generally higher in more mesic grasslands of the study area (Lyseng et al. 2018), which in turn, were those with the greatest susceptibility to biomass changes under reduced precipitation (Souther et al. 2020). Notably, variation in defoliation regime did not alter either ANPP or root biomass sensitivity to drought, suggesting these responses may remain regardless of changes in land use activity across studied grasslands.

In summary, the resistance of these northern temperate grasslands to four consecutive years of extreme drought has important implications for management practices. However, our findings show that growing-season defoliation suppressed ANPP, specifically when coupled with drought, while root biomass remained more resistant to change. Importantly, the timing and intensity of defoliation early in the season did not influence this pattern, and therefore the manipulation of individual grazing management practices may not offer any flexibility to sustain plant productivity under drought. Perhaps the biggest conclusion we can draw from these findings is that defoliation in drought years, no matter when or how much defoliated, is likely to increase ecosystem vulnerability, specifically aboveground shoot mass, to drought.

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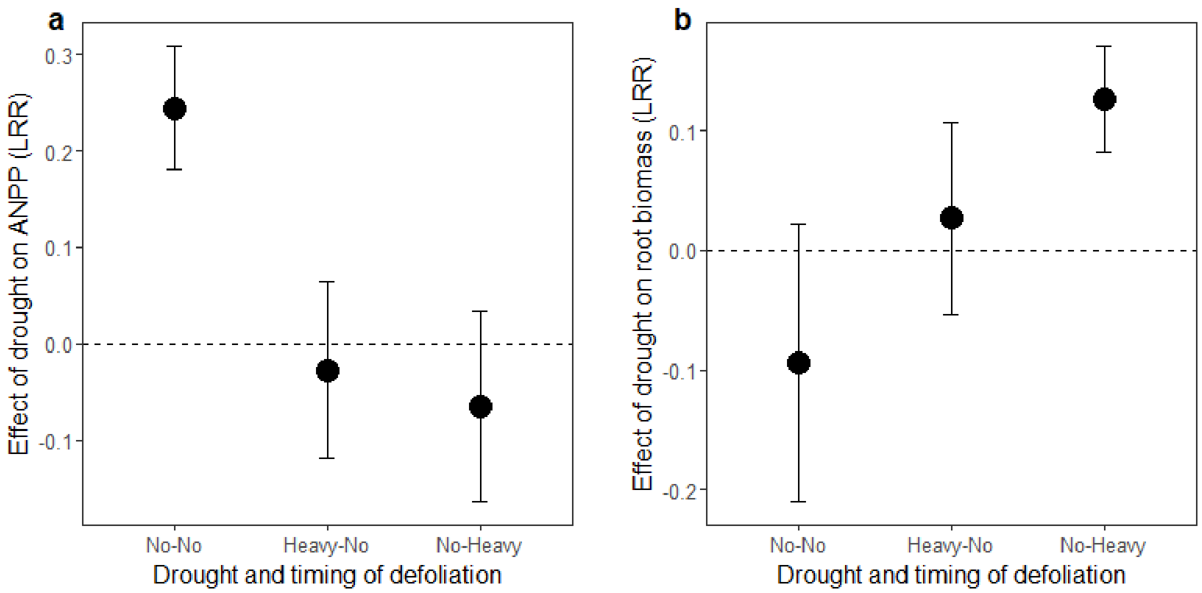

Figure 4-1. Effects of drought (No-No) and defoliation timing (early, Heavy-No; late, No-Heavy) on a) aboveground net primary productivity (ANPP) and b) root biomass in the top 15 cm soil across seven sites (mean ± SE) under drought conditions. Effects of drought on ANPP and root biomass were quantified by the log response ratio (LRR = ln[reduced precipitation / ambient precipitation]).

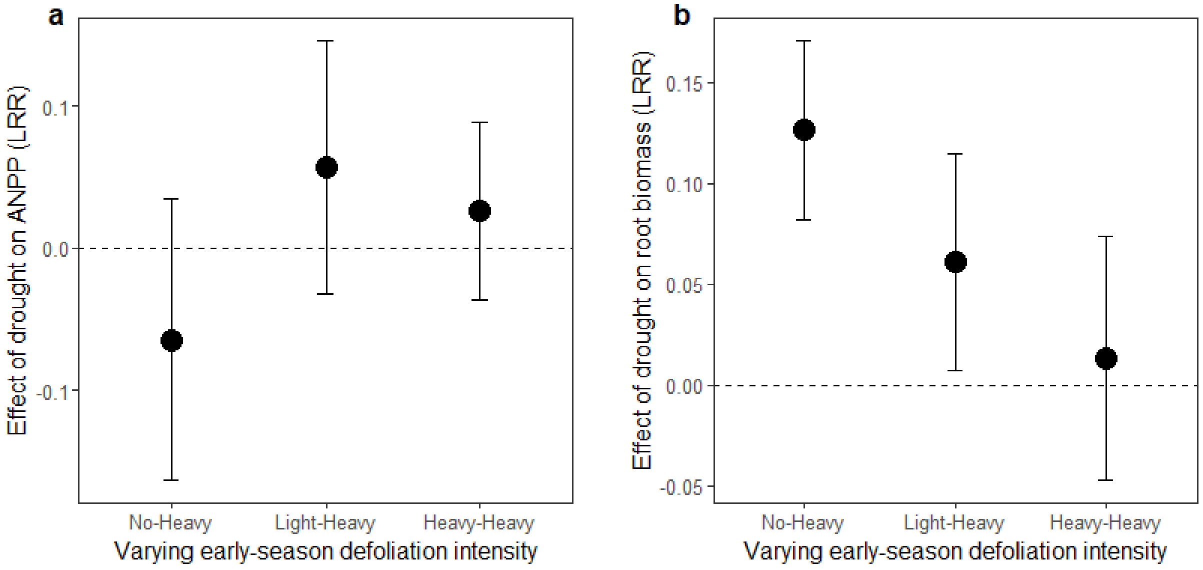

Figure 4-2. Effects of varying early-season defoliation intensity (none, No-Heavy; light, Light-Heavy; heavy, Heavy-Heavy) on a) aboveground net primary productivity (ANPP) and b) root biomass in the top 15 cm of soil across seven sites (mean ± SE) under drought conditions. Effects of drought on ANPP and root biomass were quantified by the log response ratio (LRR = ln[reduced precipitation / ambient precipitation]). The No-Heavy (none), Light-Heavy (light), and Heavy-Heavy (heavy) treatments indicate the varying early-season defoliation intensity.

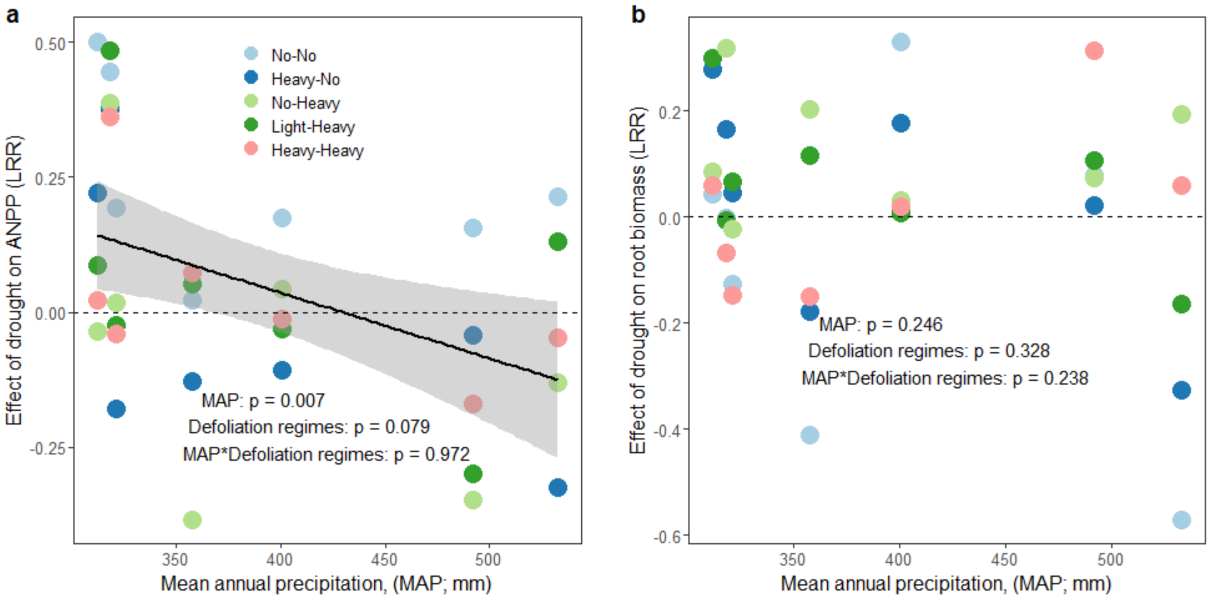

Figure 4-3. Effects of defoliation (intensity by frequency/timing) regimes on a) aboveground net primary productivity (ANPP) and b) root biomass in the top 15 cm of soil along the precipitation gradient of study sites. Effects of drought on ANPP and root biomass (No-No) were quantified by the log response ratio (LRR = ln[reduced precipitation / ambient precipitation]). The Heavy-No (early) and No-Heavy (late) treatments represent the variation in the timing of defoliation, whereas the No-Heavy (none), Light-Heavy (light), and Heavy-Heavy (heavy) treatments indicate the varying early-season defoliation intensity, respectively.

Chapter 5: Root length dynamics vary with soil depth in response to drought and defoliation

Abstract

Climate models project a greater likelihood for multi-year drought to occur over grassland ecosystems, and interactions with other co-occurring factors, such as grazing, are expected to lead to substantial and long-lasting changes to ecosystem structure and function. Yet, there is limited understanding of how variations in precipitation and defoliation alter plant root length dynamics and whether root responses to these common drivers of the system are generalizable across communities. We used minirhizotrons from a four-year field experiment conducted at two northern temperate grasslands to test whether extreme drought (45% reduction in rainfall) and variations in defoliation (timing and intensity) altered root length dynamics. We found that root length dynamics were varied due to nearly every factor manipulated or measured in this study, suggesting highly plastic responses to variations in precipitation and defoliation. However, treatment effects and their interactions varied between sites or between soil depths within the individual site. In general, four years of extreme drought decreased the total length, production, and lifespan of roots in the absence of defoliation, particularly in the shallow soil depth at both sites. Notably, we found evidence of generalization in many aspects of root length dynamics between the two sites when exposed to both drought and defoliation, but when differences existed, they occurred under the ambient precipitation or at the deeper soil depth. For example, root length production was increased with increasing defoliation intensity early in the season in the drier site under the ambient precipitation, while a progressive decline in root length was observed in the wetter site. Further, we showed that the mortality and lifespan of roots were the aspects of root length dynamics that caused the observed shifts in total root length resulting

from drought and defoliation. Overall, our results show the rapid changes in root length in the face of co-occurring global change drivers, and the extent to which variations in defoliation maintain root length under drought depends on environmental conditions.

Introduction

Climate models project a greater likelihood for multi-year drought to occur in the future over the Great Plains of North America (Zhao et al. 2020, Zhang et al. 2021), and interactions with other co-occurring factors, such as grazing, can lead to substantial and long-lasting changes to ecosystem structure and function (Komatsu et al. 2019, Ma et al. 2020b, Avolio et al. 2021). However, the combined effects of drought and defoliation on root length dynamics are least understood compared to shoot responses (Ma et al. 2020a, Batbaatar et al. 2021a, Batbaatar et al. 2021b, Slette et al. 2021). Predicting grassland responses to both precipitation reduction and grazing requires an understanding of root responses (Frank 2007, Wilcox et al. 2017, Ma et al. 2020a, Slette et al. 2021), given that a significant proportion of total photosynthates is allocated to roots (Gherardi and Sala 2020), making up to 85% of plant biomass in semi-arid temperate grasslands (Coupland and Johnson 1965). Yet, this remains one of the least understood questions, with significant implications for nutrient cycling, carbon sequestration, and storage (Eissenstat et al. 2000, Bardgett et al. 2014, Erktan et al. 2018a).

Differences in ecosystem sensitivity to drought can be attributed to plant root systems (Frank 2007, Ma et al. 2020a), aside from varying site characteristics, precipitation patterns, methodology, or lack of generalized assessment of imposed drought relative to natural precipitation variability (Carlyle et al. 2014, White et al. 2014a, Hoover et al. 2018, Slette et al. 2019). For example, shifts in root biomass distribution to the deeper soil profile are expected in response to a reduction in soil surface water to maximize water capture for stable shoot growth (Comas et al. 2013, Zwetsloot and Bauerle 2021). Alternatively, modifications in root systems have been linked to increased water use efficiency mediated by supporting more shoot biomass per unit root under drought (Frank et al. 2002, Frank 2007, Ma et al. 2020a). This ability of plants to acquire and efficiently use limited resources is better understood when root length dynamics rather than standing root biomass are considered as aspects of root length dynamics, such as appearance (e.g., birth), disappearance (e.g., death), and lifespan, can change without resulting changes to the root biomass (Wilson 2014, Mueller et al. 2018, Ma et al. 2020a, Weigelt et al. 2021). For example, several lines of evidence demonstrated that decreases in root length production due to experimentally altered abiotic conditions were compensated by either change in root diameter or lifespan, with no detectable changes to root biomass (Pilon et al. 2013, Mueller et al. 2018, Ma et al. 2020a). Thus, measurements of root length dynamics will be critical to explain why ecosystems differ in their ability to withstand water deficits, given their sizeable influence on overall nutrient and carbon dynamics in grasslands (Eissenstat et al. 2000, Bardgett et al. 2014, Wilson 2014, Erktan et al. 2018a).

Grassland ecosystems are historically subjected to defoliation by diverse herbivore groups (McNaughton 1979, Milchunas and Lauenroth 1993, Anderson 2006) and are currently managed for and grazed by livestock (Asner et al. 2004, Filazzola et al. 2020). Because of the long-term association of grazers and grasslands (Milchunas et al. 1988), defoliation has considerable potential to modify root length dynamics directly through the timing and intensity of plant biomass removal (Filazzola et al. 2020, Ingrisch et al. 2020, Ma et al. 2021) or indirectly through alterations of edaphic conditions (Willms et al. 1986, Batbaatar et al. 2021a). For example, stimulatory effects of grazing relative to non-grazed treatments on root length production have been attributed to the timing of plant biomass removal (Frank et al. 2002, Frank 2007). By contrast, light and heavy defoliation intensity early in the season exacerbated the negative effects of drought on root length, with no detectable effects on other aspects of root length dynamics across prairies of western Canada (Ma et al. 2020a). Understanding of how changes to defoliation timing and intensity shape root length dynamics under drought is necessary not only to effectively manage these systems, but also to shed light on whether responses of root length dynamics to the combined effects of co-occurring global change drivers are generalizable in direction and magnitude across different ecosystems.

Here, we report the interactive effects of extreme drought (ambient and reduced precipitation) either with timing (early vs late) or intensity (none, light, heavy) of defoliation on root length dynamics using four years of observations in two northern temperate grasslands, with differences in climatic and vegetation conditions in Alberta, Canada. We used identical protocols at both sites to determine which aspects of root length dynamics, if any, were generalizable across communities and co-occurring factors (Filazzola and Cahill 2021). We expected that root length dynamics in the drier site would be less responsive to the combined effects of drought and defoliation than the wetter site (Milchunas et al. 1988, Reich 2014, Bergmann et al. 2020). Specifically, we tested three hypotheses derived from other field experiments conducted in the region. First, we would expect more root length in the deeper soil depth associated with drought (Hasibeder et al. 2015, Arndal et al. 2018). Given that C_3 dominated communities cope with growing season drought by prioritizing growth early in the season when water is abundant due to winter inputs and low spring temperature (Smoliak 1986, Knapp et al. 2020), removing leaf area early in the season would reduce root length more than defoliating late in the season. Finally, we predicted that drought effects on root length dynamics would increase progressively with increases in the intensity of defoliation early in the season in both sites (Ma et al. 2020a).

Methods and materials

Study sites

We established a fully randomized factorial experiment at seven temperate grasslands across the northern edge of the Great Plains (sensu Filazzola and Cahill 2021) to better generalize the combined effects of drought and defoliation on ecosystem structure and function (Batbaatar et al. 2021b). Due to logistical reasons (see root tracing section), in this study, we present root length responses from two of the seven sites located at two research ranches operated by the University of Alberta in Alberta, Canada. The climatic, vegetation and soil properties differ between the two sites (Batbaatar et al. 2021b). The Mattheis Ranch receives 321 mm of mean annual precipitation (MAP) and has a mean annual temperature (MAT) of 3.6 °C (1901-2015). The Kinsella Ranch is wetter and colder than Mattheis (MAP = 401 mm; MAT = 1.9 °C). The Mattheis site is within the dry mixed-grass ecoregion, dominated by *Bouteloua gracilis*, *Hesperostipa comota*, and *Koeleria macrantha* on sandy loam soil. The Kinsella site falls within the aspen parkland ecoregion, a savanna habitat characterized by mixed-grass prairie and patches of trembling aspen (*Populus tremuloides*), primarily dominated by *Pascopyrum smithii*, *Festuca hallii*, and *Poa pratensis* on clay loam soil. Although both sites have a history of grazing by large mammals (e.g., deer, antelope, elk, moose, and bison), with a modern history of cattle grazing, large grazers were excluded from each site year before the experiment began in 2016.

Experimental design and treatments

We established a fully randomized factorial experiment at each site, crossing two precipitation (ambient and reduced) treatments with five levels of defoliation, altering the timing and intensity of plant biomass removal. Treatment combinations were applied to 2.5 × 2.5 m plots, separated by at least one meter, for four consecutive growing seasons (2016-2019) and replicated five times at each site (n = 50). Only central 50 × 50 cm subplots of the plots were dedicated for non-destructive sampling to minimize edge effects.

We used rainout shelters (Gherardi and Sala 2013) designed to intercept 45% of ambient rainfall to impose drought conditions greater than site-specific precipitation variability throughout the growing seasons (approximately May 1 to October 1), except for the first year, 2016, when shelters were built in early July. The reduced precipitation treatment was achieved using a roof comprised of ten V-shaped transparent acrylic slats evenly spaced to cover 45% of tall wooden frames. Because shelters were sloped from 120 cm height at the top to 90 cm at the low end, these roofs have minimal effects on plot microclimatic or plant physiological processes (Loik et al. 2019, Batbaatar et al. 2021b).

We manipulated the timing of defoliation (June or September) together with intensity (no, light, and heavy) to create five defoliation treatments. We also note that these variations in defoliation also correspond to common management decisions used in livestock operations in the region (Adams et al. 2013, France et al. 2020). To test whether the timing of defoliation and drought interacted to affect root length dynamics, we examined two treatments that clipped vegetation once a year at the intensity of 3 cm (heavy) stubble height at different times of the year: either heavy defoliation in June with no defoliation in September (Heavy-No) or no defoliation in June with heavy defoliation in September (No-Heavy). To test how varying early-season defoliation intensity affected the responses of roots to drought, we examined three treatments that clipped vegetation at the same intensity of 3 cm stubble height in September but were preceded by a different defoliation intensity (either no defoliation (none), 7 cm (light) or 3 cm (heavy)) in June, resulting in No-Heavy, Light-Heavy, and Heavy-Heavy treatments, respectively.

All defoliation treatments were applied annually starting from 2017, the first growing season after rainout shelters were erected. A mower set at the appropriate height was used to remove vegetation from plots. Vegetation from a central 50 × 50 cm subplot was hand clipped and removed to minimize the disturbance associated with mowing.

Root observation

To monitor root length dynamics, we installed one transparent acrylic minirhizotron tube (90 cm long, 4.5 cm inner diameter) into the center of each plot at approximately a 30° angle from the soil surface in June 2016. Although tube depths varied among plots, an average depth was 30 cm at each site. This depth allowed for the sampling of most roots, as the bulk of root mass in temperate grasslands is found in the upper 30 cm of soil (Coupland and Johnson 1965, Lauenroth 2000). Image gatherings began in May 2017, ten months after the installation, but we recognize recovery after soil disturbance can persist for years (Milchunas et al. 2005, Hendricks et al. 2006). However, the disturbance was consistent among all treatments and thus not biased by treatment imposition. We also captured reference images on each minirhizotron tube in 2016 (2.5 months after the installation) to identify the pre-existing roots, allowing subsequent measurements to be only on those initiated after tube installation and treatment imposition (see root tracing section). Root images were acquired with a Bartz BTC 100X video camera system (Bartz Technology Corporation, Carpinteria, USA) at 1.35 cm intervals along the length of minirhizotron tubes every month for each growing season (approximately from May 15 to September 15) at each site. From a total of 15 sessions during the experiment, we gathered 27826 and 25212 images in total for Mattheis and Kinsella, respectively.

Root tracing

For logistical reasons, we subset the acquired images on each tube by selecting every 4th image for each sampling date, resulting in ca. 7200 images at Mattheis and ca. 6500 images at Kinsella to be processed. The number of selected frames (8-10 frames per tube) varied slightly among tubes due to variation in tube depths; however, the proportion of images analyzed corresponded to approximately 25% of each tube's observed area. All selected images were digitally analyzed using WinRHIZO TRON MF software (Regent Instruments Inc., Quebec, Canada) and divided into 0-15 cm (shallow) and 15-30 cm (deep) depth increments.

We recorded the date of appearance (e.g., birth) and disappearance (e.g., death) of each root that was present in May 2017 or later but was not present on reference images captured in 2016. Our approach allowed us to circumvent two common limitations of minirhizotrons. First, roots that recolonized tube-soil interface during the equilibrium period can confound treatment effects on roots, thereby likely to increase the risk of overestimating the total root length, root length production, and root length mortality (Hendricks et al. 2006, Freschet et al. 2021a). We did not digitize roots that recolonized tube-soil interface during the equilibrium period (10 months in our case); thus, our estimate reduces the bias, thereby better reflecting the responses of roots to treatment effects. Finally, roots recorded on the first image session are often excluded from the survival analyses due to their uncertain birth date associated with tube installation, leading to the loss of valuable data on root responses (Milchunas et al. 2005, Mueller et al. 2018). The use of reference images captured in 2016 (2.5 months after the tube installation) as a baseline for root tracing allowed us to accurately determine the birth date of each individual root observed, thereby enabling cohorts of roots produced in 2017 to be incorporated for the survival analysis.

We measured root length on individual roots that were present on each sampling date. To estimate the total root length at each plot, we summed all individual root lengths for each session. To determine the demographic cause of changes in total root length, we also estimated the lifespan of each individual root, root length production (the sum of both new and existing root growth for each session), and root length mortality (the sum of previously existing root length that disappeared in each session). To remove the bias due to variation in tube depths, we further weighted three root length variables by the total number of frames selected for each soil depth increment on tubes.

Statistical analyses

All statistical analyses were performed on the data from Mattheis and Kinsella separately using R (R Core Team 2019) in order to identify whether there was consistency in results between sites rather than why site differences existed (sensu Filazzola and Cahill 2021). We used the packages lme4 (Bates et al. 2015) to fit mixed-effects models, and survival and coxme packages (Therneau 2020, Therneau 2021) to conduct the survival analyses. We constructed custom contrast matrices using emmeans package (Lenth 2019) for linear mixed-effects models, and *linearHypothesis()* function from car package (Fox and Weisberg 2019) for mixed-effects survival models to test our hypotheses. Statistical significance was set at α = 0.05 for all analyses. All codes can be available at https://dataverse.library.ualberta.ca/dataverse/Baka.

We used the repeated-measure mixed-effects models to determine whether the combined effects of reduced precipitation and defoliation treatments on the total root length, root length production, and root length mortality varied between soil depths. We used the plot level observations as each treatment replicates (n = 5) for mixed-effects models; thus, the dependent variables were the mean total root length, root length production, and root length mortality in each plot for each year. In the mixed-effect models, fixed effects were the main and interactive effects of reduced precipitation, defoliation, and soil depth (shallow and deep), while plot and year (repeated measure) were included as random factors. To satisfy the assumptions of normality and equal variance of residuals, we used square-root transformation on the mean annual total root length, root length production, and root length mortality.

At each site, mixed-effects Cox proportional hazard models (Cox 1972) were used to determine the main and interactive effects of treatments on fine-root survival at shallow (0-15 cm) and deep (15-30 cm) soil depths separately. We limited the survival analyses to cohorts of roots produced only in 2017 since the lifespan of these roots reflects treatment effects the longest. Roots that were still alive at the end of the study or disappeared due to frames shifts (43% of roots in Mattheis and 34% of roots in Kinsella) were censored. In the Cox proportional hazard models, fixed effects were the main and interactive effects of reduced precipitation and defoliation treatments, while the plot was included as a random factor. The assumptions of the Cox proportional hazard models were assessed visually by log-minus-log survival plots (Vittinghoff et al. 2012). Further, the lifespan of roots in the models was stratified by birth months (May-September).

To determine whether drought effects on total root length, root length production, and root length mortality varied between shallow and deep soil depths, we tested the reduced precipitation × soil depth interaction term in the absence of defoliation (No-No). Next, we created a contrast matrix using Heavy-No and No-Heavy treatments to test whether the combined effects of drought and timing of defoliation on root length dynamics varied by soil depths. Finally, we created a contrast matrix involving three treatments, including No-Heavy, Light-Heavy, and Heavy-Heavy. These treatments differed by the intensity of defoliation early in the season (none, light, heavy) and enabled us to test whether the combined effects of drought and varying early-season defoliation intensity on root length dynamics varied between shallow and deep soil depths.

Results.

In general, the amount of root length produced during the experiment was up to four times higher at Kinsella than Mattheis (lower rainfall) (Figures 5-1, 5-2, 5-3). Root length growth doubled at the shallow soil depth at Kinsella relative to the deep depth, while it remained similar across the soil profile at Mattheis (Figures 5-1, 5-2, 5-3). The mean and median root lifespan across all treatments and years was greater in Mattheis (334 and 306 days) than in Kinsella (254 and 87 days). Overall, during the experiment, we observed similar patterns in root length dynamics in response to treatments in the shallow soil depth at both sites, while divergence in the responses was observed in the deep soil depth between sites (Figures 5-1, 5-2, 5-3).

Total root length, root length production, and root length mortality were all affected by a significant three-way interaction of precipitation, defoliation, and soil depth at both sites (Table 5-1; Appendix-5; S1-3). The lifespan of roots was not different across treatments in the shallow soil depth at both sites, while the individual (Mattheis) and interactive (Kinsella) effects of drought and defoliation altered root lifespan in the deeper soil depth (Table 5-2). Together, these results indicate substantial sensitivity in root length dynamics to global change drivers, with responses depending on environmental conditions, defoliation, and soil depth. Below, we will disseminate these results in accordance with our specific hypotheses.

Does drought result in more root length at deeper soil depth?

In the absence of defoliation, the four consecutive years of extreme drought generally led to decreases in total root length and root length production in the shallow soil depth at both sites (Figure 5-1). These reductions in root length associated with drought were only evident at Mattheis in the deeper soil depth (Figure 5-1).

At both sites, there was a comparable reduction in root length mortality under reduced precipitation in the shallow soil depth relative to the ambient precipitation, while an increase in root length mortality was observed in the deep soil depth (Figure 5-1c). At both sites, drought increased overall root lifespan (7% in Mattheis and 16% in Kinsella) compared to the ambient precipitation in the shallow soil depth. By contrast, we observed substantial decreases in root lifespan under drought relative to ambient precipitation in the deep soil depth, to a much greater extent at Mattheis (120%) than Kinsella (19%) (Table 5-2).

Together, these results showed that drought decreased the length and production of roots, particularly in the shallow depth, and such shifts in root length were caused by altered mortality and lifespan of roots associated with drought.

Does late-season defoliation relative to early-season have less impact on root length dynamics under drought?

The timing of defoliation and drought interacted to affect root length dynamics only at Kinsella (Figure 5-2). At Mattheis, early- and late-season defoliation treatments had comparable effects on total root length and root length production under both ambient and reduced precipitation treatment across the soil profile over the course of the experiment (Figure 5-2a, 5-2b). At Kinsella across years, total root length and root length production were increased more by late-season defoliation than by early-season defoliation in the shallow depth under the ambient precipitation (Figure 5-2a, 5-2b). However, the effects of defoliation timing on total root length and root length production were comparable in the deep soil depth under the ambient precipitation or across the soil profile under reduced precipitation (Figure 5-2a, 5-2b).

Early- and late-season defoliation had similar impacts on root length mortality and lifespan under the ambient and reduced precipitation treatment across the soil profile at Mattheis (Figure 5-2c; Figure 5-4), though both treatments decreased the lifespan of roots relative to the non-defoliated control treatment (Table 5-2), particularly under the ambient precipitation in the deep soil depth (Figure 5-4). At Kinsella, early- and late-season defoliation had similar impacts on root length mortality or lifespan under both ambient and reduced precipitation treatments in the shallow soil depth (Figure 5-2c, Figure 5-4). However, root length mortality and lifespan were impacted by a significant drought × defoliation timing in the deep soil depth (Figure 5-2c, Table 5-2). There was greater root length mortality or decreases in root lifespan with early- than late-season defoliation under the ambient precipitation. In contrast, late-season defoliation resulted in more root length mortality or shortened root lifespan under the reduced precipitation (Figure 5-2c, Figure 5-4).

Do the effects of increased early-season defoliation intensity on root length dynamics increase progressively under drought?

Throughout the experimental period, root length dynamics were affected by the combined effects of drought and defoliation intensity at both sites. However, the total root length and root length production responses to varying early-season defoliation intensity varied between sites (Figure 5-4a, 5-4b). At Mattheis, the total root length and root length production increased with increasing intensity of defoliation early in the season at the shallow soil depth under the ambient precipitation, while light defoliation intensity resulted in the lowest total root length and production under reduced precipitation at the deep soil depth, relative to none or heavy intensity of defoliation (Figure 5-4a, 5-4b). By contrast, at Kinsella, with increased defoliation intensity early in the growing season, total root length and root length production decreased at the shallow depth under the ambient precipitation or deep soil depth under the reduced precipitation treatment (Figure 5-4a, 5-4b).

Root length mortality and lifespan were not varied among defoliation intensity under the ambient and reduced precipitation treatments across the soil profile at either site (Figure 5-3c, Figure 5-5, Table 5-2). However, relative to ambient conditions, varying early-season defoliation intensity increased the lifespan of roots in general under the reduced precipitation at the deep soil at Mattheis but decreased it at Kinsella.

Discussion

Here we demonstrate that co-occurring factors strongly affect root length dynamics at two northern temperate grasslands that differed in climatic and vegetation conditions. However, the direction and magnitude of effects are largely site-specific depending on the interactions between drought, defoliation, and soil depth. Overall, the responses of root length dynamics to the combined effects of drought and defoliation in the shallow soil depth were similar between Mattheis and Kinsella, but the responses diverged between sites in the deep soil depth. Further, our results identified key differences between the two sites in how variations in defoliation affects root length dynamics under drought conditions. Below, we discuss each of the three hypotheses we tested in detail.

Drought effects on root length dynamics varied with soil depth between Mattheis and Kinsella in the absence of defoliation. Across the years of the experiment, drought reduced root length at both sites, agreeing with findings from previous research (Ma et al. 2020a, Zwetsloot and Bauerle 2021). This was especially evident at the deep soil depth in Mattheis but not in Kinsella, contradicting our hypothesis of more root length at deeper soil under drought conditions. Likewise, at Mattheis, we found substantial decreases in root lifespan associated with drought in the same soil depth, while drought prolonged the lifespan of roots in the upper soil surface. Together these results indicated that water acquisition from deeper soil depth was limited, and we attribute the observed decreases in root length to changes in the lifespan of existing roots. Since the balance between carbon costs and benefits of resource acquisition determines the optimal root lifespan (Eissenstat et al. 2000, McCormack and Guo 2014), plants may have deployed a drought avoidance strategy by decreasing root proliferation or shortening root lifespan in the deeper soil depth (Comas et al. 2013). Alternatively, plants can maintain the absorptive capacity of root systems by proliferating throughout the upper soil layers rather than vertically (Weigelt et al. 2021). The latter strategy is most efficient in terms of foraging for water associated with scattered rainfall events during drought (Weigelt et al. 2021), given that drought comes in many forms (Knapp et al. 2015b). In this scenario, plants would need to extend the lifespan of existing roots in order to minimize the carbon costs or maintain root length production to the level of non-stressed conditions (Eissenstat et al. 2000, McCormack and Guo 2014). Although both drought avoidance and water foraging strategies were observed in the current study, we postulate that water foraging strategy may explain the observed convergence between Mattheis and Kinsella in root length responses to drought in the shallow soil depth.

Our findings only partly supported the hypothesis that root length dynamics would be less affected by late- than early-season defoliation under drought. As expected, late- rather than early-season defoliation markedly increased root length production in the shallow soil depth under ambient conditions but only in Kinsella. The increased absorptive capacity of root systems may reflect the compensatory root growth responses (Frank et al. 2002, Ma et al. 2021) or enhanced nitrogen availability associated with defoliation (Piñeiro et al. 2010). The latter of the two could be the likely mechanism in our study given that early-season defoliation resulted in elevated root length mortality and shortened root lifespan under the ambient condition, while root length loss was greater with late- than early-season defoliation under drought. More importantly, the effects of early- and late-season defoliation treatments on root length dynamics were not different between Mattheis and Kinsella under drought conditions, but when differences existed, they occurred under the ambient conditions or in the deeper soil depth at Kinsella as discussed above. This shows the fundamental differences between the two systems when subject to single stress of defoliation, yet both systems show convergence when simultaneously exposed to drought and defoliation.

Our expectations of a progressive decrease in root length production with increasing defoliation intensity under drought were the only case in one of the two sites, particularly in the deep soil depth. Contrary to our expectations and findings from the previous study (Ma et al.

2020a), root length production was stimulated by heavy defoliation intensity in Mattheis. The observed site- and depth-specific changes in root lifespan may explain the contrasting root length responses to defoliation intensity under drought and underlie differences in ecological strategies between the two sites for dealing with combined effects of co-occurring factors. For example, the increased root lifespan in response to drought and defoliation in Mattheis indicates a slow absorptive root strategy, promoting carbon storage in root tissues for future growth (Reich 2014, Weigelt et al. 2021). Reduced length and lifespan of roots in Kinsella under the combination of drought and heavy defoliation intensity reflect a fast absorptive root strategy (Reich 2014, Weigelt et al. 2021), suggesting that the combined effects of these treatments may lead to a substantial reduction in the absorptive capacity of root systems. In this context, defoliation intensity should be reduced in drought years to enhance the long-term stability of the mesic systems.

We conclude that drought, variations in defoliation, and soil depth interacted to affect root length dynamics; however, treatment effects and their interactions vary between the two sites or between soil depths within the individual site in the northern temperate grasslands of western Canada. We also conclude that the mortality and lifespan of existing roots are the aspects of root length dynamics that caused the observed shifts in total root length resulting from changes in precipitation and defoliation. Most importantly, Mattheis and Kinsella exhibited general convergence in many aspects of their root length dynamics to common drivers of these grasslands, but when differences existed, they occurred under the ambient condition or at the deep soil depth. Considering that multiple factors interact to alter ecosystems dramatically (Komatsu et al. 2019, Avolio et al. 2021), our findings are valuable for developing predictive frameworks of ecosystem responses to co-occurring global change factors and drawing broad conclusions on vulnerabilities of natural systems that are subjected to multiple stressors.

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Table 5-1. Statistical outputs for repeated-measure mixed-effects models of total root length, root length production, and root length mortality at individual sites. Plot and year were included as random effects to account for spatial and temporal autocorrelations in the mixed models.

Response	Mattheis			Kinsella		
	df	F	P	df	F	P
Total root length						
Drought	1, 53.1	0.36	0.552	1, 55	0.52	0.474
Defoliation	4, 53.1	1.34	0.267	4, 55	2.35	0.065
Depth	1, 233	3.59	**0.059**	1, 238	67.61	**<0.001**
Drought × Defoliation	4, 53.1	0.87	0.486	4, 55	0.99	0.421
Drought × Depth	1, 233	2.19	0.14	1, 238	1.11	0.293
Defoliation × Depth	4, 233	3.34	**0.011**	4, 238	5.67	**<0.001**
Drought × Defoliation × Depth	4, 233	6.41	**<0.001**	4, 238	7.11	**<0.001**
Root length production						
Drought	1, 57.4	0.26	0.612	1, 58.2	0.2	0.654
Defoliation	4, 57.4	1.80	0.141	4, 58.2	3.37	**0.015**
Depth	1, 233	14.65	**<0.001**	1, 238	26.7	**<0.001**
Drought × Defoliation	4, 57.4	1.49	0.218	4, 58.2	1.64	0.175
Drought × Depth	1, 233	5.15	**0.024**	1, 238	0.31	0.579
Defoliation × Depth	4, 233	3.99	**0.004**	4, 238	4.65	**0.001**
Drought × Defoliation × Depth	4, 233	6.42	**<0.001**	4, 238	4.73	**0.001**
Root length mortality						
Drought	1, 49.3	0.2	0.653	1, 59.8	1.74	0.193
Defoliation	4, 49.3	0.52	0.719	4, 59.8	1	0.414
Depth	1, 233	14.06	**<0.001**	1, 238	63.12	**<0.001**
Drought × Defoliation	4, 49.3	0.19	0.942	4, 59.8	0.87	0.49
Drought × Depth	1, 233	0.84	0.361	1, 238	2.54	0.112
Defoliation × Depth	4, 233	1.78	0.133	4, 238	3.36	**0.011**
Drought × Defoliation × Depth	4, 233	2.69	**0.031**	4, 238	6.65	**<0.001**

$P \leq 0.05$ values are shown in bold.

Table 5-2. The output of mixed-effects Cox proportional hazard models for root lifespan. The main and interactive effects of drought and defoliation were tested separately at shallow and deep soil depth. Plot was included as a random effect to account for spatial autocorrelation. A hazard ratio < 1 indicates an increase in root lifespan, while a hazard ratio > 1 shows a decrease in root lifespan.

	Mattheis		Kinsella	
	Hazard ratio	P	Hazard ratio	P
Shallow soil depth (0-15 cm)				
Drought (reference = ambient)	0.93	0.81	0.84	0.58
Heavy-No (reference = No-No)	0.76	0.35	1.34	0.34
No-Heavy	1.2	0.56	0.82	0.51
Light-Heavy	1.27	0.44	1.11	0.74
Heavy-Heavy	1.4	0.26	0.86	0.62
Drought × Defoliation	-	0.93	-	0.56
Deep soil depth (15-30 cm)				
Drought (reference = ambient)	3.20	**0.04**	1.19	0.6
Heavy-No (reference = No-No)	1.73	0.34	3.21	**< 0.001**
No-Heavy	2.72	0.08	0.99	0.97
Light-Heavy	2.65	0.08	0.77	0.46
Heavy-Heavy	3.73	**0.02**	1.59	0.15
Drought × Defoliation	-	0.50	-	**< 0.001**

$P \leq 0.05$ values are shown in bold.

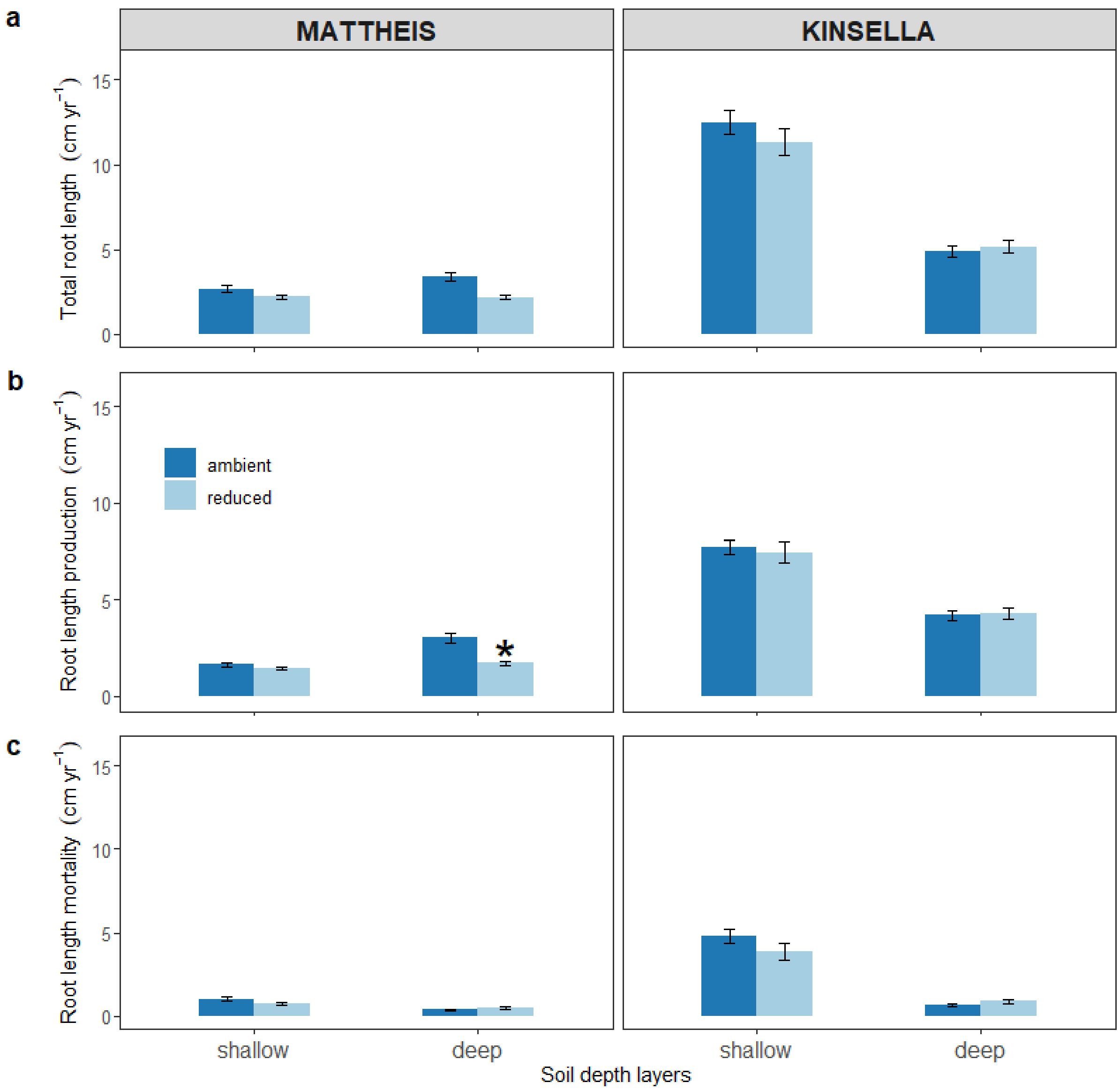

Figure 5-1. Effects of drought on the mean annual total root length (a), root length production (b), and root length mortality (c) at shallow (0-15 cm) and deep (15-30 cm) soil layers across 2017-2019 in the absence of defoliation (data from non-defoliated control treatment). Error bars represent ± 1 SE (n = 15).

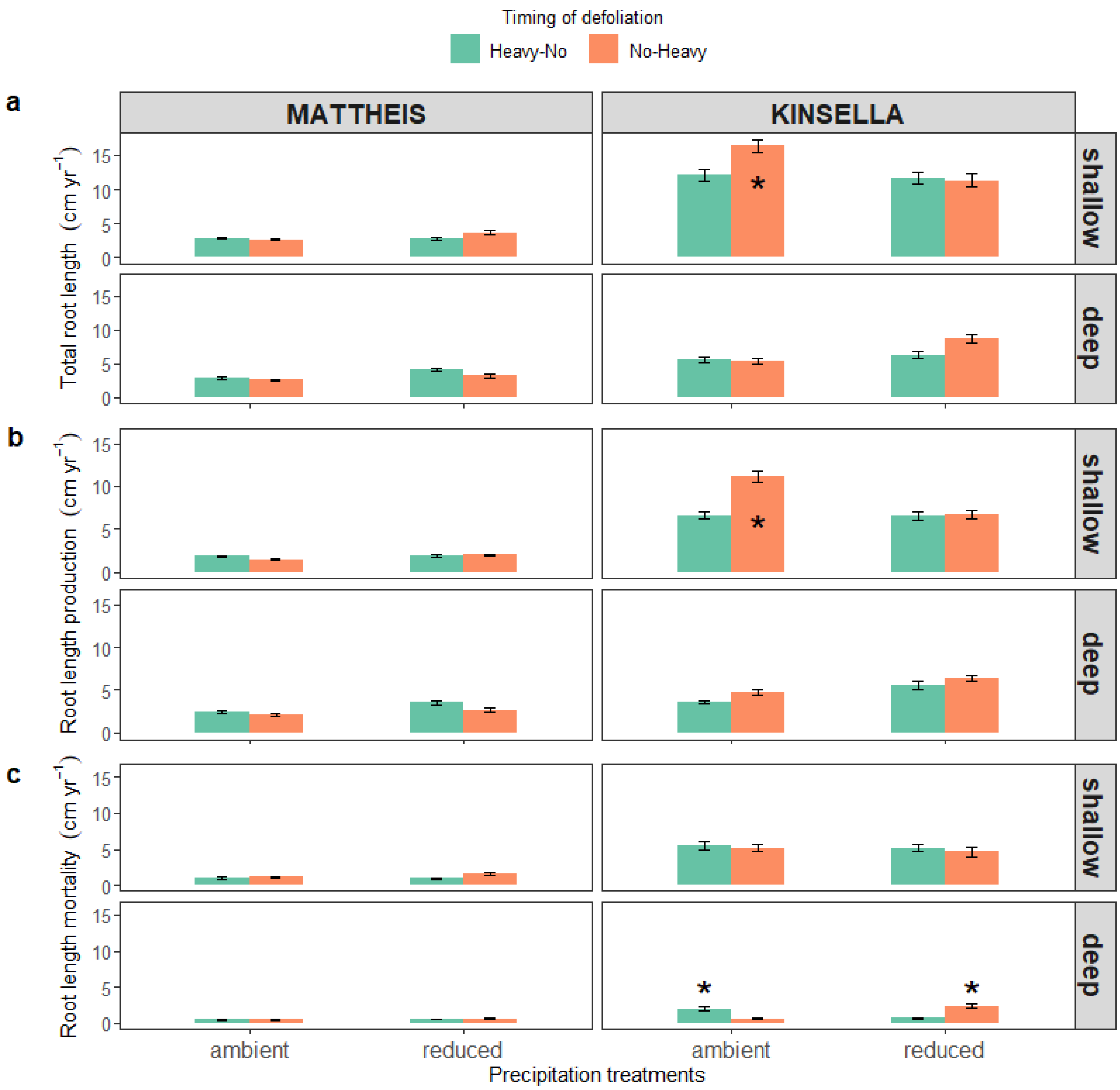

Figure 5-2. The combined effects of reduced precipitation and defoliation timing on (a) the mean annual total root length, (b) root length production, and (c) root length mortality at each soil depth across 2017-2019. Error bars represent ± 1 SE (n = 15).

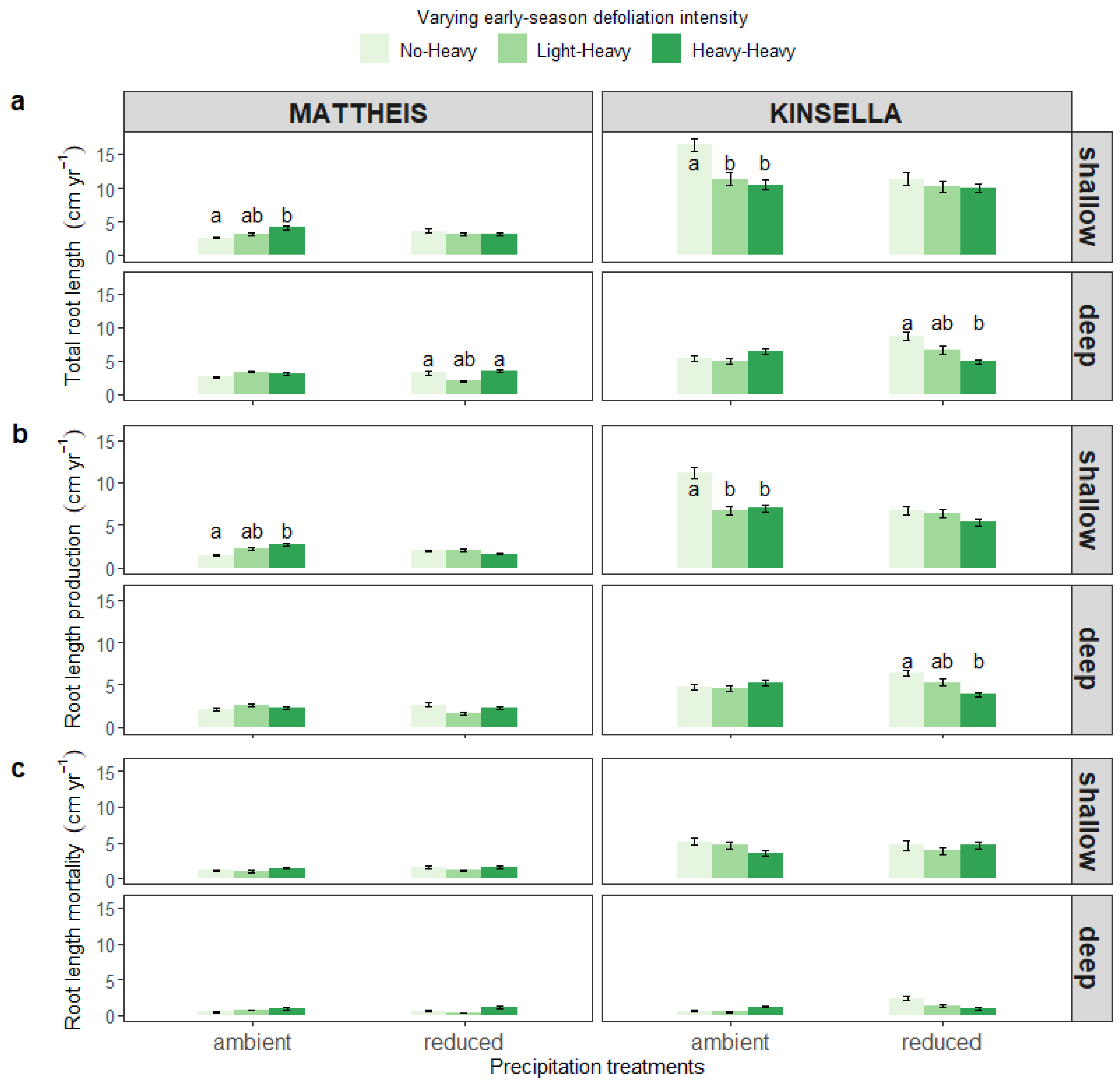

Figure 5-3. The combined effects of reduced precipitation and varying early-season defoliation intensity on the mean annual (a) total root length, (b) root length production, and (c) root length mortality at each soil depth across 2017-2019. Error bars represent ± 1 SE (n = 15).

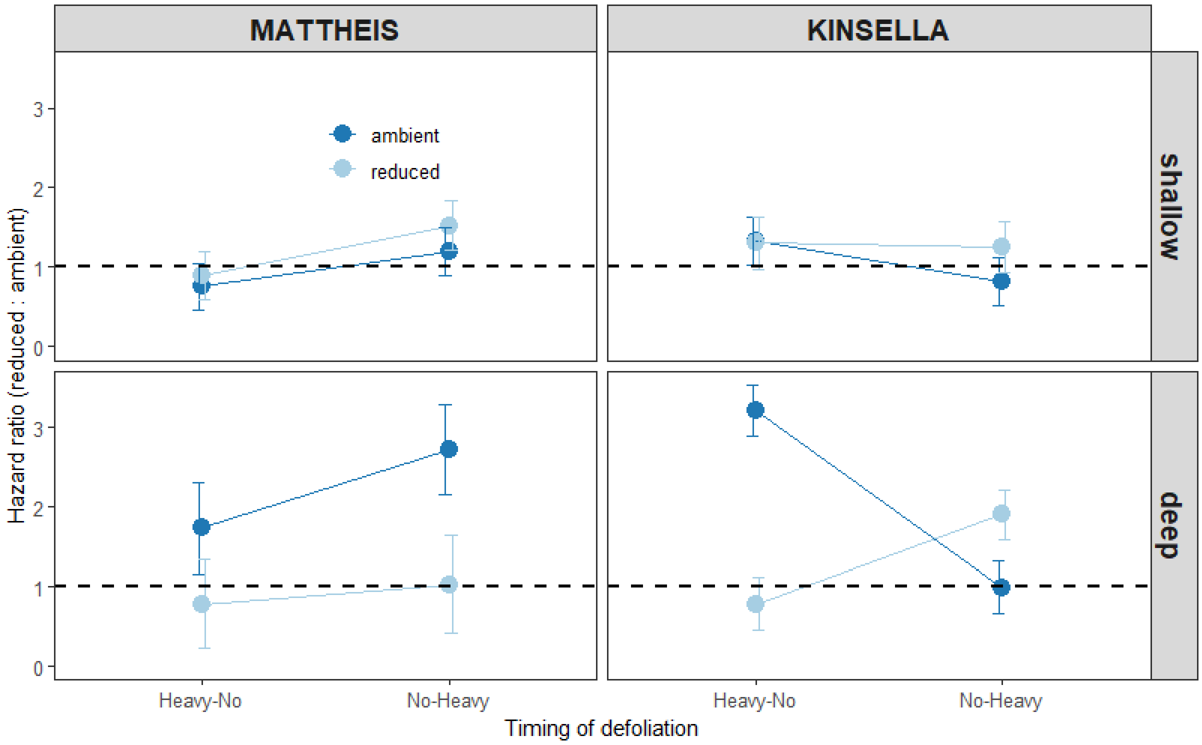

Figure 5-4. Hazard ratios for the interactive effects of drought and defoliation timing calculated at each soil depth separately using the Cox proportional hazard model for the lifespan of roots. Reference is reduced to ambient precipitation ratio in the absence of defoliation. Error bars represent ± 1 SE. A hazard ratio < 1 indicates an increase in root lifespan, while a hazard ratio > 1 shows a decrease in root lifespan.

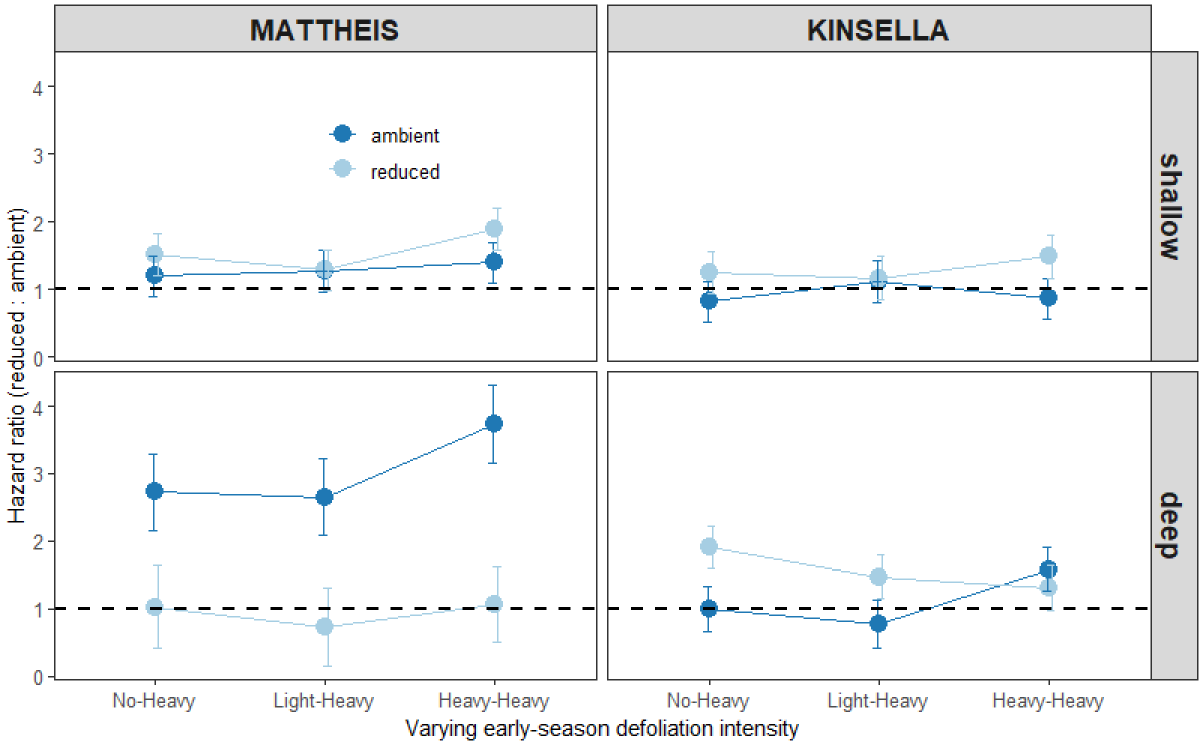

Figure 5-5. Hazard ratios for the interactive effects of drought and varying early-season defoliation intensity (none, light, and heavy) calculated at each soil depth separately using the Cox proportional hazard model for the lifespan of roots. Reference is reduced to ambient precipitation ratio in the absence of defoliation. Error bars represent ± 1 SE. A hazard ratio < 1 indicates an increase in root lifespan, while a hazard ratio > 1 shows a decrease in root lifespan.

Chapter 6: Conclusions and implications

The objectives of my thesis were to (1) assess the sensitivity of the northern temperate prairie ecosystem to the independent and combined effects of variation in precipitation and defoliation, (2) to gain a comprehensive understanding of how drought and defoliation shape ecosystem structure and function, and (3) to determine how root length dynamics respond to drought and defoliation. To accomplish this, I utilized long-term observational data in combination with a four-year factorial experiment, where I experimentally imposed four years of extreme drought and altered the timing and intensity of aboveground plant biomass removal at seven native temperate grasslands. In the remainder of this chapter, I will address these three overarching objectives using results from previous chapters and discuss broader implications of this dissertation in how it may inform future theoretical, empirical, modelling, and management efforts in the face of climate change.

In Chapter 2, using spatially extensive long-term records of plant production and climate data, I was able to examine how grazing influenced ecosystem sensitivity, measured as aboveground net primary productivity (ANPP), to precipitation. I found that grazing increased the sensitivity of ANPP to year-to-year fluctuation in annual precipitation across 31 grazed grasslands relative to paired non-grazed counterparts. Notably, I showed arid grasslands were more sensitive to interannual variability in precipitation when grazed than were in mesic grasslands. Further, I examined changes to components of biomass (e.g., grass, forb, and litter) to determine mechanisms behind the ecosystem responses. I found that changes induced by grazing in the contribution of grass and forb biomass to total ANPP and litter led to increased ecosystem sensitivity to precipitation when grazed. This chapter illustrates that the current use of the relationship between precipitation and ANPP as a benchmark in ecosystem models (Estiarte et al.

2016, Knapp et al. 2017b) is limited in predicting ecosystems sensitivity to projected changes in precipitation variability when exposed to the combination of two or more global change drivers. This scenario is likely in many systems in this era of anthropogenically driven global change (IPCC 2021). Conversely, an increased amount of litter reduced ecosystem sensitivity to interannual variability in precipitation. This has important implications in assessing ecosystem vulnerability to less predictable weather in the future, especially from a producer's perspective. Litter is known to regulate the availability of resources, chiefly soil moisture (Willms et al. 1986, Naeth et al. 1991, Deutsch et al. 2010, Hilger and Lamb 2017, Vandandorj et al. 2017), and thus making it an easily assessed proxy for ecosystem resiliency for land manager (Adams et al. 2016).

To address objectives 2 and 3 of my thesis, I sought to examine the combined effects of drought and defoliation on ecosystems across the northern edge of the Great Plains. To do this, I traded within site replicates of treatments for more sites to be included in my field experiment and treated sites as a form of replication in my analyses *sensu* Filazzola and Cahill (2021), allowing better generalization of the findings across the region. In Chapter 3, I examined the effects of drought duration on ecosystem structure and function in the absence of defoliation. This enabled me to test a hypothesis of whether drought impacts were progressive through time by examining a treatment × year interaction, a challenge in past experiments due to their limited duration (Hoover et al. 2018, Wang et al. 2021). I found that drought impacts were not compounding through time, contradicting expectations that the effects of prolonged drought would intensify over time (Zhang et al. 2019, Orth et al. 2020, Felton et al. 2021) and the results of other precipitation manipulation experiments conducted in the Great Plains of North America (Wilcox et al. 2017, Hoover et al. 2018). Drought affected species composition more often than species richness, evenness or community productivity. Contrary to expectation, I found that ANPP increased under drought above the ambient treatment in the fourth year of the experiment. Further, I explicitly demonstrated that variation in the abundance of dominant species led to shifts in community composition rather than the carry-over effects of prior year climatic and soil conditions (Sala et al. 2012) or the interrelationship of the response of different ecosystem components (Smith et al. 2009). These findings show that many ecosystem properties within these grasslands appear to be relatively resistant to consecutive years of water deficit. My results are consistent with several other multi-year drought experiments within grasslands that found a high degree of resistance to multi-year drought (Heitschmidt et al. 2005, Evans et al. 2011, Jentsch et al. 2011, Estiarte et al. 2016).

In chapter 4, I examined the sensitivity of both shoot and root responses to co-occurring factors of drought and defoliation. In particular, incorporating root responses into models in the future has high potential to improve understanding of the combined effects of global change drivers on ecosystems (Wilcox et al. 2017, Ma et al. 2020a, Slette et al. 2021). I found that the sensitivity to the combined effects of drought and defoliation differed between above- and belowground plant biomass across seven temperate grasslands. Specifically, defoliation in drought years, regardless of the timing or intensity of biomass removal, increased the sensitivity of aboveground biomass to drought, while root biomass remained insensitive to the combined effects across sites. In line with the previous experiment conducted in the region (White et al. 2014a), I also found that ANPP was more sensitive to drought in mesic grasslands than in arid grasslands. These results suggest that ecosystem sensitivity models might be overestimating the negative effects of co-occurring factors, given that many are based on aboveground biomass but not belowground (Estiarte et al. 2016, Knapp et al. 2017b), which make up more than half of grasslands biomass (Coupland and Johnson 1965, Sims and Singh 1978). Although I cannot identify the mechanisms behind this differential sensitivity, it could be due to improved root system efficiency (e.g., aboveground biomass produced per unit root length) at the community level (Ma et al. 2020a). Indeed, I found evidence of increased root system efficiency under reduced precipitation (Appendix 4-S4) by examining the relationship between root and shoot biomass from non-defoliated controls as a proxy measure of root system efficiency (Frank 2007, Ma et al. 2020a). However, I view this relationship as an interesting hypothesis for further testing.

Although root biomass is useful for predicting the effects of drought and defoliation on ecosystem services such as forage production and carbon sequestration, the efficiency of plant root systems to acquire growth-limiting soil resources is better reflected by root length than mass (Wilson 2014, Mueller et al. 2018, Ma et al. 2020a, Weigelt et al. 2021). In Chapter 5, using minirhizotrons, I examined the response of root length dynamics (e.g., root length, production, mortality, and lifespan) to drought and variation in defoliation at two of my seven study sites. Total length, production, mortality, and lifespan of roots were affected by every factor manipulated or measured in this study, consistent with highly plastic responses found in the previous study (Ma et al. 2020a). In general, I found that four years of extreme drought decreased the total length, production, and lifespan of roots in deeper soil at both grasslands in the absence of defoliation across years. Most importantly, I found evidence of generalization in many aspects of root length dynamics in response to the combined effects of drought and defoliation, which are common stressors in these grasslands (Koerner and Collins 2014, Kohli et al. 2020). I further showed that the mortality and lifespan of existing roots were the aspects of root length dynamics that caused the observed shifts in total root length resulting from drought and variation in defoliation. These findings are valuable for developing predictive frameworks of ecosystem responses to co-occurring global change factors and indicate substantial sensitivity in root dynamics to global change drivers, with responses depending on environmental conditions, defoliation, and soil depth.

In summary, my work highlights the highly responsive nature of temperate grassland ecosystems to the variation in precipitation and defoliation. There are three general conclusions from this dissertation. First, the relationship between precipitation and ANPP may be inappropriate to be used as a benchmark in ecosystem models for predicting ecosystem sensitivity, particularly if a system is exposed to the combination of two or more global change drivers. In fact, my results clearly show that grazing increases ANPP sensitivity to interannual variability in precipitation, especially in arid grasslands. This conclusion is further reinforced by the results of Chapter 4, whereby defoliation, regardless of what type, reduces ANPP during drought years. Second, the response of root biomass to the combined effects of precipitation and defoliation does not always mirror that of ANPP (Chapters 4 & 5), which is often not incorporated in ecosystem models. Third, evidence from my multi-site experiment shows that northern temperate grasslands are highly resistant to four consecutive years of extreme drought (Chapters 3-5). Although dominant species are important in their own right as a determinant of the strong resistance of these systems, they only partially explain how carbon sequestering abilities of ecosystems will be affected by global change drivers. I found highly plastic responses in root length dynamics to variation in precipitation and defoliation, which also explain the ability of these systems to withstand water deficits, given their sizeable influence on overall nutrient and carbon dynamics in grasslands (Eissenstat et al. 2000, Bardgett et al. 2014, Wilson 2014, Erktan et al. 2018a). The findings from my research highlight that root responses should be taken into account for predicting the magnitude and direction of changes in grassland ecosystems to variation in precipitation and defoliation.

The findings from my dissertation are generalizable across different ecosystems as I found highly consistent responses in root and shoots among seven sites studies, despite their differences in climate and vegetation. This can have significant implications both in terms of improving the development of better predictive frameworks as well as maintaining ecosystem goods and services. In terms of ecosystem models, my findings suggest that the relationship between precipitation and ANPP should be used with caution as a benchmark for ecosystem models when predicting the sensitivity of a system subjected to the combination of multiple factors. Further, root responses should also be incorporated into the models as I found differential sensitivity of shoot and root biomass in response to the combined effects of drought and defoliation. Especially, a deeper understanding of root growth and development in the context of drought and grazing will not only add information to the growing body of literature on plant responses to multiple stressors but will also inform rangeland managers to help reduce the potential risks to ecosystem function and structure that are associated with increased water limitation from an extended drought. Therefore, my findings highlight that roots can maintain carbon sequestration during drought, thereby increasing carbon storage and minimizing risks associated with forage loss. However, reducing defoliation intensity is necessary to maintain healthy roots, particularly in mesic systems in drought years.

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Appendices

Appendix 2-S1. Characteristics of the 56 study sites. Values of mean annual temperature (MAT) and mean annual water-year precipitation (MAP) are given as means ± SD (in parentheses) over 9-28 years. ANPP sensitivity is determined by the slopes of the relationships between ANPP and MAP. Shading highlights the 31 grasslands that experienced high precipitation variability during the periods of observation. MAP CV: the coefficient of variation for water-year precipitation for each site. adj.R2: variation explained by each model, ID #: site-specific identification numbers; Year: length of records.

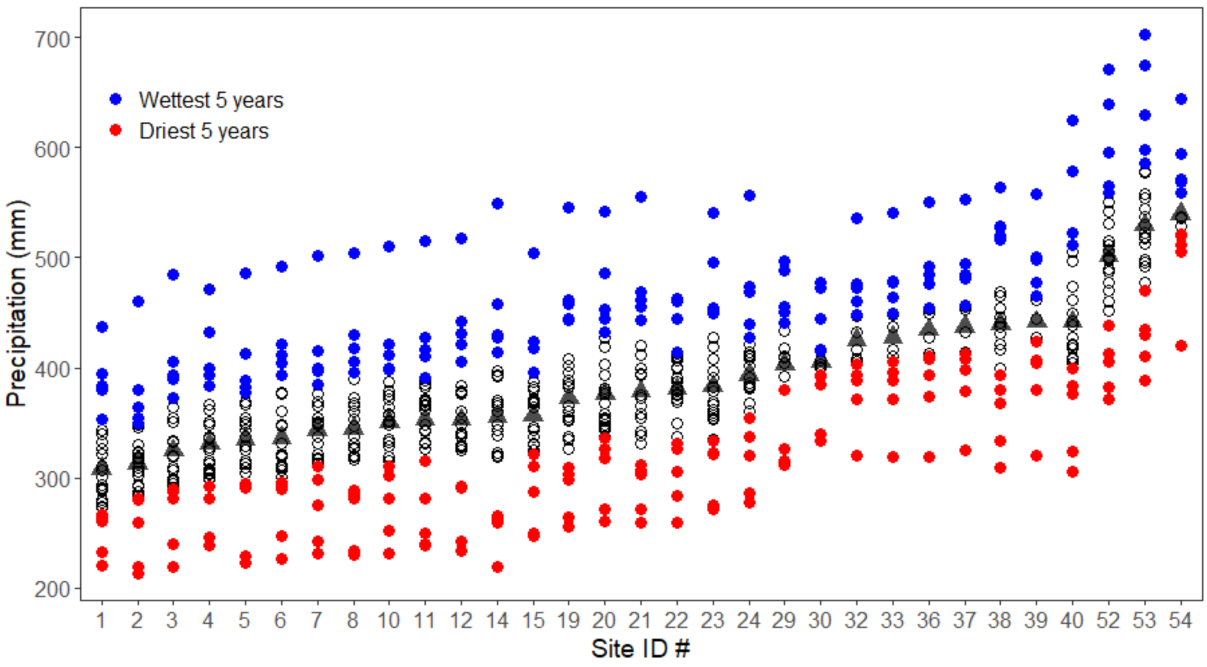
ID #	Year	MAT (°C)	MAP (mm)	MAP CV		ANPP sensitivity		adj.R^2	
				116 year	Observed	Non-grazed	Grazed	Non-grazed	Grazed
1	27	5.5 (0.8)	308.2 (49.9)	0.20	0.16	0.008	0.021**	0.03	0.26
2	27	4.6 (0.8)	313.2 (48.4)	0.20	0.15	0.012	0.026*	0.02	0.14
3	27	4.6 (0.8)	324.8 (53.4)	0.20	0.16	0.009	0.023*	-0.01	0.18
4	27	4.6 (0.8)	331.2 (51.9)	0.19	0.16	0.014***	0.021***	0.39	0.53
5	26	5.1 (0.8)	335.4 (52.8)	0.20	0.16	0.019*	0.028**	0.15	0.30
6	27	4.9 (0.8)	336.6 (55.3)	0.20	0.16	0.008	0.018**	0.00	0.31
7	25	4.9 (0.8)	343.7 (55)	0.20	0.16	0.004	0.005	-0.03	-0.02
8	26	5.3 (0.8)	344.9 (58.8)	0.20	0.17	0.01	0.018*	0.05	0.18
9	13	5.7 (0.6)	345.5 (53.7)	0.22	0.16	-0.006	0.007	-0.01	0.00
10	27	5.2 (0.8)	350.9 (54.4)	0.20	0.16	0.011	0.02**	0.05	0.33
11	28	4.6 (0.8)	352.5 (57.5)	0.20	0.16	0.014	0.017	0.01	0.04
12	25	5.1 (0.8)	353.2 (61.3)	0.20	0.17	0.014	0.016*	0.10	0.21
13	13	5.2 (0.7)	353.5 (37)	0.20	0.10	0.016	0.032	0.15	0.23
14	23	5.2 (0.8)	356.1 (74.4)	0.23	0.21	0.014*	0.029**	0.13	0.36

15	27	4.5 (0.8)	356.1 (51.6)	0.19	0.14	0.008	0.024	-0.03	0.06
16	15	4.1 (0.7)	357.9 (46)	0.19	0.13	0.009	0.023	-0.01	0.21
17	12	5.4 (0.7)	358.3 (46.6)	0.19	0.13	0.004	0.011	-0.08	0.09
18	10	5.1 (1)	366 (58)	0.20	0.16	0.003	0.011	-0.05	0.25
19	25	5.7 (0.8)	372 (65.2)	0.20	0.18	0.013**	0.01*	0.26	0.13
20	27	4.2 (0.8)	376.1 (60.8)	0.19	0.16	0.009	0.01*	0.09	0.16
21	25	5.7 (0.8)	378.4 (66.3)	0.20	0.18	0.007	0.017**	0.05	0.35
22	26	5.4 (0.8)	380.5 (50.4)	0.20	0.13	0.028*	0.035**	0.20	0.31
23	27	5.1 (0.8)	382.9 (60.5)	0.19	0.16	0.005	0.01*	0.03	0.12
24	25	4.6 (0.8)	393.6 (57.8)	0.19	0.15	0.01	0.012	0.00	0.06
25	15	2.9 (0.7)	394.6 (43.3)	0.17	0.11	0.016	0.023	0.03	0.11
26	14	3.6 (0.6)	395.5 (36.8)	0.16	0.09	0.014	0.008	-0.02	-0.07
27	14	3.2 (0.7)	396.8 (35.6)	0.15	0.09	0.025	0.032	0.00	0.00
28	14	3.5 (0.6)	397.9 (37.6)	0.16	0.09	-0.006	-0.009	-0.07	-0.07
29	16	5.5 (0.6)	403.3 (58.9)	0.21	0.15	0.017	0.011	0.13	-0.01
30	14	3.4 (0.6)	405.9 (40.2)	0.16	0.10	0.001	0.007	-0.08	-0.07
31	14	3.3 (0.7)	408.4 (37.3)	0.15	0.09	0.016	0.01	-0.02	-0.07
32	15	2.9 (0.7)	425.3 (50.4)	0.16	0.12	0.038*	0.042**	0.36	0.39
33	15	2.8 (0.7)	427.6 (51.8)	0.16	0.12	0.021	0.017	0	0.06
34	14	3.2 (0.7)	428.1 (42.9)	0.15	0.10	0.024	0.027	0.09	0.10
35	14	4.8 (0.6)	428.5 (39.9)	0.19	0.09	0.013	0.009	0.05	0.00
36	15	2.7 (0.7)	434.3 (54.6)	0.16	0.13	0.024*	0.018	0.25	0.13
37	15	2.7 (0.7)	437.5 (53.9)	0.16	0.12	0.027*	0.021	0.22	0.15
38	21	2.4 (1)	439.9 (65.1)	0.17	0.15	0.016*	0.021*	0.18	0.17
39	15	2.9 (0.7)	441.6 (55.3)	0.15	0.13	0.028*	0.034*	0.25	0.30
40	27	3 (0.8)	441.7 (69.7)	0.19	0.16	0.001	0.003	-0.04	0.00

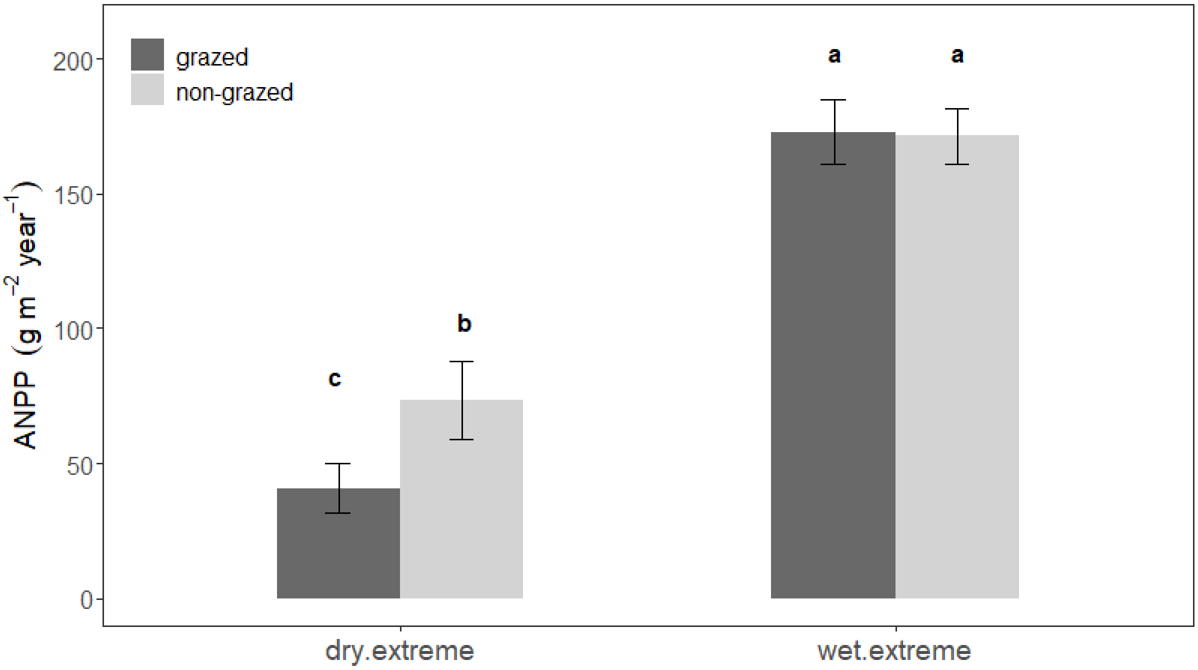
41	12	2.9 (0.7)	442.2 (46.6)	0.16	0.11	0.02	0.006	0.01	-0.08
42	12	2.7 (0.7)	443.5 (56.6)	0.16	0.13	0	0.008	-0.10	-0.06
43	13	2.7 (0.7)	445.2 (55.4)	0.16	0.12	0.001	0.012	-0.09	-0.03
44	13	2.7 (0.7)	445.4 (55.7)	0.16	0.13	0.008	0.012	-0.04	0.00
45	13	2.7 (0.7)	445.8 (50.5)	0.16	0.11	-0.001	0.002	-0.09	-0.09
46	14	2.8 (0.7)	445.9 (45.4)	0.16	0.10	0.004	0.004	-0.08	-0.08
47	12	2.5 (0.8)	449.5 (57.8)	0.16	0.13	0.005	0.008	-0.08	-0.06
48	12	2.5 (0.8)	452.3 (60.5)	0.17	0.13	0.023	0.016	0.17	0.04
49	14	2.9 (0.7)	457.4 (47.5)	0.15	0.10	0.006	0.022	-0.06	0.13
50	9	3.3 (0.7)	463.2 (54.4)	0.15	0.12	-0.01	0.005	-0.06	-0.12
51	14	3.1 (0.7)	473.8 (46.8)	0.15	0.10	0.003	0.001	-0.08	-0.08
52	26	3.7 (0.7)	501.8 (70.6)	0.19	0.14	0.012*	0.014**	0.14	0.26
53	25	3.1 (0.7)	529.6 (75.1)	0.18	0.14	0.008	0.017**	0.10	0.26
54	14	3.8 (0.6)	539.6 (49.6)	0.19	0.09	0.013*	0.007	0.30	-0.01
55	12	3.2 (0.5)	564.2 (46.9)	0.18	0.08	-0.018	0.002	0.19	-0.10
56	14	2.9 (0.6)	591.6 (51.9)	0.18	0.09	0.002	-0.006	-0.07	-0.04

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

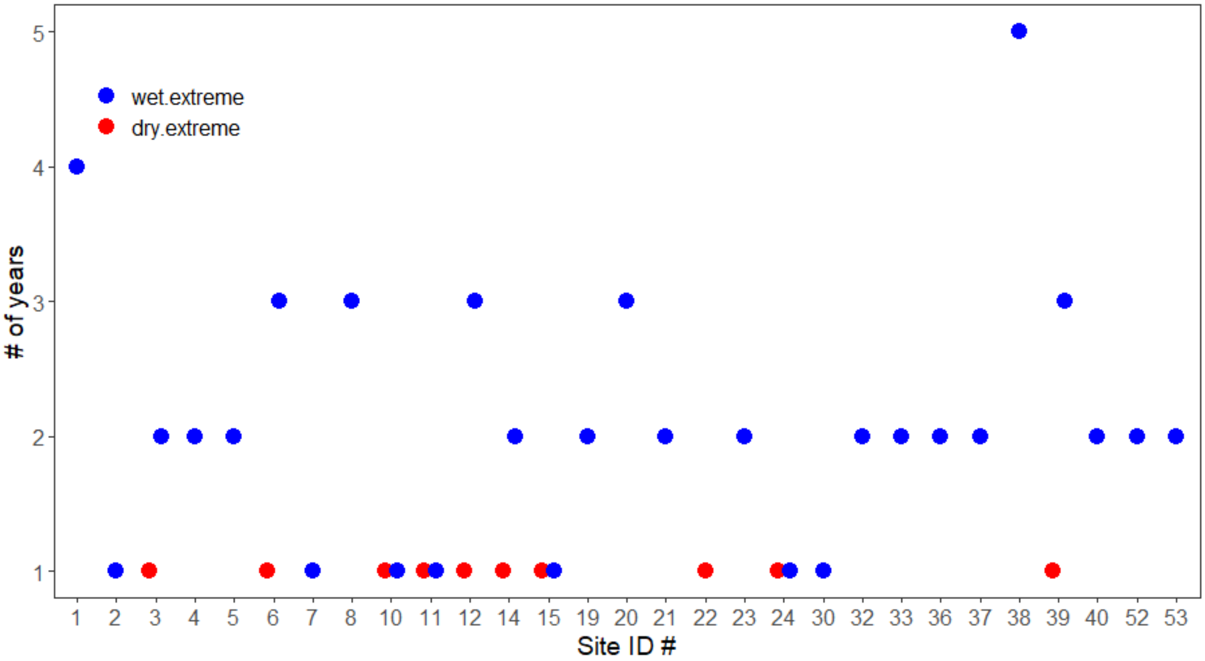
Appendix 2-S2. Total water-year precipitation at 31 sites that experienced high variability in precipitation during the period of observation. Sites are arranged from the most arid to the most mesic according to their long-term average water-year precipitation (black triangles). Site IDs correspond to Appendix 2-S1. Open circles represent water-year precipitation in most years at a given site, while colored circles represent the five driest (red) and wettest (blue) years, respectively.



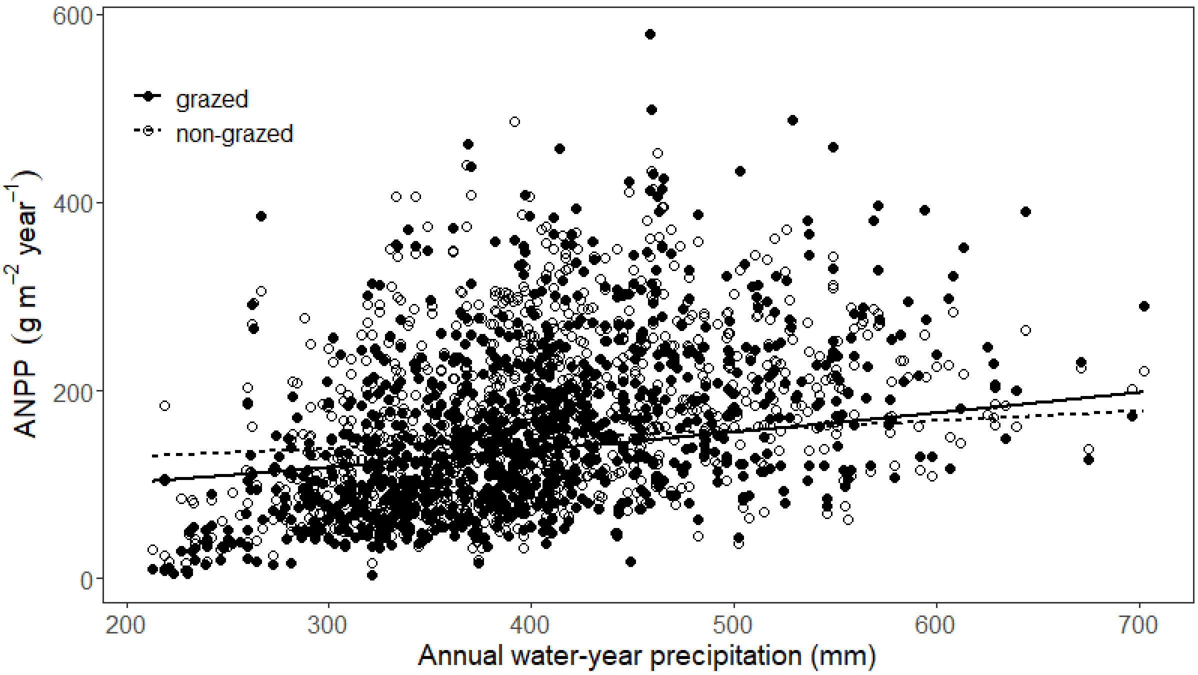
Appendix 2-S3. Effects of grazing on ANPP (means ± SE) during extreme dry and wet years of 31 sites that experienced substantial variability of precipitation during the period of observation (see Appendix 2-S2). ANPP was affected by extreme water-year precipitation ($F_{1,17.05} = 72.87$, $p < 0.0001$), exposure to grazing ($F_{1,98.62} = 9.17$, $p = 0.0031$) and their interaction ($F_{1,98.62} = 8.25$, $p = 0.0045$). Bars sharing a letter are not statistically different ($p > 0.05$).



Appendix 2-S4. The observed number of extreme dry and wet years of 31 sites that experienced substantial variability of precipitation during the period of observation.



Appendix 2-S5. Relationship between annual water-year precipitation and total aboveground net primary production (ANPP) across 56 grasslands with paired grazed and non-grazed locations (Appendix 2-S1). ANPP was affected by water-year precipitation ($F_{1,1465.4} = 23.44$; $p < 0.0001$), exposure to grazing ($F_{1,1995.4} = 24.96$; $p < 0.0001$), and their interaction ($F_{1,1995.4} = 15.23$; $p < 0.0001$). Lines are linear mixed effect models fit for each of the grazed and non-grazed locations.



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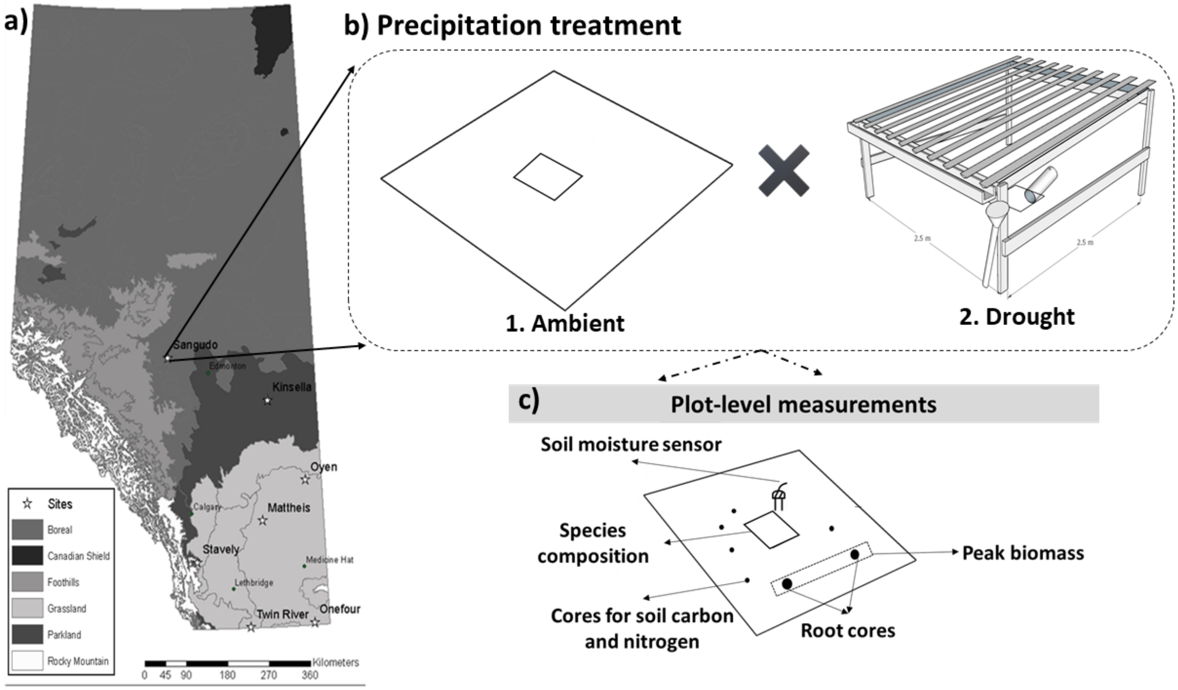
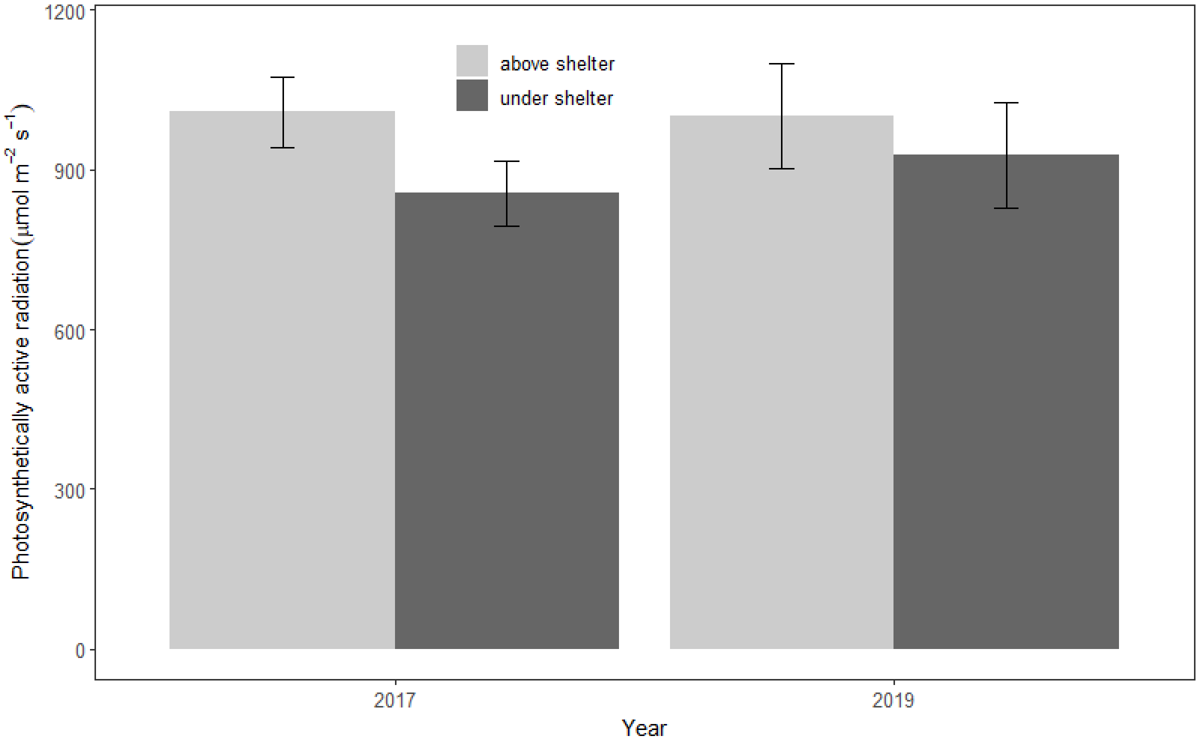
Appendix 3-S1. Characteristics of seven study sites across the grassland ecoregion of Alberta, Canada. Mean annual precipitation (MAP) and temperature (MAT) are based on 115-years (1901-2015) of data obtained from ClimateNA v6.3 software. The annual (PPT) and growing season precipitation (GSP) for the ambient treatment during the experiment are based on data obtained from weather stations nearby the study sites. Values in parentheses are standard deviations. Sites are numbered from arid (1) to mesic (7) according to MAP. The percentage reduction in rainfall for each site was determined by site-specific precipitation variability and was calculated as: 100-((MAP 1st percentile / MAP) *100).

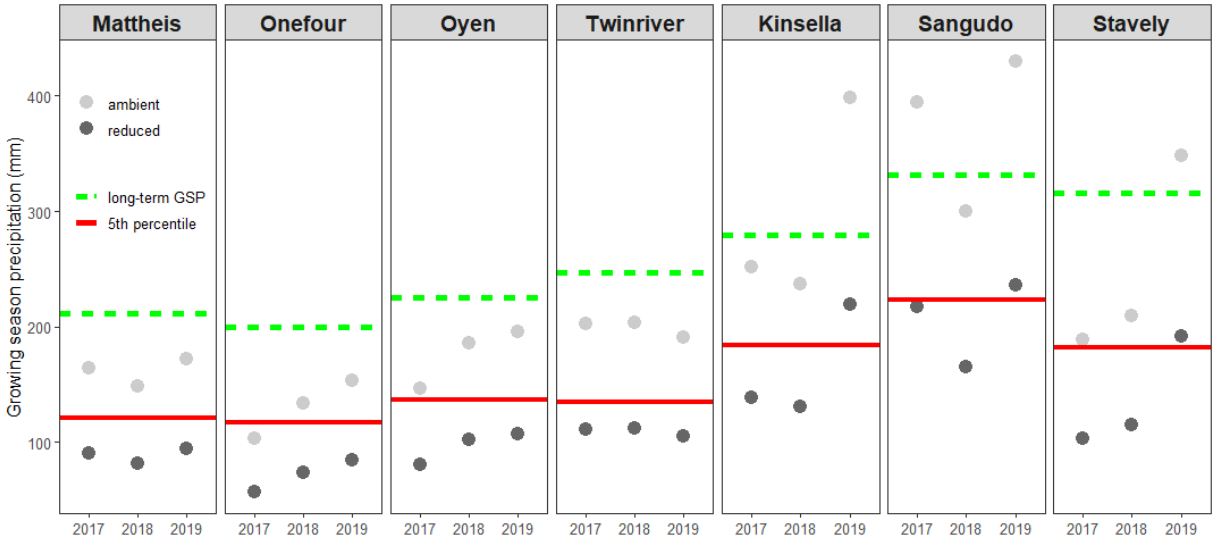
		Mattheis	Onefour	Oyen	Twin river	Kinsella	Sangudo	Stavely
Site ID		1	2	3	4	5	6	7
MAP (mm)		312 (±64)	318 (±73)	321 (±67)	358 (±87)	401 (±68)	492 (±74)	533 (±110)
MAT (°C)		3.6 (±1.04)	4.9 (±1)	32.7 (±1.1)	4.7 (±0.9)	1.9 (±1.1)	2.2 (±0.9)	3.4 (±0.9)
MAP 1st percentile		182	199	204	216	253	316	315
% Reduction to get to the 1st of MAP		42	38	36	40	37	36	41
Soil texture		Sandy loam	Clay loam	Loam	Clay loam	Clay loam	Loam	Silty clay loam
Dominant plant species		*Bouteloua gracilis,* *Hesperostipa comata,* *Koeleria macrantha*	*Bouteloua gracilis,* *Hesperostipa comata,* *Pascopyrum smithii*	*Hesperostipa curtiseta,* *Avenula hookeri,* *Elymus lanceolatus*	*Festuca idahoensis,* *Elymus lanceolatus,* *Hesperostipa comata*	*Festuca hallii,* *Pascopyrum smithii,* *Poa pratensis*	*Elymus repens,* *Trifolium repens,* *Taraxacum officinale*	*Festuca campestris,* *Danthonia parryi,* *Poa pratensis*
PPT (mm)	2017	283.7	202.6	255.9	398.9	414.6	544.7	403.5
	2018	237.4	213.8	252.1	361.9	322.8	433.9	351.7
	2019	252.2	242.7	252.5	306.0	479.8	581.5	473.0
GSP (mm)	2017	164.1	103.0	147.0	202.6	251.6	394.9	188.6
	2018	148.5	133.5	185.8	203.9	237.4	300.0	209.8
	2019	172.5	153.1	195.4	190.7	398.1	429.9	348.3



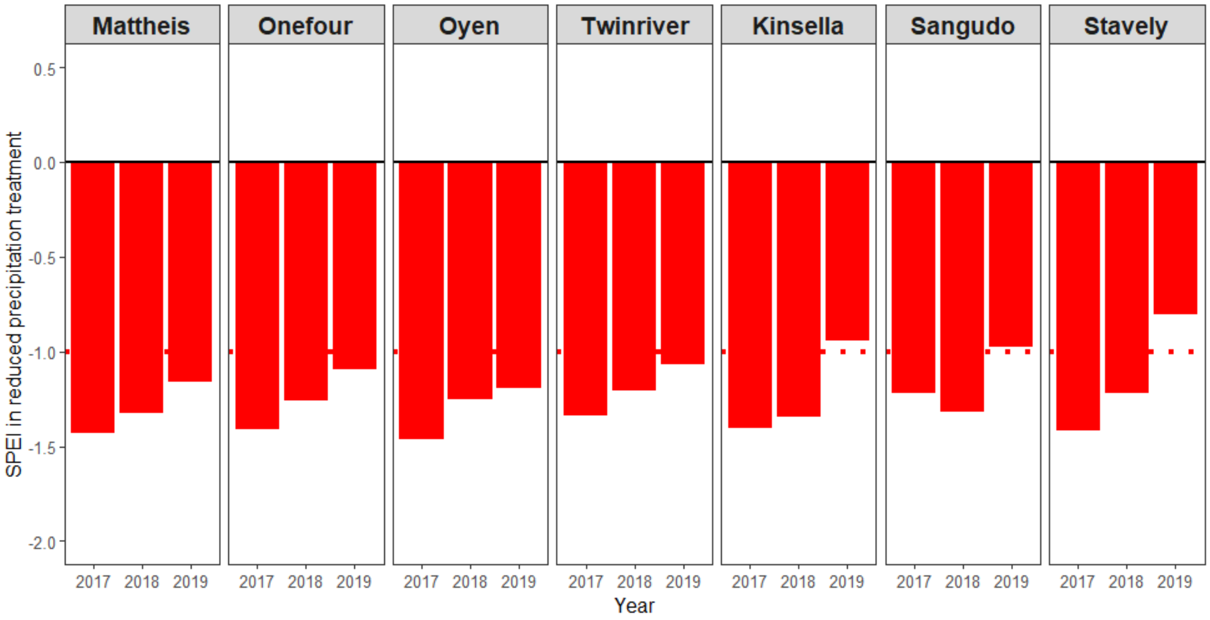
Appendix 3-S2. The location of study sites, b) experimental design at each study site, and c) description of plot-level measurements.



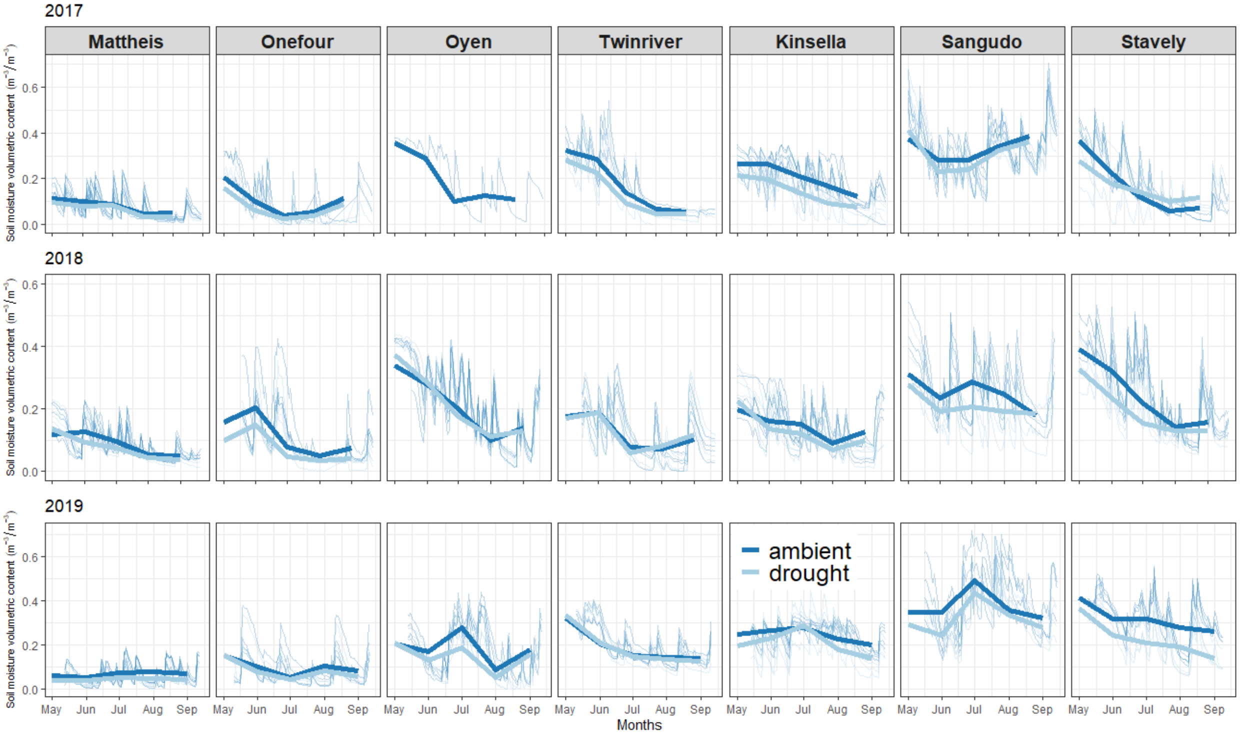
Appendix 3-S3. Comparison of light conditions above and under rainout shelters (mean ± SE). We used a Li-Cor model 2994 linear quantum sensor to measure photosynthetically active radiation under and above the shelter twice over the course of the experiment. Measurements were taken at noon under cloud-free days between mid May and July in 2017 and 2019.



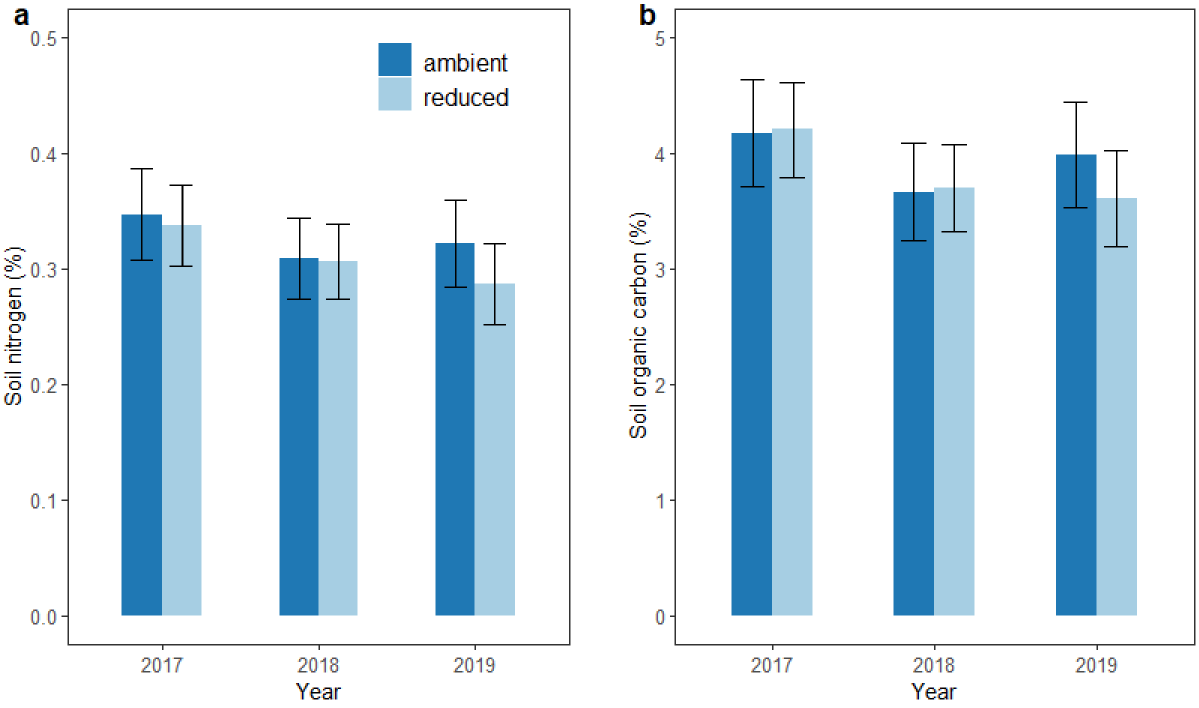
Appendix 3-S4. Growing season precipitation (GSP) of treatments relative to historical drought magnitude (5th percentiles) across all site × year combinations.



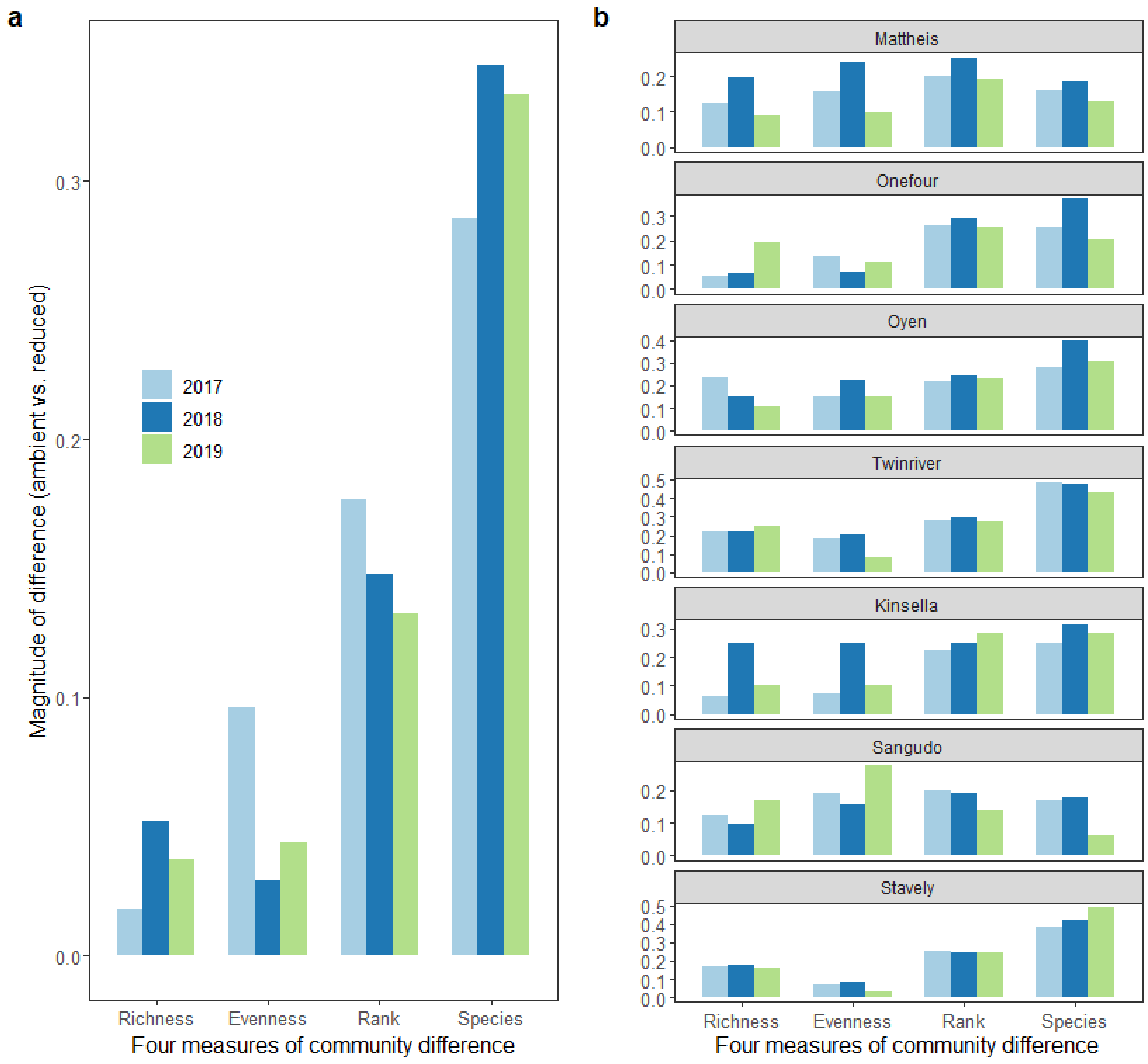
Appendix 3-S5. The estimated drought severity of reduced precipitation treatments across all site × year combinations. Drought severity was estimated by the Standardized Precipitation-Evapotranspiration Index (SPEI). Positive SPEI values indicate water surplus, and negative values indicate water shortage relative to the reference period. The dotted line indicates severe drought conditions.



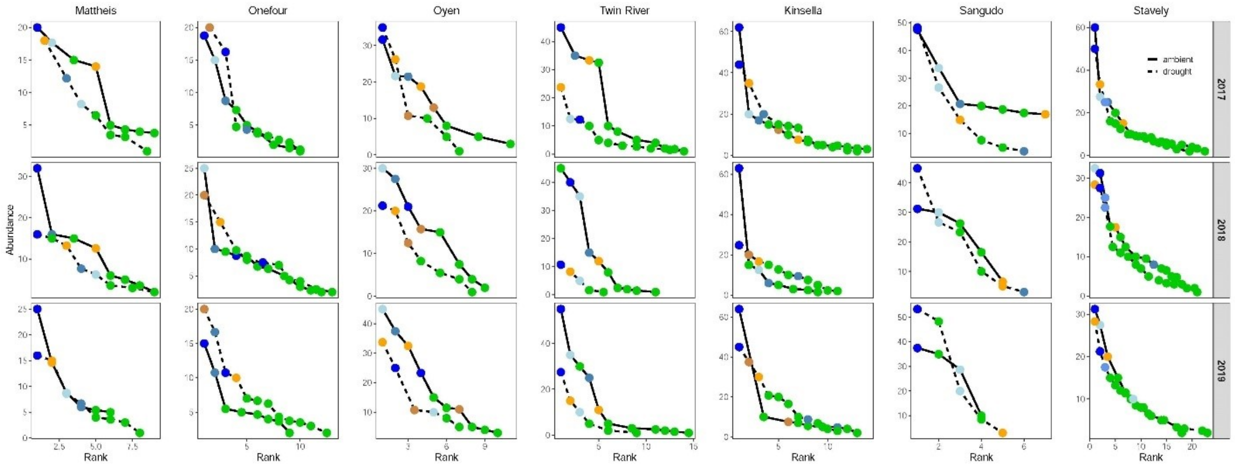
Appendix 3-S6. Daily (thin lines) and monthly (broad lines) average dynamics of growing season volumetric soil moisture content (0-5 cm) for each of the ambient and reduced precipitation treatments during the experimental years.



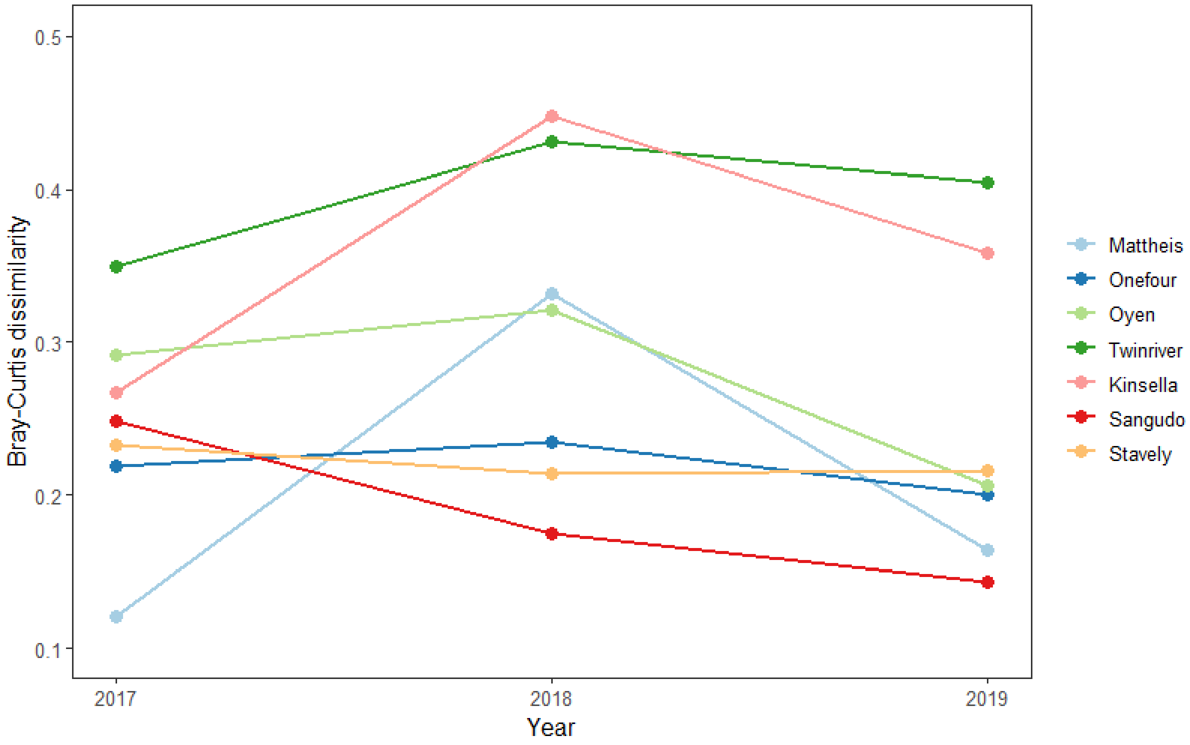
Appendix 3-S7. Mean (±SE) of a) total soil nitrogen and b) soil organic carbon within the top (0-15 cm) soil layers for ambient and reduced precipitation treatments during the experimental years.



Appendix 3-S8. The magnitude of each community difference measure contributing to the observed differences in species composition between the ambient and reduced precipitation treatments across the seven sites (a) and within each site (b) during the experiment. Rank indicates differences in the ordering of species abundance between ambient and reduced precipitation treatments, while species indicates species replacement component of beta-diversity (Avolio et al., 2019; Carvalho et al., 2012). Four measures of community difference were calculated using the RAC_difference() function within the R package codyn (Hallett et al., 2020).



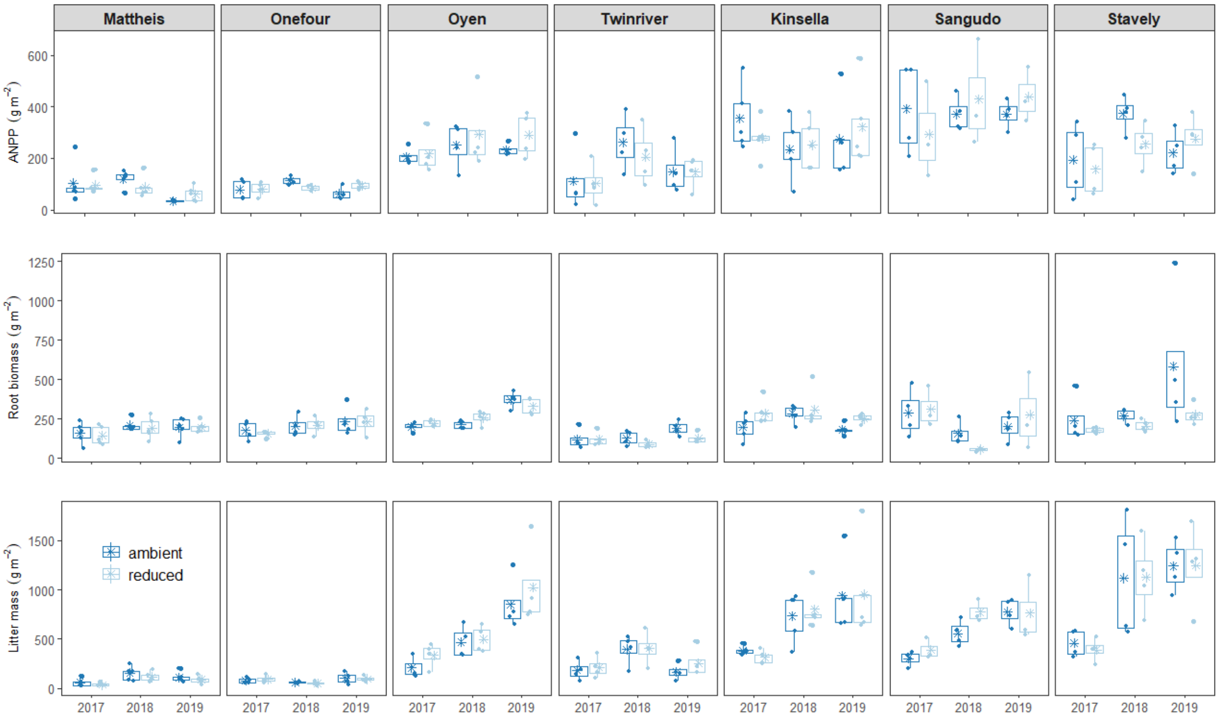
Appendix 3-S9. Rank abundance curves of all species in the ambient and reduced precipitation treatments for each site and year. The top three species of the ambient treatment are the shades of blue, whereas the top three species within the reduced precipitation treatment are the shades of orange. All other species are green.



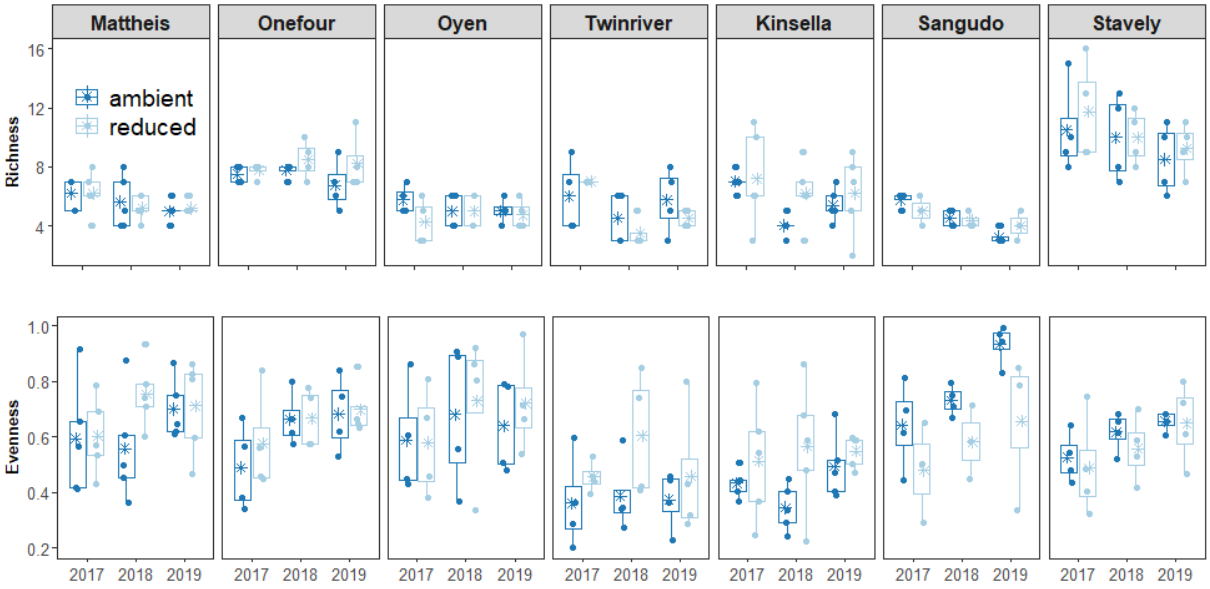
Appendix 3-S10. Differences in community composition between the ambient and reduced precipitation treatments at each site over the course of the experiment. Bray-Curtis dissimilarity index bounds between 0 (identical) and 1 (different).

Appendix 3-S11. SIMPER results for the most influential species contributing to divergence in community composition between the ambient and reduced precipitation (PPT) treatments across the seven sites. Species are listed in order of their contribution to the average Bray-Curtis dissimilarity between treatments, with a cut-off when the cumulative percent contribution reaches 70%. Shading indicates the species that increased (grey) or decreased (none-shaded) in abundance with drought.

Plant species	Growth form	Ave. abundance in ambient PPT	Ave. abundance in reduced PPT	Contribution (%)	Cumulative (%)
Poa pratensis	grass	21.94	13.67	14.21	16.94
Carex_spp	grass	4.83	8.44	5.71	23.74
Hesperostipa comata	grass	5.34	4.38	5.46	30.25
Elymus lanceolatus	grass	5.67	4.11	5.29	36.56
Bouteloua gracilis	grass	4.77	3.57	4.87	42.36
Elymus repens	grass	5.17	5.06	4.84	48.12
Hesperostipa curtiseta	grass	2.59	4.31	3.77	52.62
Taraxacum officinale	forb	3.76	2.93	3.25	56.49
Artemisia frigida	forb	2.37	3.72	3.13	60.23
Pascopyrum smithii	grass	2.72	3.37	2.91	63.70
Festuca idahoensis	grass	1.56	2.32	2.80	67.03
Koeleria macrantha	grass	2.10	1.78	2.33	69.81
Festuca campestris	grass	1.33	3.10	2.08	72.29



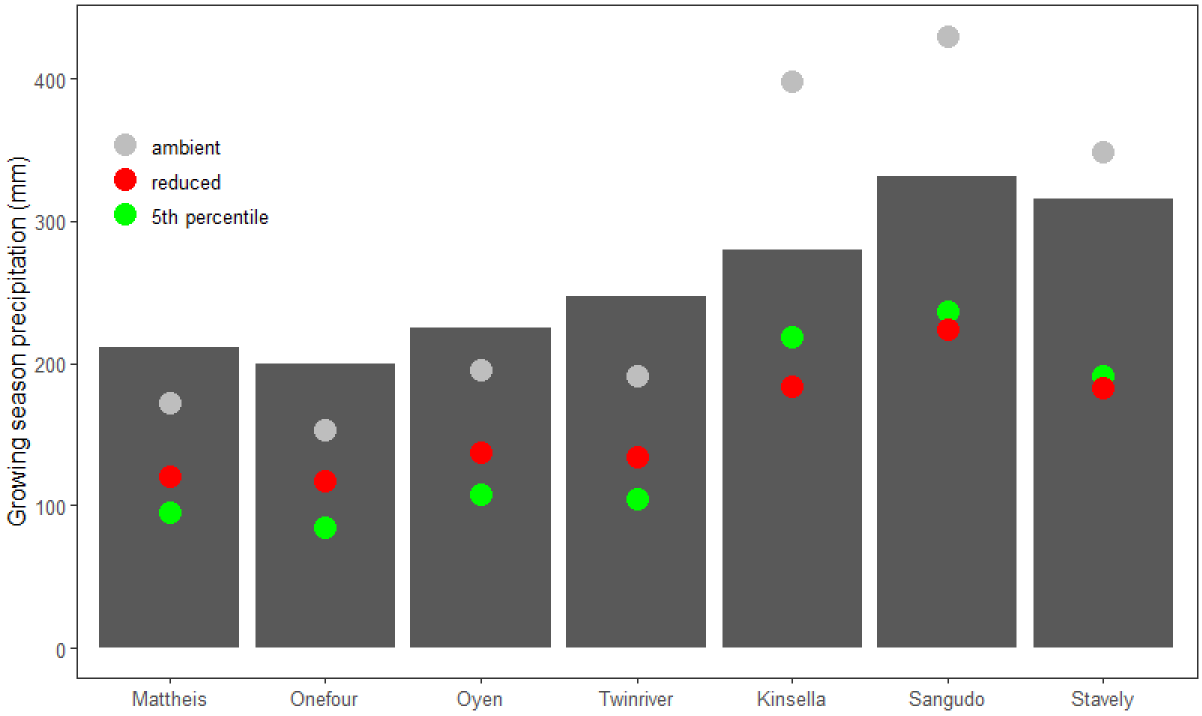
Appendix 3-S12. Site level responses of aboveground net primary productivity (ANPP, top panel), standing root biomass to 15 cm depth (middle panel), and litter mass (bottom panel) to the combined effects of treatment and year. Dots are replicates of each treatment, while star (*) indicates the mean of treatments.



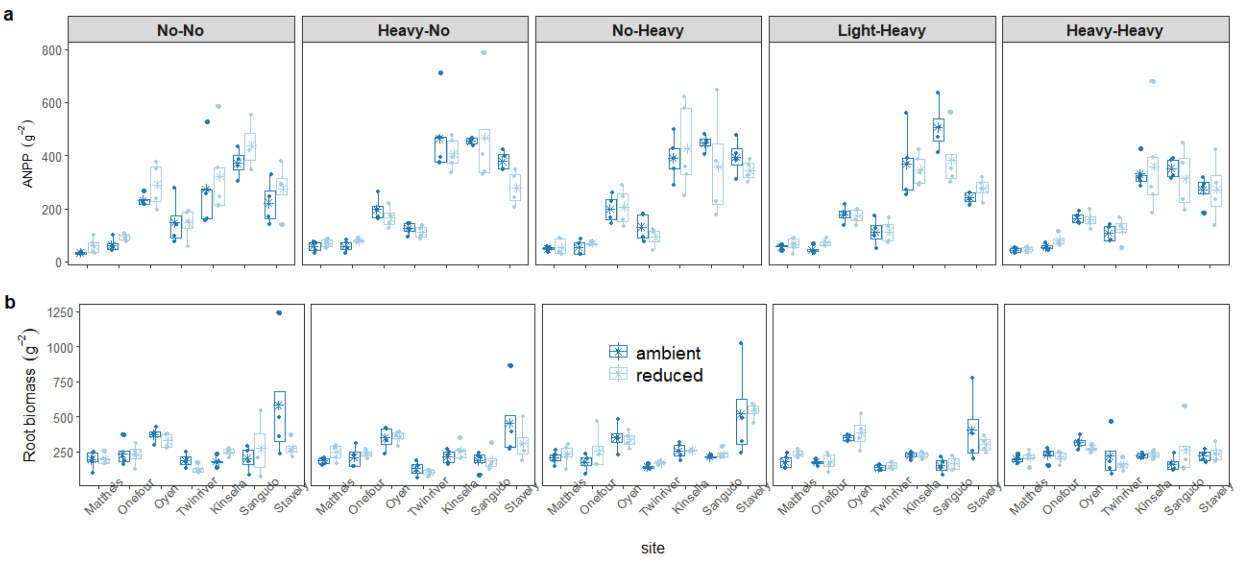
Appendix 3-S13. Site level responses of species richness (top panel) and evenness (bottom panel) to the combined effects of treatment and year. Dots are replicates of each treatment, while star (*) indicates the mean of treatments.

Appendix 4-S1. Characteristics of the seven grassland sites examined across Alberta, Canada. Mean annual precipitation (MAP ± SD) and temperature (MAT ± SD) are based on 115-years (1901-2015) obtained from ClimateNA v6.3 software (Wang et al. 2016). Aboveground net primary productivity (ANPP ± SE) and root biomass to 15 cm depth (± SE) are average values of replicates from the ambient treatment over the study period (2016-2019, inclusive).

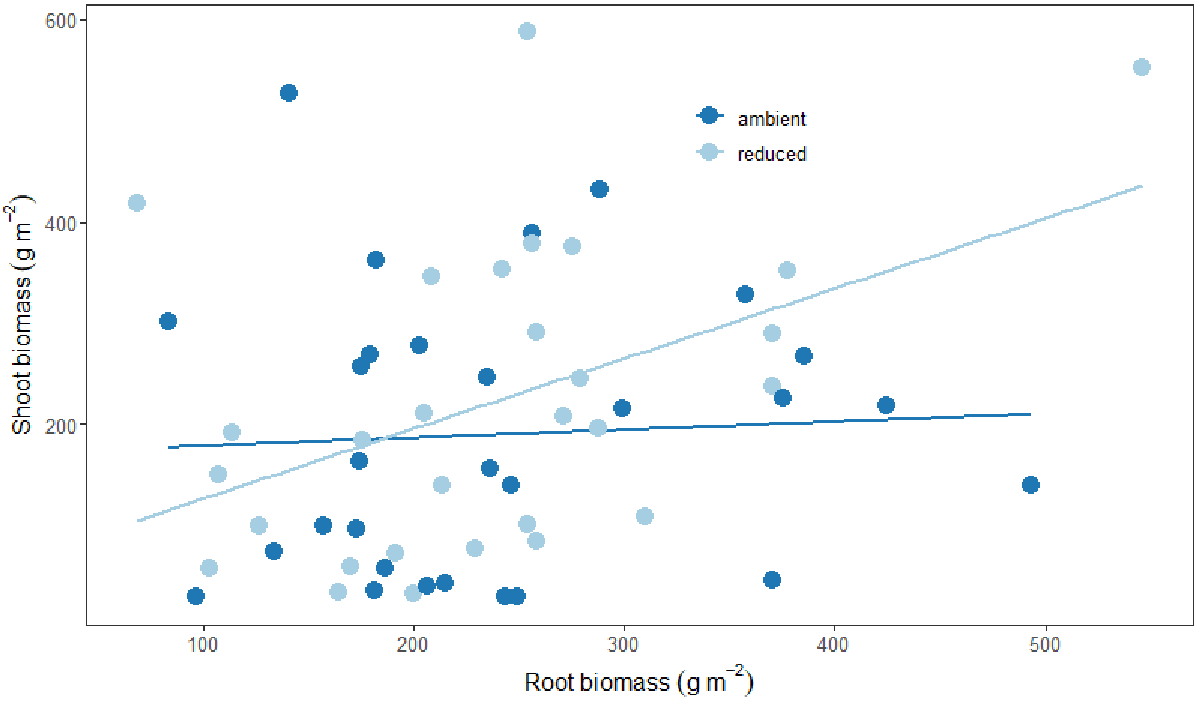
Site	MAP (mm)	MAT (°C)	Soil texture	Dominant plant species	ANPP (g m^{-2})	Root biomass (g m^{-2})
Mattheis	312 (±64)	3.6 (1.04)	Sandy loam	*Bouteloua gracilis,* *Hesperostipa comata,* *Koeleria macrantha*	119.8 (20.1)	234.3 (24.6)
Onefour	318 (±73)	4.9 (1)	Clay loam	*Bouteloua gracilis,* *Hesperostipa comata,* *Pascopyrum smithii*	87.2 (7.5)	257.5 (29.5)
Oyen	321 (±67)	32.7 (1.1)	Loam	*Hesperostipa curtiseta,* *Avenula hookeri,* *Elymus lanceolatus*	251.7 (16.9)	408.7 (75.1)
Twin river	358 (±87)	4.7 (0.9)	Clay loam	*Festuca idahoensis,* *Elymus lanceolatus,* *Hesperostipa comata*	197.5 (30.9)	182.3 (22.1)
Kinsella	401 (±68)	1.9 (1.1)	Clay loam	*Festuca hallii,* *Pascopyrum smithii,* *Poa pratensis*	326.1 (32.3)	373.3 (67.3)
Sangudo	492 (±74)	2.2 (0.9)	Loam	*Elymus repens,* *Trifolium repens,* *Taraxacum officinale*	382.5 (22.7)	346.8 (64.7)
Stavely	533 (±110)	3.4 (0.9)	Silty clay loam	*Festuca campestris,* *Danthonia parryi,* *Poa pratensis*	316.5 (38.1)	448.4 (74.3)



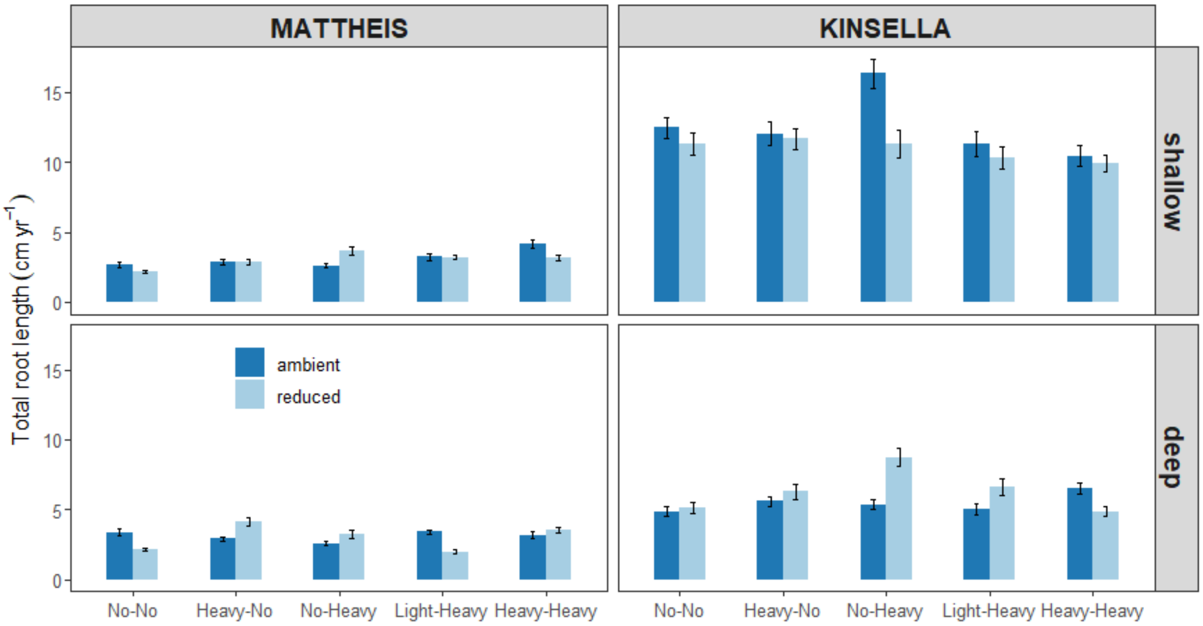
Appendix 4-S2. Growing season precipitation of each site in the final year of the experiment relative to long-term site averages (bars) and historical drought magnitude (5th percentile).



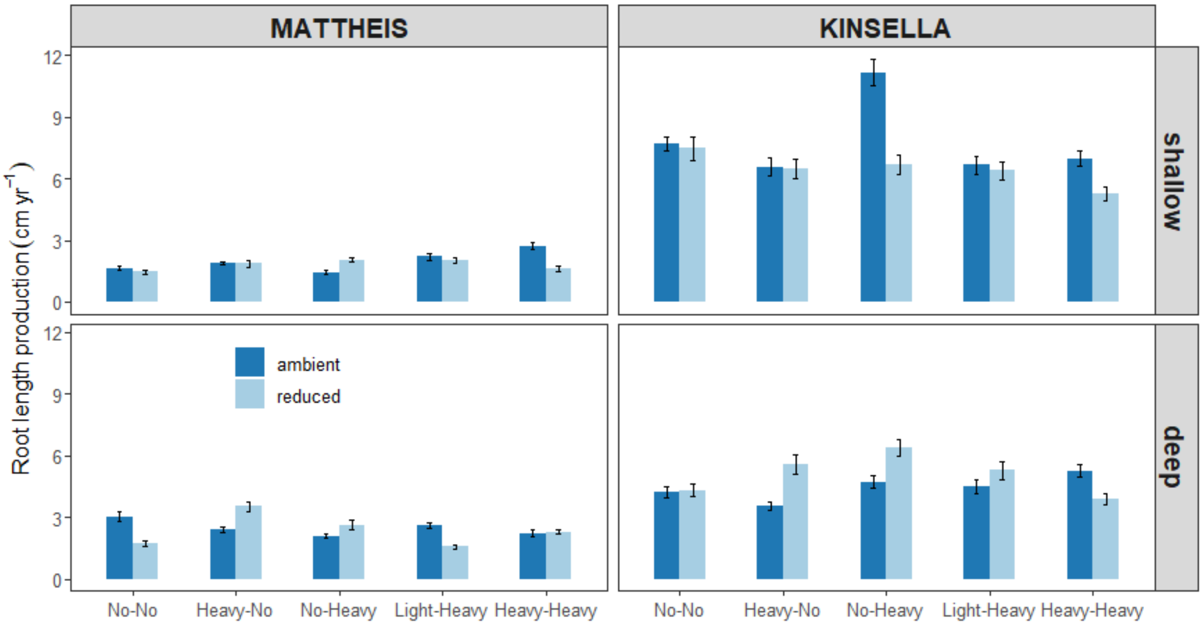
Appendix 4-S3. Site level responses of aboveground net primary productivity (ANPP, a) and standing root biomass (b) to 15 cm depth of soil to the combined effects of drought and defoliation in the final year of the experiment. Dots are replicates of treatment combinations, while star (*) indicates the mean of treatments. The non-defoliated control treatment (No-No) represents drought effects in the absence of defoliation. The Heavy-No (early) and No-Heavy (late) treatments represent the variation in the timing of defoliation, whereas the No-Heavy (none), Light-Heavy (light), and Heavy-Heavy (heavy) treatments indicate the varying early-season defoliation intensity, respectively.



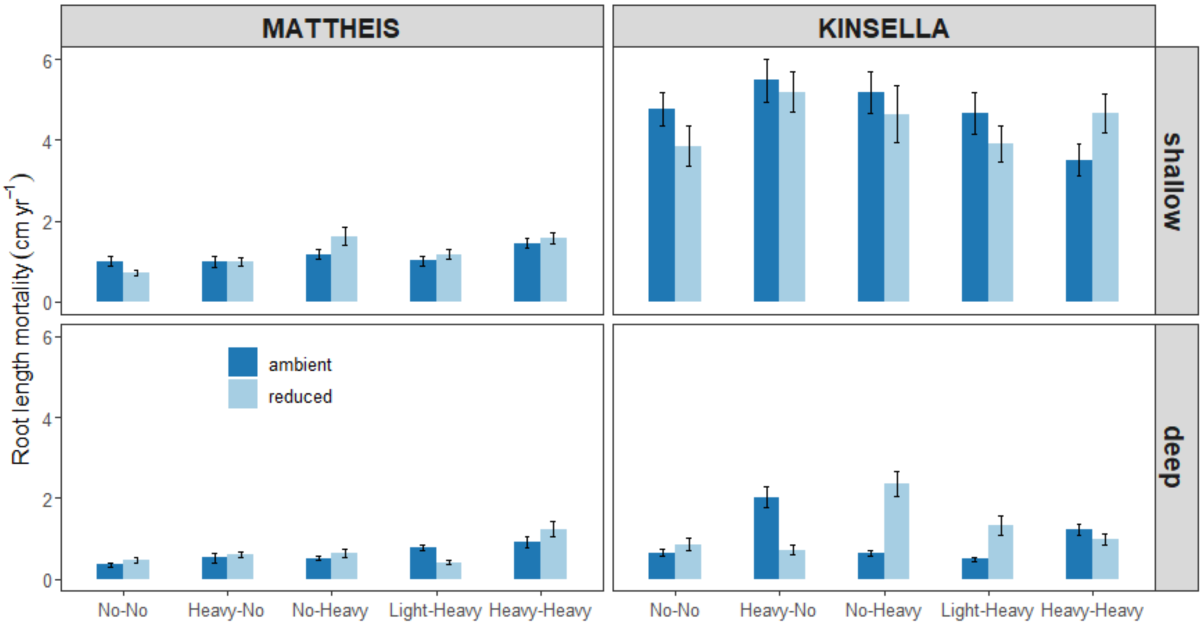
Appendix 4-S4. Aboveground biomass as a function of standing root biomass across all non-defoliated treatments. The slope of the relationship is a proxy measure of root system efficiency, i.e., aboveground biomass produced per unit of root biomass (as per Ma et al. 2020). Lines show the relationship under ambient and reduced precipitation. Each point represents a plot (n=59). A single outlier was removed from the ambient precipitation treatment.



Appendix 5-S1. Interactive effects of drought, defoliation, and soil depth on the mean annual total root length at the individual site across years (2017-2019). Error bars represent ± 1 SE (n = 15).



Appendix 5-S2. Interactive effects of drought, defoliation, and soil depth on the mean annual root length production at the individual site across years (2017-2019). Error bars represent ± 1 SE (n = 15).



Appendix 5-S3. Interactive effects of drought, defoliation, and soil depth on the mean annual root length mortality at the individual site across years (2017-2019). Error bars represent ± 1 SE (n = 15).