ERA FINAL REPORT

Project:
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Identification of regionally appropriate grazing systems for the reduction of greenhouse gasses in Alberta, now and in the future

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EXECUTIVE SUMMARY

The Canadian prairies store large amounts of carbon in grassland soils and have the potential to sequester more carbon and reduce atmospheric greenhouse gases (GHG). These ecosystems are also broadly used for cattle grazing. However, climate change threatens the economic stability of cow-calf production through increased drought risk that reduces forage availability and ultimately profitability, and conversion of grasslands to cropland will release large amounts of stored carbon to the atmosphere. Thus, cattle management that manipulates grassland ecosystems in a way that increases soil carbon will be beneficial for reduction of GHG, but new management must also increase the stability of forage production to ensure cow-calf operations are profitable. Thus, the purpose of this project was to examine whether different grazing systems, tested as different timing and intensity of plant defoliation, are beneficial for GHG reduction and forage production under both ambient and drought conditions at different locations within Alberta's grasslands.

To do this we established an experiment at seven grassland locations in Alberta that represented different dominant vegetation types, ranging from dry to wet grasslands. At each location we established a replicated experiment in which we altered the rainfall landing on experimental plots, and simulated grazing by clipping the vegetation. We imposed an extreme drought using rainout shelters – an established design for conducting drought studies that simply intercepts rainfall and diverts it off site. We had five different clipping treatments including no-clipping, clipped in fall only, clipped heavily in spring and fall, clipped lightly in spring and heavily in fall and a spring only clipping. Clipping was done by hand or lawn mower. Experimental sites were established in 2016 and treatments were applied through to the end of summer in 2019. Many components of the ecosystem were measured including: plant biomass, plant community composition, root growth and mass, greenhouse gas flux at the soil surface, soil microbial community activity, soil carbon content and normalized difference vegetation index (NDVI, a measure of plant stress).

We found that most responses were site specific although a few general trends emerged. As expected, drought and more intense clipping generally reduced plant biomass. Wetter grasslands had a larger reduction in plant biomass production compared due to drought than dry sites; however, dryer sites were more sensitive to drought when defoliated. When examining patterns among response variable there were few correlations, meaning that assumptions about matched response (e.g. plant biomass and soil carbon responding similarly) need to be made with caution. We found that GHG emissions from soils were differentially affected by one clipping treatment, the treatment where vegetation was clipped lightly in spring and heavily in fall increased carbon dioxide emission under drought conditions while all other clipping treatments reduced emissions, this effect was more pronounced at drier sites compared to wetter sites. Similar to the plant responses, we found that microbial activity was idiosyncratic across sites but was largely resistant to change. One site, located in the aspen parkland, was very responsive to the treatments and the underlying reason for this has not yet been determined. Enzymes that did respond tended to be associated with the breakdown of carbon compounds and generally were reduced by the drought treatment, and had a positive relationship with soil moisture.

This project has demonstrated that Alberta's grasslands are largely resilient to change, but that under some circumstances regions may be susceptible to variation in grazing management, particularly under drought that will lead to the potential loss or slower accumulation of soil carbon. In particular, further investigation grazing management in the drier regions of the province, in collaboration with producers using a variety of grazing management, could identify specific management practices to improve carbon sequestration and enhance cow-calf production.

PROJECT DESCRIPTION

INTRODUCTION AND BACKGROUND

Grasslands are important sinks for carbon and can help reduce atmospheric greenhouse gases which offsets climate change. At the same time, these ecosystems are broadly used for grazing livestock. Thus, given public desire for agricultural systems that reduce GHG and the potential for carbon offsets, the identification of grazing practices to enhance soil carbon would be beneficial for the publics and cow/calf producers. Meanwhile, the Canadian prairies have experienced the driest and wettest years on record in the past decades and more extreme weather is predicted. Changes in rainfall affect forage production and can reduce cow/calf profitability, but previous research has shown that some grasslands are more sensitive to drought than others (Carlyle 2014; White 2012, 2013). Different grazing systems have been proposed to help increase carbon storage and minimize forage loss, but these processes need to be evaluated under conditions of increased drought in order to provide more reliable predictions for future efficacy.

Rainfall is the primary driver of grassland productivity (Sala 1984) and thus carbon capture, but rainfall patterns are changing across Alberta and more extreme droughts are predicted (Solomon 2007). These changes create uncertainty around the biological processeses that regulate carbon storage, and the profitability of cow-calf operations which if converted to cropland are expected to lose as much as half of their soil carbon. Studies in Western Canada have shown that some grasslands are sensitive to the combined effects of drought and grazing while others are not, but the reason for this variation is not understood (White 2013, Carlyle 2014). The best grazing management system (i.e. season-long or rotational grazing) for these systems is highly debated (Briske 2008). One study in Alberta indicates that the optimal grazing system depends on rainfall, vegetation and the timing of defoliation (Broadbent et al. 2019), but these patterns have not been examined at a large scale.

Furthermore, precipitation and grazing can interact to affect carbon sequestration and this effect is likely to change along environmental gradients (Derner 2007). We have shown that grazing can increase soil carbon storage (Hewins et al. 2018, Bork et al. 2020) and that these effects may be linked to grazing induced changes in the plant community (Bork et al. 2019). However, a mechanistic understanding of how grazing, and drought, drive carbon and nutrient cycling in these systems is incomplete so we have examined the effects of these factors on extracellular enzymes in soils and GHG flux out of soils. Extracellular enzymes are sensitive to both changes in plant vegetation due to grazing (Chuan et al. 2020), the effects of defoliation itself and changes in water availability (Hewins et al. 2016), changes in EEA are important as they are the proximate driver of the breakdown of dead plant material in ecosystems.

Of particular importance for minimizing the impact of drought and regulating soil carbon are plant roots. The link between increasing grazing intensity and less root mass is known, but we will provide new knowledge by linking root growth to above ground production under drought and different grazing systems. The use of root cameras enables regular monitoring of root growth in experimental conditions and can relate production to carbon storage (MacDougall 2011) and relate specific root properties, such as their length to ecosystem change (Ma et al. 2020). This data will allow us to create a measure of root health, and while measurements of roots are impractical for producers we will then link this data to above ground plant measurements that are accessible to producers such as normalized difference vegetation index (NDVI), which can indicate plant vigor and productivity (Paruello 1997). By linking above and below ground processes, we will develop a better understanding of forage and GHG responses to the effects of reduced rainfall and defoliation.

ORIGINAL PROJECT OBJECTIVES FROM AGREEMENT:

The project goal is to use an experimental approach to understand the role of grazing systems in maximizing carbon storage and minimizing GHG emissions from Alberta’s grasslands. The recommendations from this research will aid producers in identifying the grazing system that is best suited for the landscape in which they operate and define management practices that will help them to increase soil carbon and reduce GHG. Specifically, the following questions will be addressed:

1. What are the resulting changes in GHG and soil organic carbon (SOC) due to changes in grazing management?
2. Are the effects of management intensive grazing (MIG) on SOC and GHG emissions universal across all grassland types? If not, can best management practices be developed at regional level? And, what are the manageable environmental factors that determine GHG flux?
3. The success of MIG is dependent on plant growth which is typically related to precipitation; so, does reduced rainfall alter the balance of GHG emissions? Are these processes likely to be a significant carbon sink in the future when droughts are expected to be more common?
4. What are the controls (e.g. environment, grazing, microbial community) on GHG flux from these systems?

With this information we can inform the appropriate management strategies for the reduction of GHG with grazing in Alberta’s grasslands.

PROJECT OUTCOMES AND LEARNINGS (INCLUDING, AS APPROPRIATE)

OVERALL PROJECT ACHIEVEMENTS

The project successfully established and completed a 4-year (2016-2019) field study that experimentally manipulated drought and grazing in small plots at 7 locations through out Alberta’s grasslands. In doing so we achieved a number of goals and the research taught us much about the ecosystem.

Summary of practical outcomes:

Employment and training: Two PhD students were directly trained in the completion of this project, through separate funding two MSc students additionally used the experimental infrastructure for other studies. During the course of the project one research associate assisted and 23 undergraduates worked on the project in temporary full-time or part-time positions assisting with lab and field work.

Communication: The project was communicated through 1 published journal publication and 6 more are in preparation, 2 PhD theses and 2 MSc theses, 14 presentations to industry, 4 scientific presentations and 2 interviews published in trade magazines (full listing of all communications are provided later in this report).

Summary of research outcomes:

- The rainout shelter treatments were effective at reducing soil moisture, and during the period of the experiments, when considering ambient rainfall, we were able to induce an extreme droughts at the study sites.
- A challenging outcome is that many responses were site specific and could not be related to broader environmental factors, which would support more general findings. However, there were a number of general findings that are highlighted in the bullets that follow.
- Plant communities were largely resistant to both drought and grazing, although both treatments reduced forage production. Counter intuitively, drought led to increased above ground plant biomass but often had the opposite effect on roots which highlights the need to consider the response of the entire plant.
- Plant biomass was affected by defoliation but the timing and intensity of defoliation mattered less than just the presence of defoliation.
- Arid sites tended to be more responsive to treatments than mesic sites
- Soil C did not respond to treatments, this is not surprising given the short-term nature of the study and the known slow response of this variable. However, indicators of carbon turnover did indicate responses, e.g., extracellular enzymes used by soil microbes and plants to break down carbon was altered by defoliation and drought
- A light spring defoliation, followed by a heavy fall defoliation led to increased CO2 flux under drought conditions – especially at more arid sites. Other studies have suggested that similar grazing patterns increase plant growth which may explain the increased respiration. This pattern requires further investigation and is likely associated with increased plant growth meaning that that net effect on soil C is not clear.
- Soil CO2 flux was controlled primarily by enzyme activity, soil temperature and to some degree soil moisture.
- Other GHG, methane and nitrous oxide, did not vary in response to treatments.
- Soil microbial communities were reduced by drought treatments and affected by the various defoliation treatments.
- NDVI was not responsive to treatments, this requires some further investigation as it was not expected.

EXPERIMENTAL PROCEDURES/METHODOLOGY

Study sites: The experiment was conducted at seven grassland locations across a climate gradient in Alberta, Canada: Mattheis, Onefour, Twin-river, Oyen, Kinsella, Sangudo and Stavely (Table 1). These sites represent multiple ecoregions including the parkland, foothills and mixed dry grassland (Downing and Pettapiece 2006). All sites were composed of native perennial grasses and forbs, with a moderate shrub component in Stavely. Grazing was excluded from all sites prior to initiation of the experiment, but all were moderately grazed historically.

Table 1: Description of the seven study sites used in the project. Soil characteristics are the mean from 5 samples collected at the site in 2016. Mean annual precipitation and temperature are from Environment Canada.

Site	Mean Annual Precipitation (mm)	Soil texture	pH (1:2)	Soil bulk density (g/cm3)	Mean Annual Temperature (°C)	Dominant vegetation
Kinsella	400-500	Clay loam	5.73	1.47	2.26	*Poa pratensis, Agropyron dasystachyum, Pascopyrum smithii*
Sangudo	500-600	Loam	6.71	1.49	2.28	*Elymus repens, Trifolium repens*
Stavely	450-550	Silty clay loam	5.96	0.93	3.91	*Festuca campestris, Danthonia parryi, Poa pratensis*
Oyen	300-350	Loam	5.57	1.39	3.18	*Agropyron dasystachyum, Avenula hookeri, Hesperostipa curtiseta*
Twinriver	350-400	Clay loam	6.51	1.33	5.12	*Agropyron dasystachyum, Artemisia frigida, Festuca idahoensis*
Mattheis	350-400	Sandy loam	6.21	1.94	4.45	*Hesperostipa comate, Artemisia frigida, Koeleria macrantha*
Onefour	300-350	Clay loam	6.47	1.82	4.99	*Agropyron dasystachyum, Hesperostipa comate, Artemisia frigida*

Experimental Design: The experiment at all sites was designed in a five by two full factorial design with five defoliation treatments to simulate grazing and two levels of drought treatments (Figure 1). Rainfall was reduced by 45% using rainout shelters (Gherardi and Sala, 2013) in the drought plots, and the other plots experienced ambient conditions. We determined that a reduction of 45% was sufficient to create an extreme reduction in precipitation (1st percentile based on historical records) and well below what would be within the normal range of precipitation based on the coefficient of variation at each site (Table 2). Defoliation treatments were applied by manual clipping and/or lawnmower with two levels of intensity and frequency combinations: heavy-heavy, light-heavy, heavy-none, none-heavy and none-none. Heavy-heavy and light-heavy plots were clipped two times a year (June and September) to a 7-7 and 3-7 cm stubble height, respectively. Heavy-none plots were clipped once a year (in June only) at 3 cm height, and none-heavy plots were clipped once a year (in September only) at 3 cm height, the none-none were never clipped and the twice clipped plots were clipped once in June and once in September. In total there were 300 plots, two sites Mattheis and Kinsella, each had 5 replicates, while the other sites each had 4 replicates. Generally, vegetation was sampled from all plots, while GHG and soil sampling was typically only done in 3 replicates, which was assigned randomly. The rainoutshelters were first installed in June 2016, they were taken down for the winter months then re-constructed for each subsequent year and in place from May through October. Baseline sampling was done in 2016 and vegetation sampling continued until 2019, while GHG sampling ended in 2018.

Table 2: Description and comparison of historic rainfall data derived from weather stations and modelling tools. Also included is an estimated reduction in order to achieve a 1 in 100 year low rainfall event.

Site name	MAP (actual)	CV precip (actual)	MAP (generated)	CV precip (generated)	r2 MAPa vs. MAPg	1% quantile MAP (generated)	% reduction to get 1% from generated
Onefour	328.33	0.32	318.89	0.25	0.88	180.00	43.55
Stavely	394.16	0.44	429.38	0.20	0.10	239.00	44.34
Lethbridge	406.57	0.26	378.21	0.25	0.63	191.00	49.50
Sangudo	497.71	0.19	519.52	0.15	0.40	312.00	39.94
Oyen	311.76	0.23	326.93	0.20	0.22	188.00	42.50
Kinsella	407.89	0.22	440.64	0.17	0.42	314.00	28.74
Mattheis	329.42	0.22	322.68	0.23	0.55	176.00	45.46

Notes:

- Site “Lethbridge” was used in this determination as it was intended to be one of our study sites, but ended up not being included in the final site selection.
- Online data sources were retrieved in February 2016.
- MAP (actual) – avg of data from weather stations. Onefour, stavely, Lethbridge data from Ag Canada employees, others from http://agriculture.alberta.ca/acis/township-data-viewer.jsp
- CV precip (actual) – std dev/ avg of MAP in each site using actual (weather station) values
- MAP (generated) - calculated using Precipitation Trends Tool: http://wp.natsci.colostate.edu/droughtnet/global-precipitation-information-resource/
- CV precip (generated) – std dev/ avg of MAP in each site using generated values
- R2 MAPa vs. MAPg – r2 calculated in excel
- 1% quartile MAP (generated) – generated data entered into the Precipitation Manipulation Tool which calculates 1% quantile values% reduction to get 1% from generated – calculated as: 100 – ((1% MAP / MAP generated) * 100)

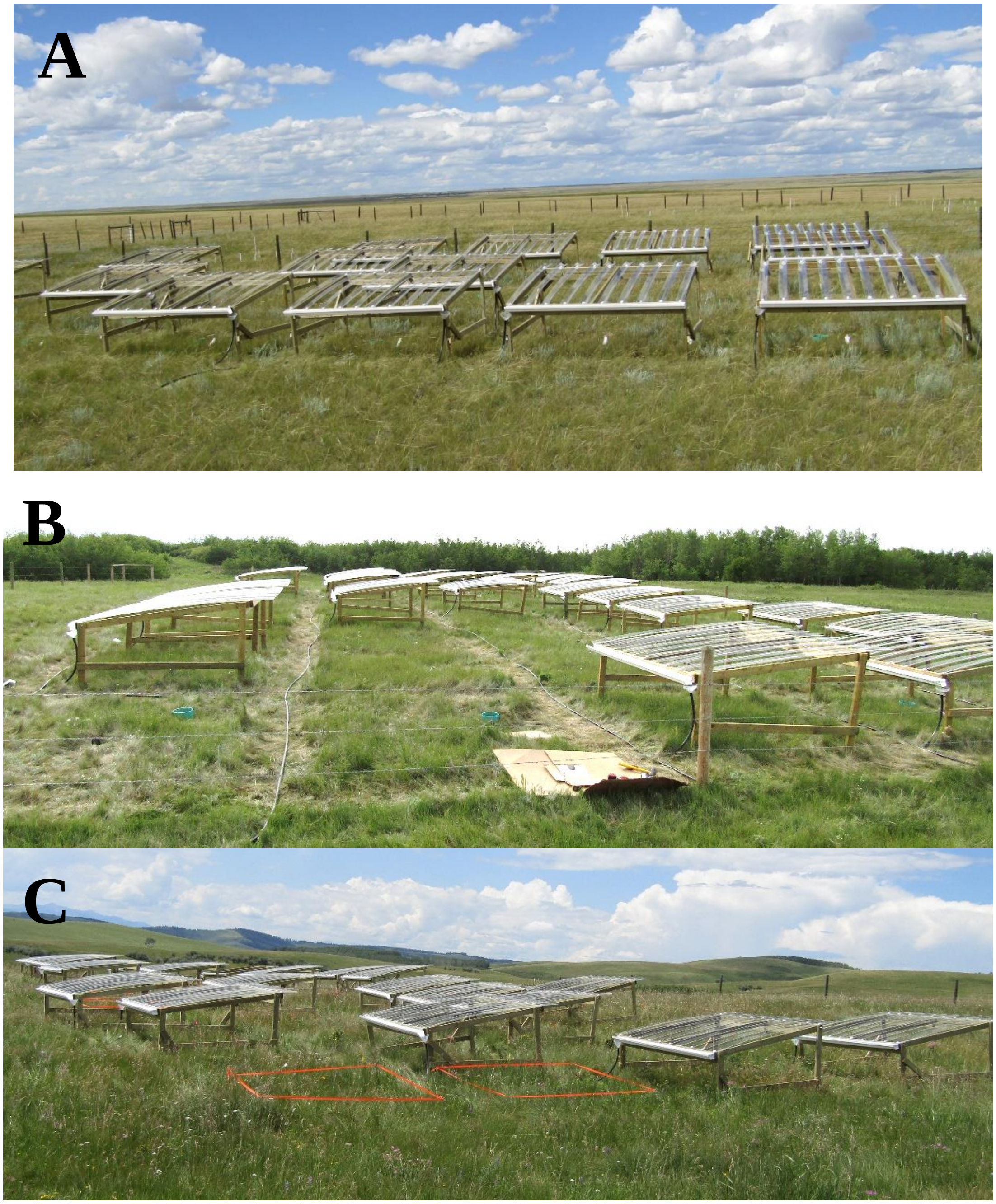


Figure 1: Photos of three of the seven study sites, a) Onefour, b) Mattheis and c) Stavely. Structures are the rainout shelters that reduced ambient precipitation by 45%.

Soil moisture and temperature: Data loggers were used to measure soil moisture and temperature through the duration of the study in order to assess the micro-climatic effects of the treatments. Sensors were placed into every single plot at 2 sites (Mattheis and Kinsella) and into the unclipped drought and ambient plots at the remaining 5 sites.

Vegetation Sampling: Baseline vegetation data was collected once in late July in 2016, then once a month from May to September in 2017, 2018, 2019, we measured community composition, light availability, and soil moisture/temperature at the center of each plot (50 x 50 cm) and rainfall at the site level. Above- and belowground biomass was harvested once during peak biomass every year. Species that comprised 80% abundance (Tecco et al. 2013) at each site were selected for trait sampling. For each species, a wide range of traits (e.g. height, specific leaf area, specific root length) were measured and collected from individuals outside the experimental plots in the summer of 2018. Community composition, species diversity (e.g. richness and evenness), and changes in response variables will be quantified as the variation from controls.

Root measurements: In the summer of 2016 at each site minirhizotron tubes (90 cm long x 5.4 cm inside diameter) were installed at the center of each plot at approximately a 30° angle to the soil surface, to a depth of 30 cm. Tubes were capped except during measurement periods. To track changes in root growth, root images were captured with a minirhizotron camera at 1.35 cm intervals along each tube every four weeks from May to September in 2017 and 2018. Additionally, two root cores were taken from each plot during peak biomass to estimate belowground biomass. *Root Tracing*: Reference images were taken from each tube in September 2016, 3 months after tube installation, which are used as a baseline when digitizing roots. Root length, diameter, condition (white or brown) and status (live vs. disappeared) of all roots that are not present in reference images but only present in digitizing images were recorded using WinRHIZO Tron.

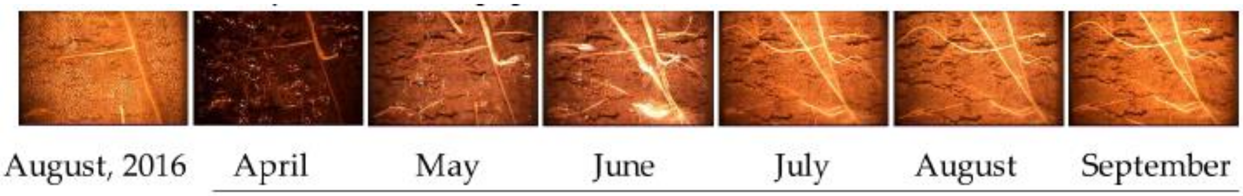


Figure 2:Example images from root imaging with the rhizotron camer. The series of images above are taken at the same at different times.

Soil sampling for C, N and general properties: In 2016, 2017, 2018 and 2019, four 15 cm deep soil samples were collected from every plot, pooled, sieved (2 mm) and dried for C and N analysis. Additionally, 5 10 cm cores were collected from each site to estimate bulk density and soil texture (methodology adapted from Blake 1986). A subsample of soil was ground to a fine powered using a ball mill then analyzed with a LECO elemental analyzer for total carbon and nitrogen content, a soil standard was run ever 10 samples.

Soil sampling and processing for EEA and Microbial Analyses: Soil samples were collected using a 3.25 cm diameter soil corer at 0-15 cm depth from each site in May, June and August of 2017 and 2018. From each plot, five samples were randomly collected and were composited at each sampling. Samples were immediately placed in cooler with dry ice and were kept frozen during transport and then were stored at -20 C until further analysis. All samples were sieved (2 mm) to remove coarse fragments and roots. Subsamples were taken from each sample to measure the gravimetric soil water content and soil pH. Gravimetric soil water content was determined by drying 40 g of soil at 100 C for 48 hours. Soil pH was determined using a 1:5 soil:deionized water suspension (Rayment and Higginson, 1992). Additionally, at the initiation of the experiment, 5 separate soil samples were collected from a depth of 0-15 cm at each site in order to describe site-level soil characteristics such as bulk density and soil texture.

Extra-cellular enzyme analysis: The following five EEAs were measured: β-glucosidase (BG), β-Cellobiosidase (CELLO) and β-xylosidase (XYLO), acid phosphatase (AP) and N-acetyl- β-glucosaminidase (NAG) (Saiya-Cork et al. 2002) (See Table 3 for a brief functional description of each). Additionally, we calculated the geometric mean of the three enzymes related to carbon cycling (BG, CELLW and XYLO) in order to obtain a gross "carbon enzyme" activity (Chuan et al. 2020). Briefly, 1 g of soil was buffered in 50 mM using sodium acetate buffer solution. The pH of the buffer solution was adjusted to the field pH of the sample using acetic acid. The enzyme activity was measured in 96-well Costar black polystyrene microplates (Corning Inc., NY, USA). After the addition of substrate, the microplates were incubated in the dark at room temperature for 4 hours. Microplates were read using a SpectraMax M3 microplate reader (Molecular Devices LLC., Sunnyvale, CA, USA) at 365 nm excitation and 450 nm emission. Final enzyme activities were calculated in nmol per gram of dry soil per hour using the equation by Saiya-Cork et al. (2002).

Table 3: Extracellular enzymes measured in this study and a brief functional description

Enzyme	Function in soil
β-Glucosidase (BG)	Cellulose degradation
β-N-acetylglucosaminidase (NAG)	Chitin degradation
Cellobiohydrolase (CELLO)	Cellulose degradation
β-1, 4-xylosidase (Xylo)	Carbon cycling: Hydrolyses xylobiose releasing xylose monomers
Acid phosphatase (Phos)	Phosphorous cycling: Releases inorganic P from phosphate esters

Phosphorous cycling: Relea

Soil greenhouse gas flux: Soil GHG emissions were measured from April to September (twice a month in April and May and once a month afterward), during 2017 and 2018 using static chambers (PVC pipes) (0.008 m^3). Gas chambers were driven at least 6 cm into the soil. The lid of the chamber was closed with a Plexiglas on the top and covered with aluminum foil tape to reflect light and maintain the ambient air temperature inside the chamber during gas sample collection. Gas samples were collected at 0, 10, 20 and 30 minutes interval using 20 mL syringe and stored in 12 mL pre-evacuated glass exetainers (Labco Ltd., Lampeter, UK). The air temperature inside the chamber during gas sampling was recorded with a temperature meter (HH806AU, Omega Engineering, CT, USA). Soil volumetric moisture content and soil temperature was also recorded. The concentration of the gases (CO2, CH4, and N2O) was determined on a gas chromatograph (Varian CP-3800, Varian Canada, Mississauga, Canada) equipped with a thermal conductivity detector, a flame ionization detector, and an electron capture detector. (Pedersen, 2011).

NDVI: Normalized difference vegetation index is a measure of plant "greenness" and can be used to indicate plant productivity or stress. We measured NDVI once per month at the same time that root imaging was done. Measurement were taken using a spectroradiometer (Apogee Instruments PS-200) with and up and down facing light meter in order to account for incoming solar radiation. Measurements were calibrated regularly in the field using a reflection standard

(Teflon). NDVI was calculated using the formula and wavelength given in Wang et al. (2016a, b).

Statistical Analyses: We used a variety of statistical approaches to analyze the data. Mixed-models were used to compare treatment effects across either site (a categorical variable) with site as the random effect. If instead, the intent was to compare across sites we simply included site as a factor in the analysis, and not as a random effect. To account for site effects, we used either "site" as a categorical variable, or we used precipitation level as a continuous variable. For responses that are likely the result of long-term site characteristics, such as vegetation responses, we used the mean annual precipitation of the site calculated over the past 30 years. If instead we were interested in more responsive variables, such as EEA, we used precipitation levels from the same year that the data was collected. Regardless, in all figures data is arranged to present the sites from driest to wettest site. There are differences in site rankings that results from this approach, for example Mattheis has the lowest long-term average, but in the years of our study Onefour had the lowest levels of precipitation. In these analyses, plot was treated as a random effect if multiple samples were analyzed through time. Owing to the large variation among sites we also compared the effects of clipping treatments on the effect size of the drought (calculated as the log-response ratio (lnRR =ln (ambient – drought)). We used ordination (NMDS) paired with permutational analysis of variance to examine multivariate responses of plant communities and suites of EEA. Data was transformed where appropriate, but we have presented untransformed data in all figures to ease interpretation. Significance was assessed at an alpha value of 0.05. All analyses were completed in R (R Core Team 2020).

ADDITIONAL RESEARCH OBJECTIVES:

This project enabled three smaller projects, for which we have reported on here as they are highly relevant.

Effect of grazing on forage sensitivity to changes in precipitation. We used long-term (14 - 28 years) precipitation and ANPP 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. Data was analyzed with a mixed model.

Biological Soil Crusts: Biological soil crusts, primarily lichen, *Selaginella densa,* and bryophytes (mosses) were quantified in all study plots in 2016 and then again in 2019. Species identification is challenging, but all samples were identified by a Lichenologist at the Royal Alberta Museum. Analyses were challenging due to high variability in initial cover of lichens across study plots and sites, thus we used the measures collected in 2016 as covariates in our analyses of 2019 data, which was done using generalized linear models and mixed models. We evaluated the response of biological soil crusts as individual species and multivariate analyses were done with ordination (NMDS). Additionally, the funding that supported this research supported work to resolve taxonomic uncertainty within a group of common grassland lichens – the details of this methodology are not included here, but preliminary results will be provided as it provides insight on the diversity of this important group of understudied organisms.

Forage quality response to drought and defoliation: Forage quality was measured as plant tissue nitrogen concentration in 2017 and 2019. These data are still being analyzed; however, some preliminary results are available. Nitrogen content was measured with a FOSS NIR forage analyzer calibrated with a curve generated for mixed grassland samples. Samples for these analyses were collected only at Mattheis and Kinsella at peak biomass (July).

RESULTS OF STUDY

Status of the technology risks at the end of the project (both retired risks and risks to be retired)

Soil organic carbon: Soil organic carbon was not affected by treatments in terms of C concentration or the change in the amount of carbon in the soil through the duration of the experiment (p>0.1). This result is not surprising given the relatively short time frame and that it is well know that carbon accumulates slowly in grassland systems. However, we anticipated this response and additionally measured other indicators of soil change processes such as plant production, including below ground root growth and turnover, microbial enzyme activity, the microbial community and greenhouse gas flux from the soil.

Efficacy of drought treatments: In order to put the treatments into context we measured soil moisture through time and also examined the annual ambient precipitation in order to quantify the nature of the drought we created with the rainout shelters. Plots receiving the drought treatment had on average lower soil moisture level at all sites in all three years of treatment; although there was variation in the degree of treatment effect and occasions when the drought plots had greater soil moisture than the ambient plots (Figure 3). Likely this is the result of complex interactions between temperature, plant and litter shading of the soil and plant transpiration rates that all contribute to the overall water balance. Additionally, despite year to year, and across site variation in precipitation levels, both growing season and annual precipitation were reduced in the drought treatments at all sites and in all years well below historical averages (Figure 4). So, we are confident that we have achieved reductions in water availability over the course of three years that would represent a severe water deficit for all of these grasslands; despite the year-to-year variation in the magnitude of this effect.

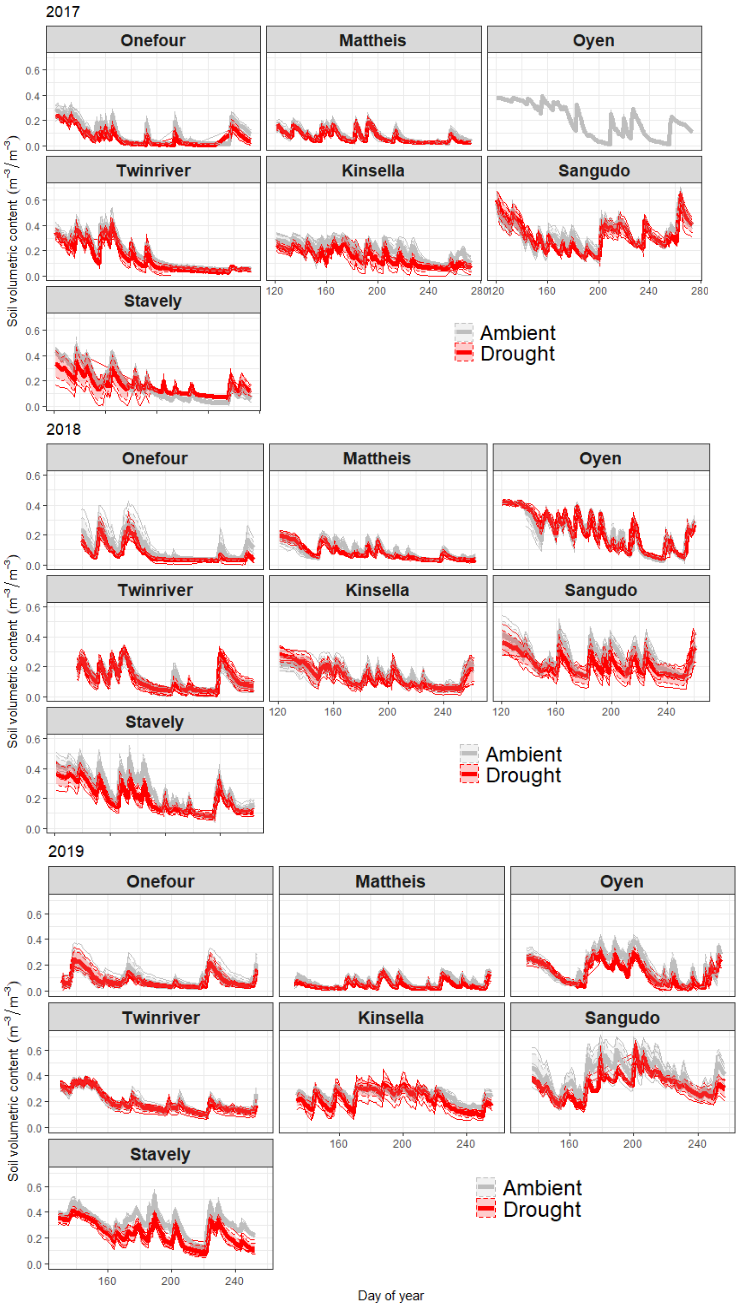


Figure 3: Soil volumentric moisture content measured through time at all study sites for 2017, 2018 and 2019, indicating that generally drought treatments had lower soil moisture content than ambient plots.

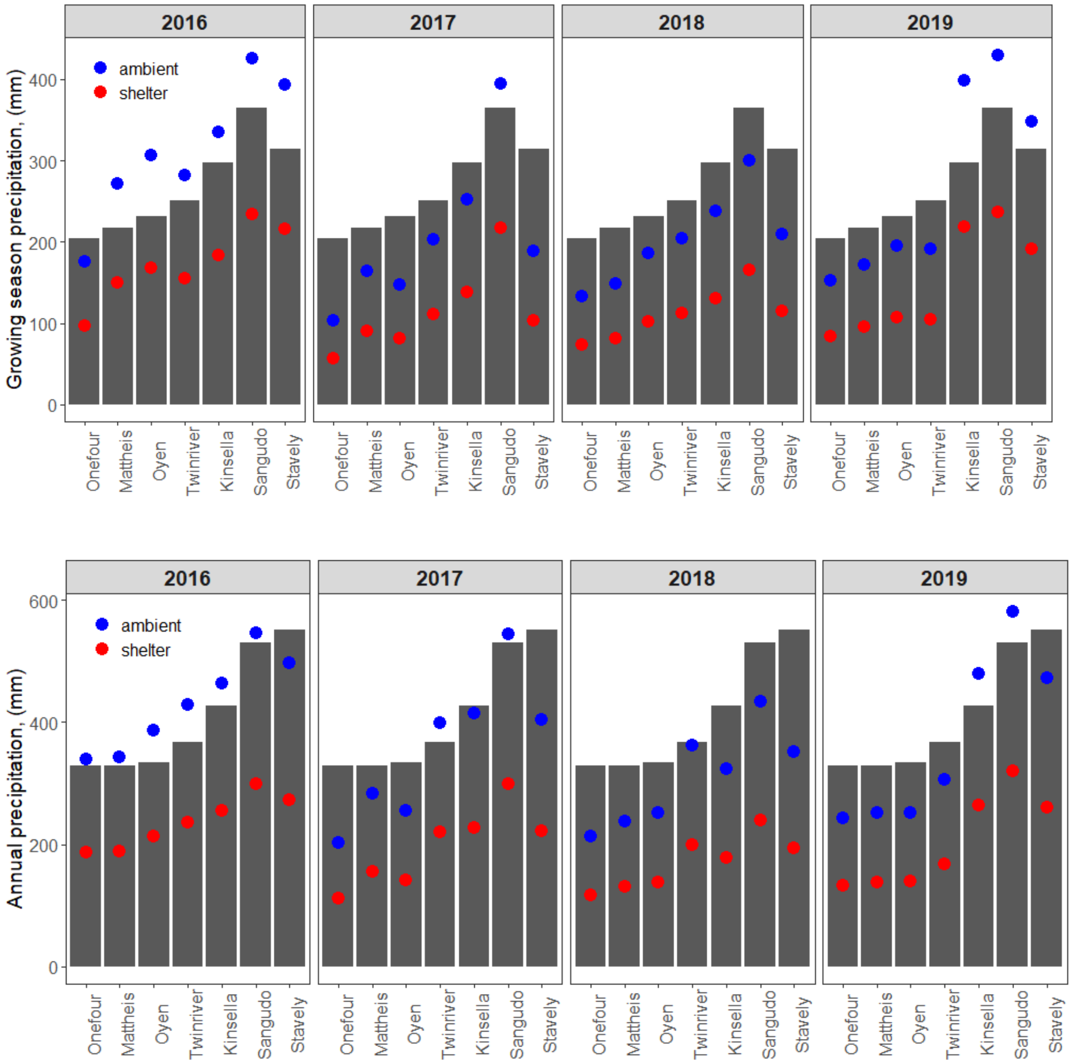


Figure 4: Growing season precipitation (top) and annual precipitation (bottom) of each site during the duration of the study. Shaded bars are the long-term mean annual precipitation for the last 100 years. The blue and red dots are the precipitation received by ambient and drought plots, respectively.

Response of Vegetation: The assembly of plant communities is often determined by multiple interacting abiotic and biotic filters (HilleRisLambers et al. 2012), including precipitation and grazing in grassland ecosystems (Milchunas et al. 1988, Sala et al. 1988, Milchunas et al. 1993). Predicting changes in plant community composition and ecosystem functioning in response to these factors, and the management challenges associated with them, has been a focus of research for ecologists and rangeland managers (Breshears et al. 2016). The need to better understand the interactive effects of shifting precipitation patterns and different grazing systems is paramount in most grasslands due to projected increase in the frequency and intensity of extreme climatic events, such as drought (Smith 2011), which will likely result in broad-scale shifts in land use (Foley et al. 2005, Knapp et al. 2008). Thus, the magnitude and direction of changes in grasslands may not be predicted from single factor studies as natural systems are almost always simultaneously subjected to multiple stressors

(Vinebrooke ta al. 2004, Crain et al. 2008). Furthermore, the effects of water availability and grazing on plant community composition and productivity in grasslands have been relatively well studied (Milchunas et al.1988, 1993, McNaughton 1985, Fleischner 1994), but their effects are often viewed as independent, neglecting potential interactions. 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. 2014, Broadbent et al 2016), suggesting an incomplete understanding of ecosystem responses to drought under different land use practices. Furthermore, the underlying mechanisms for these variable results remain poorly understood (White et al. 2012). Thus, there is a need to understand the relationship of grazing, drought, and their interaction on ecosystem function (Breshears et al. 2016)

Forage Production: We found that forage responses to drought and grazing were idiosyncratic across sites, but some generalities did emerge. Overall, drought and more intense clipping often lead to lower plant biomass in all three years of treatments ($P < 0.05$; Figure 5). Furthermore, we found that wetter sites were generally more sensitive to the effects of drought than were drier sites for most responses. However, the correlation among the response of these ecosystem properties was generally inconsistent with few positive or negative correlations among the variables' responses (Figure 6). There is no consistent ecosystem process that associated with responses of other processes (Figure 6), suggesting that responses of ecosystem processes to drought are independent from each other. In other words, drought-induced changes in one ecosystem process may not result in changes to other ecosystem process.

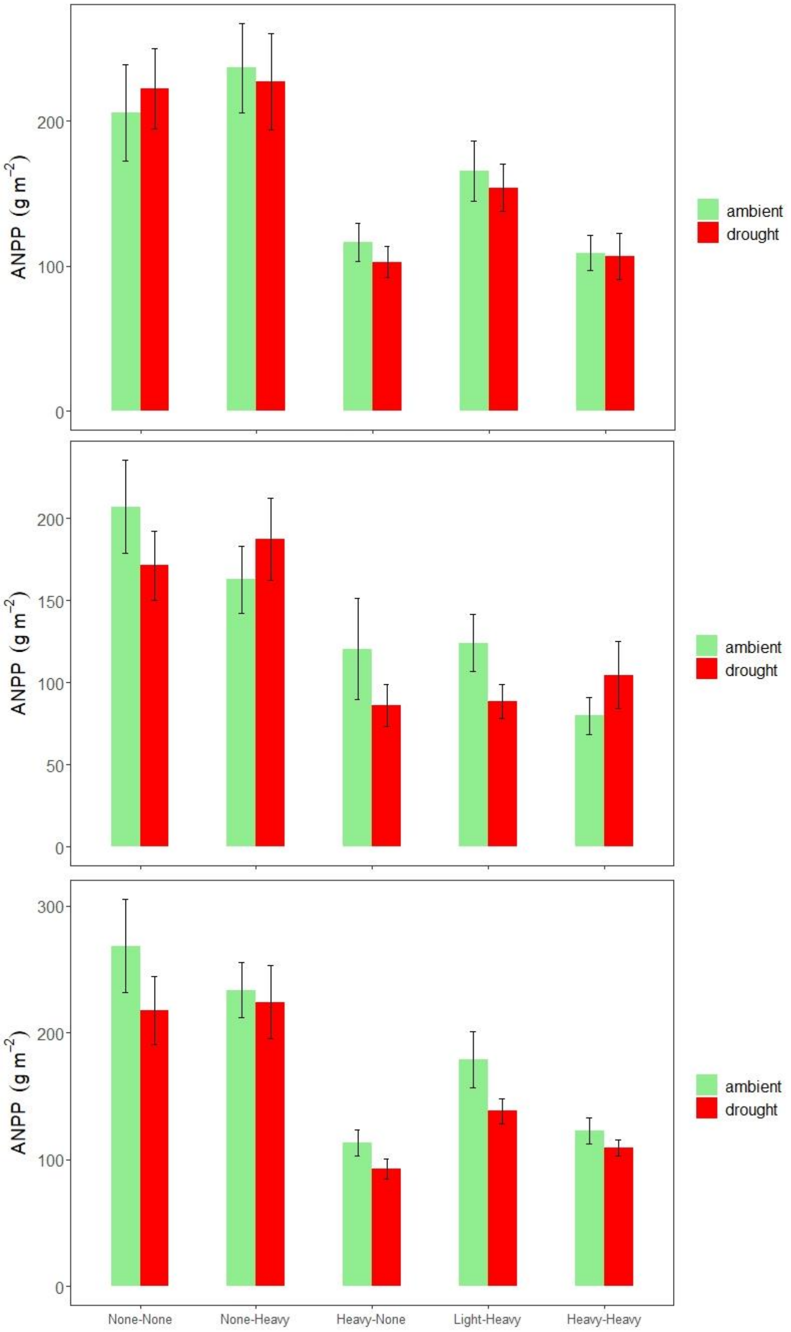


Figure 5: Mean annual biomass production average across all seven study sites in 2017, 2018, and 2019. Biomass was collected at peak production, mid-July of each year.

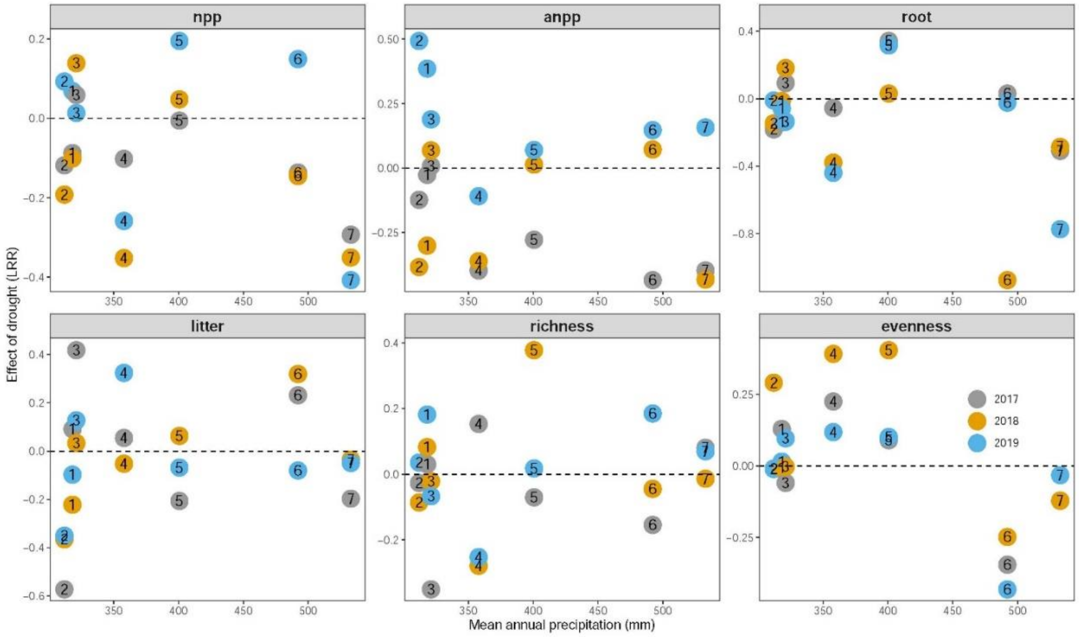


Figure 6: Response ratio (drought plots: ambient plots) of ecosystem properties measured at each site. Numbers represent the different sites, with 1 being the driest and 7 being the wettest site. Points further below the line indicate a greater negative effect of drought. Different colours represent different years.

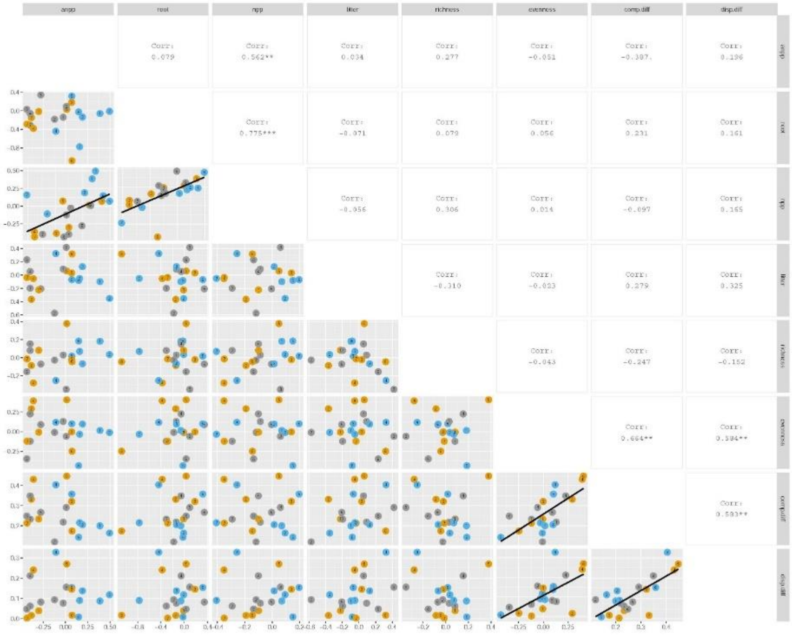


Figure 7: Correlation matrix of the sensitivities of different ecosystem properties to drought. Plots with solid lines overlaid indicate a significant correlation between measurements in their response to drought. Generally, there were few correlations indicating that ecosystem properties are not equally sensitive to changes induced by drought.

Plant species composition: Drought altered species composition, especially at wetter sites which shifted in similarity toward the more xeric sites (Figure 8a). However, the magnitude and direction of drought impact varied with years and sites, again highlighting the individualistic response of sites (Figure 8b) in which for some sites drought made plant communities less similar, while at other sites it made communities more similar. Univariate measures of species composition were not affected (Figure 8, p>0.05)), which is not surprising given the relatively low species richness contained within plant communities of the Northern Great Plains. The composition and relative abundance of species within grassland communities are the direct result of filtering effects of climatic factors (e.g. precipitation patterns) and disturbances (e.g. grazing) (HilleRisLambers et al. 2012, Milchunas et al. 1988, Lavorel and Garnier 2002). The individual effects of drought and grazing on community composition and function have been extensively studied, with negative consequences on plant productivity but varied consequences for species composition depending on their frequency and intensity (Milchunas et al. 1988, Olff and Ritchie 1998, Hover et al. 2014, Byrne et al. 2017, Juergren et al. 2017, Herrero-Jaureguie and Oesterheld 2018,). However, little attention has been given to how grasslands respond to the interactive effects of drought and grazing. This is a critical knowledge gap in our understanding because both drought and grazing can act as filters (Lavorel and Garneier 2002, Diaze et al. 2007, Debello and Sebastia 2005), selecting species with traits associated with their response to each factor. However, their interaction could determine the magnitude and direction of changes in the composition and functioning of plant communities. Trade-offs and functional correlations among traits can determine whether or not the effects of one stressor could mitigate the effects of a second stressor (Vinebrooke et al., Crain et al. 2008). For instance, a grazing system could mitigate the negative impacts of drought and maintain community composition and productivity if both drought and grazing favor species with the same traits, or it can be heavily impacted if there are trade-offs among traits (e.g. each selects species with different traits). Further, existing studies are also of limited in spatial and temporal extent (Felton and Smith 2017, Hoover et al. 2018), making it difficult to generalize patterns or determine underlying mechanisms in ecological responses to reduced water availability under different grazing systems.

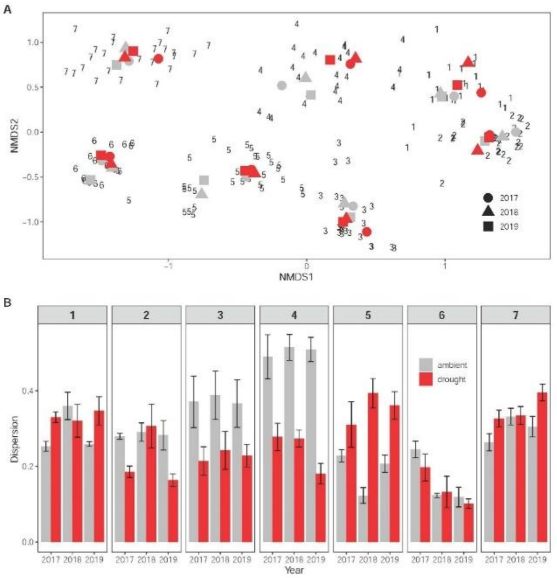


Figure 8: A) Non-metric multi-dimensional scaling ordination of experimental plant communities. Numbers represent the plots from each site, 1 being the driest site and 7 the wettest site. Red symbols represent the mean location of plots that received the drought

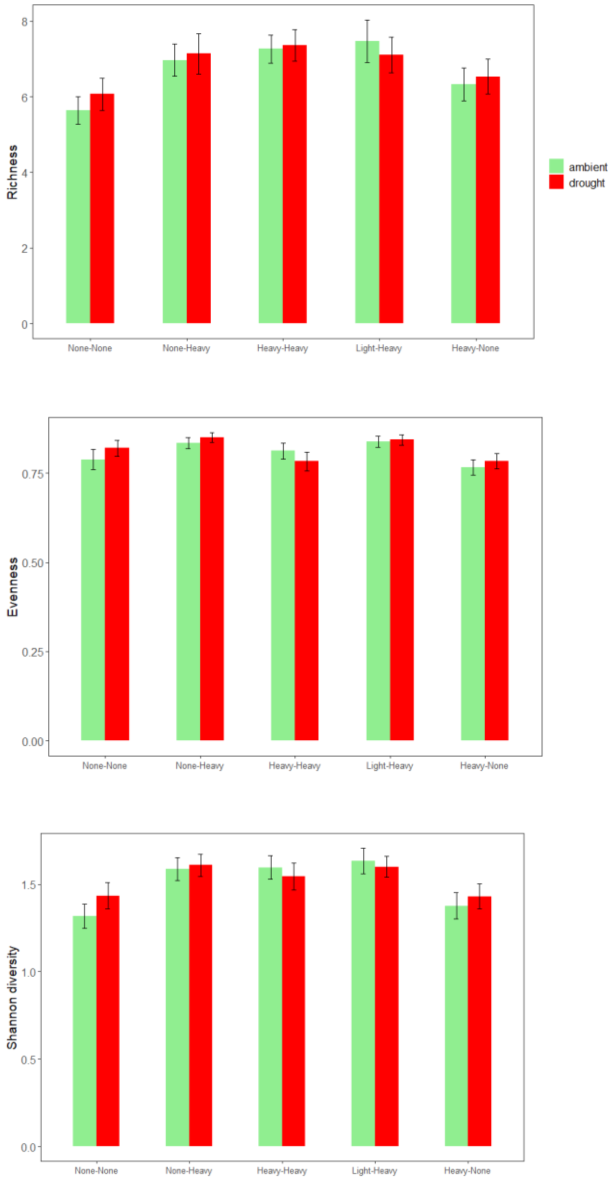


Figure 9: Mean values of measures of community composition averaged across all 7 sites. There were no effects.

Roots: Predicting grassland responses to both precipitation reduction and grazing requires an understanding of root responses (Weltzin et al. 2003), which are often overlooked by studies compared with plant shoots (Wilson 2014). Root responses are important because approximately 60-80% of plant productivity in temperate grasslands occurs belowground (Sims and Singh 1978). The response of plants to water stress and disturbance is characterized by their ability to acquire and efficiently use limited resources (McCormack and Guo 2014). Thus, changes in root production (e.g. birth, root growth) and demography (e.g. life span and turnover) in grasslands could determine how ecosystems are affected by altered precipitation or grazing (Bardgett et al. 2014, Barnett et al. 2018). Plant growth is largely a function of root growth, which has major implications for nutrient cycling as well as carbon sequestration and storage (Eissenstat et al. 2000, Bill and Jackson 2000) A growing body of research suggests that aboveground plant responses to stresses may not reflect the whole-plant response (Radville e tal. 2016, Gesche et al. 2017), which could result in divergent responses above and below ground. Similar to above ground biomass, the response of root mass to defoliation and drought was idiosyncratic across sites and treatments and no discernable overall relationship to the treatments (Figure 10) and their responses and sensitivity were not correlated (Figure 7).

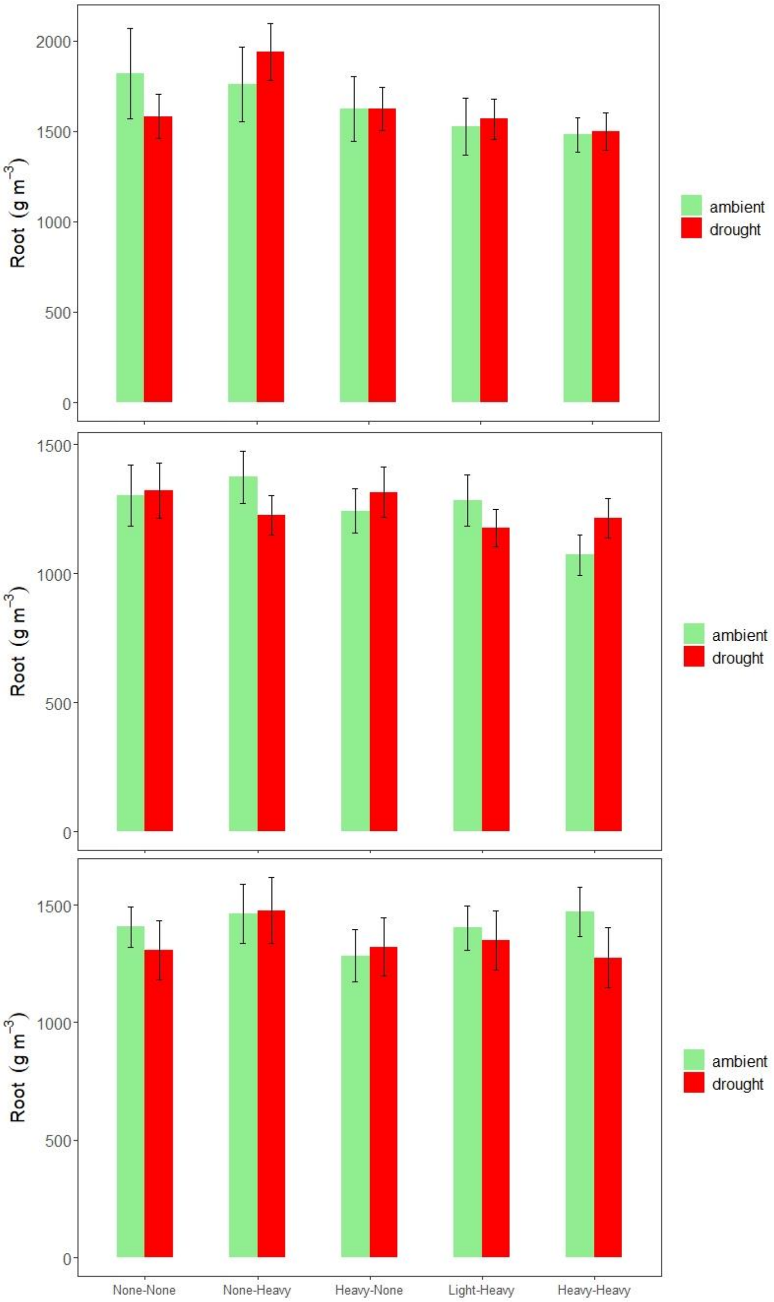


Figure 10:Mean root mass through time (top 2017, middle 2018 and 2019, bottom) averaged across all 7 sites in response to drought and clipping treatments.

However, changes in root dynamics are better reflected by root production (e.g. root birth and growth) than biomass as root growth and death occur simultaneously (Wilson 2014, Henrick and Pregitzer 1993, Partel and Wilson 2002). For example, reduced root turnover can be compensated by increased root production, with negligible changes in total root biomass but greater carbon inputs to belowground, (Mueller et al. 2018). Results from previous research show that root production varies depending on several factors, including soil depth, soil moisture and duration and magnitude of stresses (Wilcox et al. 2015, Mengtian et al. 2018, Milchunas et al. 2005, Pregitzer et al. 1993, Liu et al 2018). Drought reduces root growth and promotes more allocation of root biomass to deeper soils (Milchunas et al. 2005, Liu et al. 2018). In contrast, herbivory can promote the growth of more roots and shifts root biomass towards upper soil layer (Pucheta et al., Ruess et al. 1998). With these contradictory responses to a single factor, it was unclear how roots would respond to the interactive effects of drought and grazing. We have focused on rhizotron root tube imagery from Kinsella and Mattheis, patterns across sites were again idiosyncratic but by focusing on these two contrasting sites (relatively mesic vs xeric) we hope that some patterns may emerge (Figure 10). Installation of the rhizotron tubes is a disturbance to the roots and it is recommended that roots are allowed to recover for at least one year. None the less, we measured roots in the year following installation and through to 2019. For both sites the length of time for recover may have taken longer than 1 year (Figure 11) as we can see a steady increase in observed root length through time, with a stabilization happening in the final year. Furthermore, there is variation in the amount of root length and speed at which response plateau among defoliation and drought treatments. These patterns recurred within shallow and deep soils, but with some differences (Figure 12).

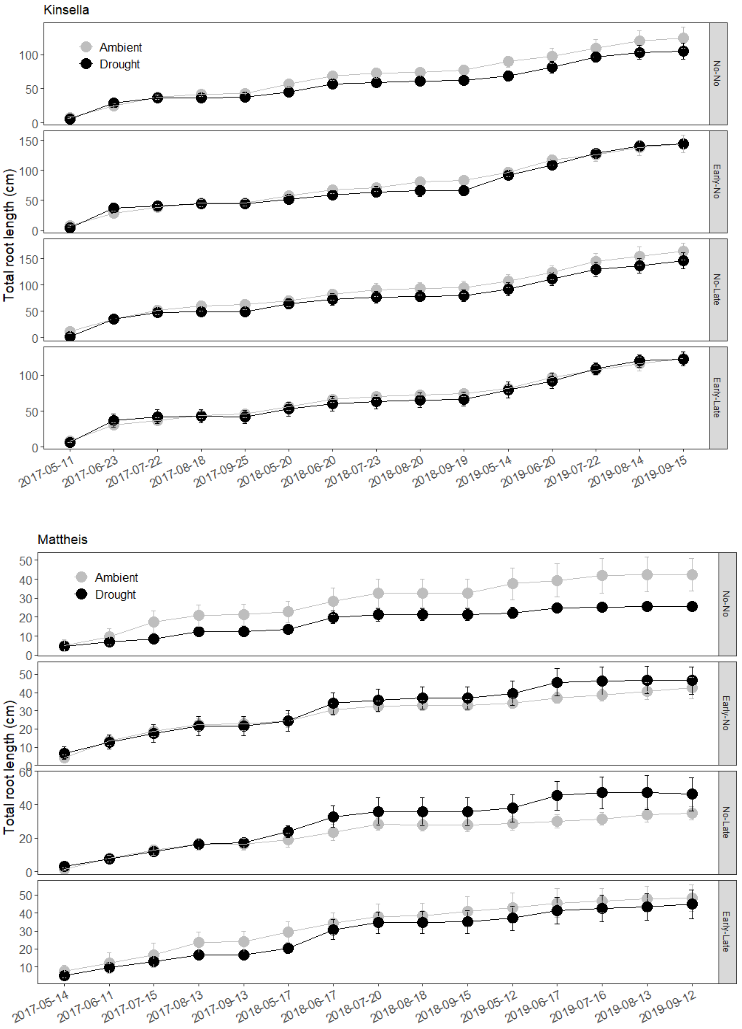


Figure 11:Mean root length through time in each of the treatment combinations at Kinsella (top) and Mattheis (bottom). The pattern suggests that it took at least a year or longer for root lengths to stabilize following tube installation in the summer of 2016.

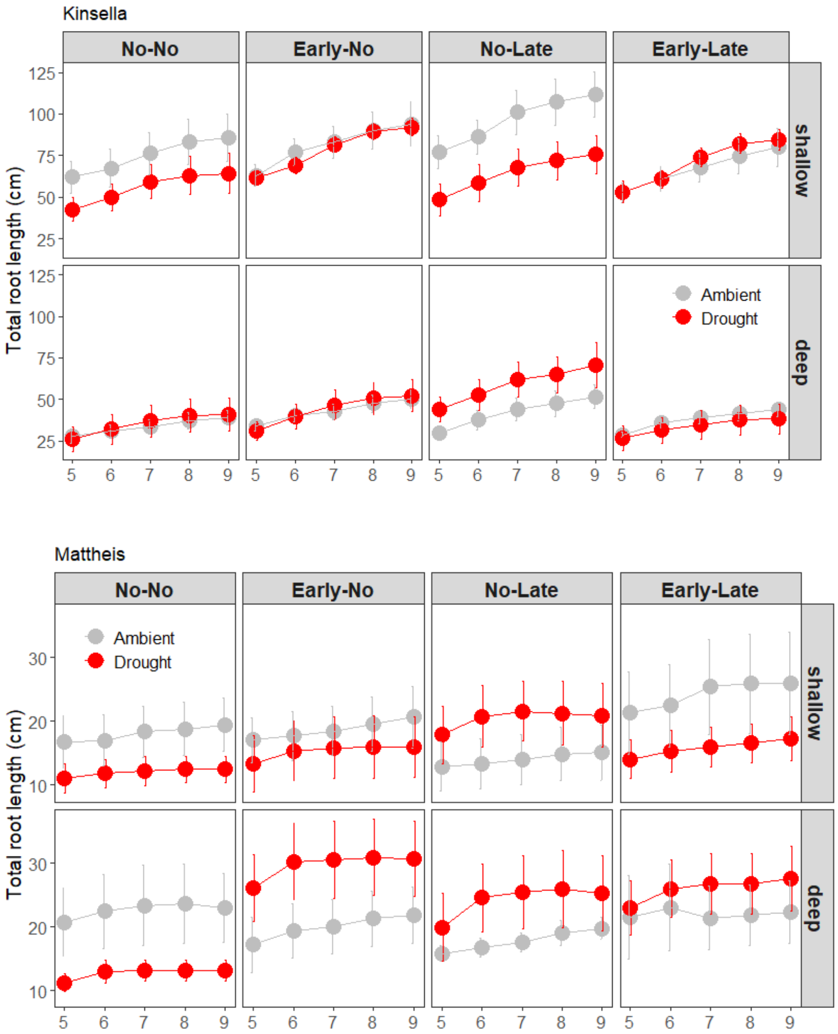


Figure 12: Mean root length in shallow and deep soils at Kinsella (top) and Mattheis (bottom). The patterns highlight variable root distributions between the two sites. The numbers on the bottom axis represent months (5 = May).

At both sites, drought reduced root length in unclipped plots in upper soils, while responses in deeper soils was muted. Furthermore, the response under different clipping treatments was variable (Figure 13). The overall length of roots was nearly 2-fold at Kinsella compared to Mathies. And not only were they affected in their total root length, the relative proportion of alive and dead roots and root tips changed in response to clipping, which presents a potential mechanism to explain patterns of soil carbon and CO2 flux and may highlight some of the sensitivities in biomass production (Figure 13, Figure 14).

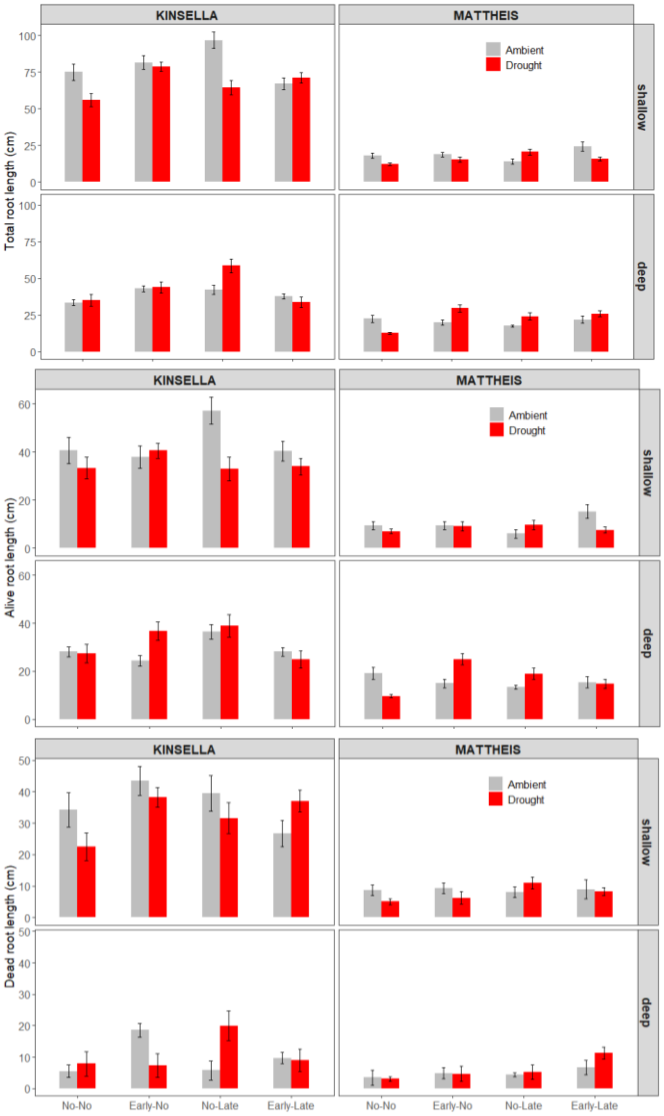


Figure 13: Mean length of total roots (top), alive roots (middle) and dead roots (bottom) across clipping and drought treatments and two soil depths at Kinsella and Matthies.

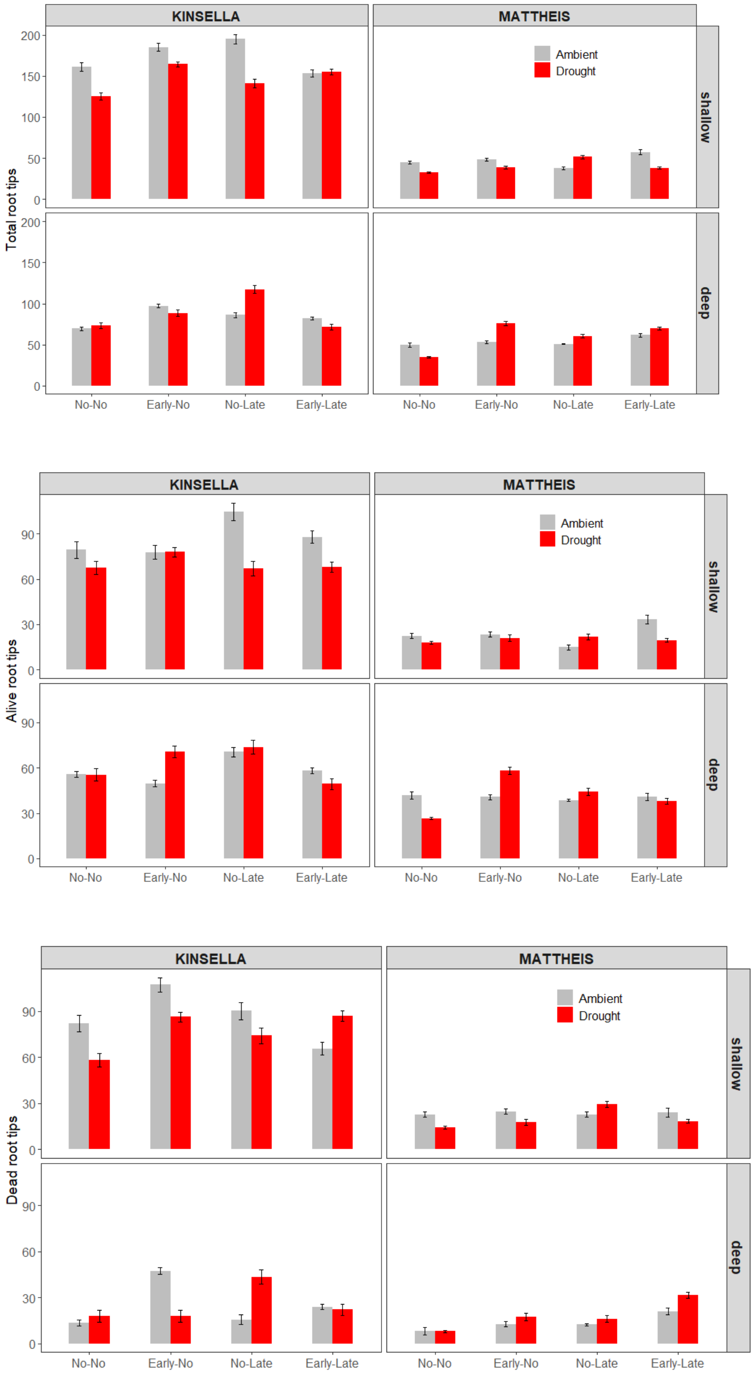


Figure 14:Mean number of root tips (top), alive root tips (middle) and dead root tips (bottom) across clipping and drought treatments and two soil depths at Kinsella and Matthies.

Furthermore, root dynamics in relation to grazing were generally opposite of the response of above ground biomass In the final year of the experiment, aboveground net primary productivity (ANPP) increased under reduced precipitation, while root biomass to 15 cm depth decreased relative to the ambient treatment (Figure 15) across the seven sites. By contrast, defoliation treatments greatly reduced ANPP ($F_{1,24}$ = 5.93, p = 0.002) but increased root biomass relative to the unclipped control treatment across sites (Figure 15), albeit the increase in root biomass was not statistically significant ($F_{1,24}$ = 1.42, p = 0.275).

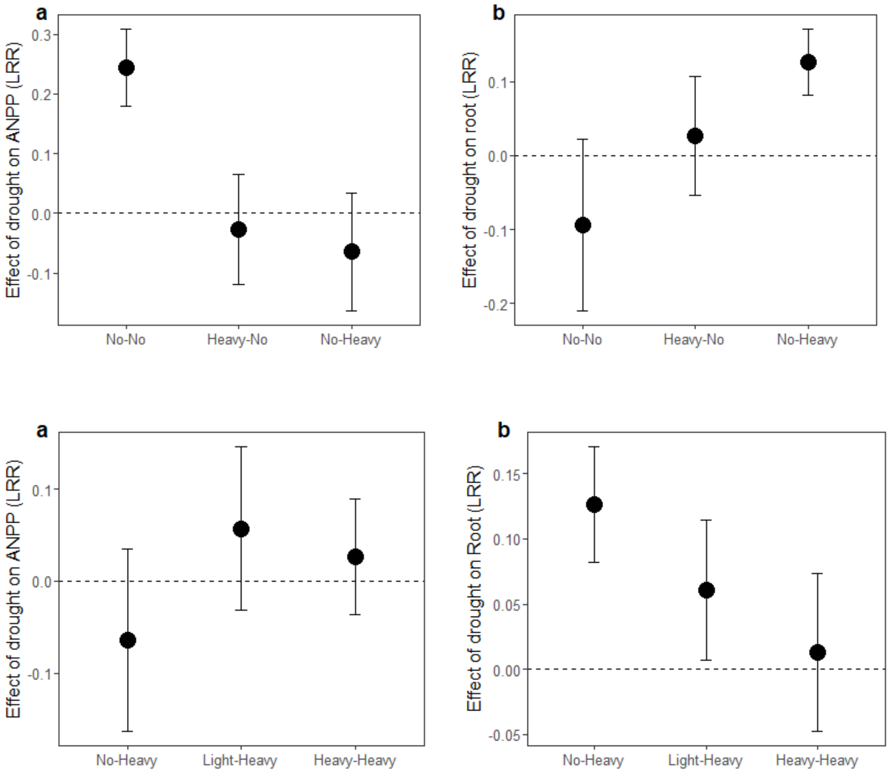


Figure 15:The effects of timing of defoliation on a) aboveground net primary productivity (ANPP) and b) root biomass in the top 15 cm soil across seven sites (mean ± SE) under drought conditions.

Across 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, despite defoliation late in the season increased root biomass more than early season defoliation (Figure 15). With an increase in the intensity of defoliation early in the season, there was a slight increase in ANPP, but root biomass declined linearly under drought conditions across sites (Figure 15). However, the observed trends in ANPP (t = 0.54, p = 0.59; t = -1.04, p = 0.31) or in root biomass (t = 0.69, p = 0.49; t = 0.5, p = 0.62) were not statistically different between defoliation treatments with varied intensity of defoliation early in the season.

The effects of defoliation treatments on ANPP or root biomass responses to drought were independent of water availability (Figure 16) as evidenced by non-significant interactions between precipitation and defoliation treatments (F4,25 = 0.13, p = 0.972; F4,25 = 1.48, p = 0.238). However, the effects of drought on ANPP were significantly associated with mean annual precipitation ($F_{1,25}$ = 8.37, p = 0.008), and the magnitude of drought impacts on ANPP was greater at mesic than arid sites, whereas, for root biomass, the relationship was not observed ($F_{1,25}$ = 1.41, p = 0.245) (Figure 17).

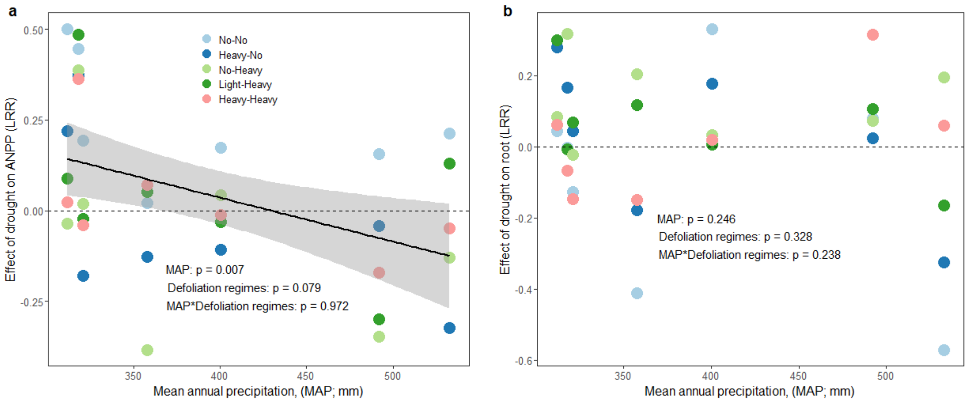


Figure 16:The effects of defoliation regimes on a) aboveground net primary productivity (ANPP) and b) root biomass in the top 15 cm soil along the precipitation gradient. The effects of drought on ANPP and root biomass were quantified by the log response ratio.

We found that sensitivity to drought differed between ANPP and standing root biomass in this multi-year and site experiment. Surprisingly, in the final year of the experiment, there was no drought-induced reduction in ANPP across the seven grasslands, contradicting previous meta-analyses (Wilcox et al. 2017, Wang et al. 2021). Decreases in root biomass in the top 15 cm soil under drought found in this study, though not statistically significant, were consistent with the findings of (Carroll et al. 2021). Differences in species strategies associated with water use may explain the observed increases in ANPP and decreases in root biomass in response to drought (Knapp et al. 2020, Ma et al. 2020). For example, to cope with drought during the growing season, communities dominated by C_3 species take advantage of higher water availability early in the season due to winter inputs coupled with lower transpiration rates (Knapp et al. 2020). Specifically, the demand of water uptake needed for shoot growth under drought conditions can meet through the improved root system efficiency (e.g., the amount of aboveground biomass produced per unit of root length investment) at the community level as a function of root length dynamics (Ma et al. 2020). We also explored the relationship between root and shoot biomasses from unclipped control treatment as a proxy measure of root system efficiency similar to (Frank 2007, Ma et al. 2020) and found evidence of increased root system efficiency under the reduced precipitation treatment (Figure 17).

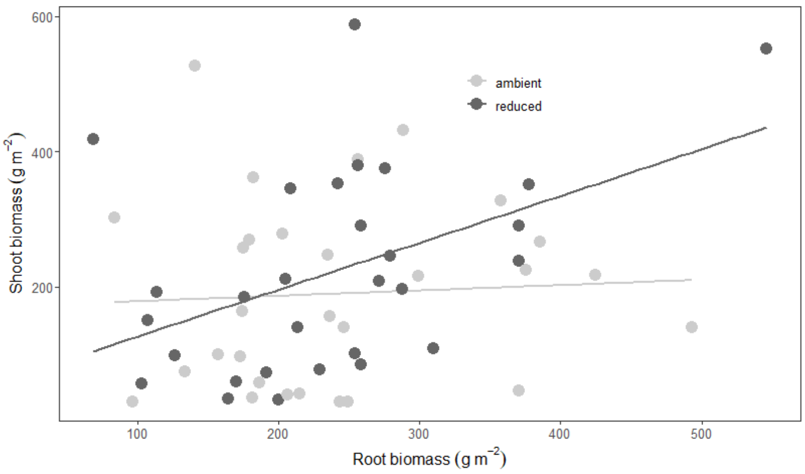


Figure 17: Aboveground biomass as a function of standing root biomass across all undefoliated treatments. The slope of the relationship is a proxy measure of root system efficiency, e.g., the amount of aboveground biomass produced per unit of root biomass (see more, Ma et al. 2020). Grey line shows the relationship under ambient precipitation while black line shows the relationship under reduced precipitation. Each point represents a plot (n=59). Note that a single outlier is removed from ambient precipitation treatment.

Compared to drought impacts, the combined effects of reduced precipitation and defoliation treatments altered the responses of ANPP and root biomass to drought. We found that defoliation treatments greatly reduced ANPP under drought conditions, whereas root biomass in the top 15 cm soil depth increased relative to unclipped communities, albeit not statistically significant. This may result from the preferential allocation of photosynthates to roots due to a higher demand for freshly assimilated carbohydrates by root systems of defoliated communities (Ma et al. 2021). However, contrary to our expectations and literature (Döbert et al. 2021, Hulvey et al. 2021), we found no evidence of shifts in the timing of defoliation or varied intensities of defoliation early in the season affected these patterns. Given that the primary pathway by which sequestration of atmospheric carbon enters the soil is through plants (Piñeiro et al. 2010), our results indicate that defoliation during the growing season, regardless of when or how, reduces plants' ability to sequester carbon under drought conditions.

We used the slope of the relationship between mean annual precipitation and plant productivity, a sensitivity metric used in previous studies (Irisarri et al. 2016, Wilcox et al. 2017, Batbaatar et al. 2021), to assess whether defoliation treatments altered the sensitivity of ANPP and root biomass to drought. Similar to the findings of White et al. (2014), we found that ANPP was generally more sensitive to reduced precipitation in mesic than arid ecosystems, while the sensitivity of root biomass to drought was independent of water availability. Defoliation treatments did not alter either ANPP or root biomass sensitivity to drought. Greater sensitivity of ANPP to the reduced precipitation in mesic ecosystems could be attributed to traits of the existing vegetation linked to drought tolerance in these grasslands (Griffin-Nolan et al. 2019). For example, non-native species representation was generally higher in more mesic grasslands of the current study area (Lyseng et al. 2018), which in turn were those with the greatest overall susceptibility to the reduced precipitation (Souther et al. 2020).

Carbon-cycling and GHG responses: Grassland ecosystems cover approximately 40% of the world’s land, storing 10% of terrestrial biomass C and about 30% of the global soil organic carbon (SOC) stock (Grosso et al., 2008). Integrated data of grassland area and its soil carbon (C) stocks are estimated to be 343 Pg C which is about 50% more than carbon stored in forests worldwide (FAO, 2015). Grasslands have a high potential for soil carbon sequestration to offset global greenhouse gas (GHG) emissions. Similar to that of forests, grasslands are known to have a soil C storage ability of significant range and are even more widespread (Lund, 2007). In addition to ecosystem goods and services, an increase in soil C stocks in grasslands can provide agronomic benefits by enhancing soil fertility, water holding capacity, soil aggregation, and a reduction in erosion (Conant and Paustian, 2002) subsequently improving the soil health. SOC pools and dynamics are influenced by the change in intensity, frequency and amount of precipitation (Canarini and Dijkstra, 2015; Zhang et al., 2013). Therefore, soil moisture plays a significant role in making grasslands a sink or source of carbon (Hoover and Rogers, 2016). However, grazing can alter plant inputs into soils and directly modify the soil environment affecting the processes that store or release soil carbon. We examined an indicator of microbial activity EEA and the flux of soil GHG to understand how defoliation and drought may interact to alter future carbon stores in grassland soils.

Extracellular enzyme activity: Extracellular enzymes in the soils play an important role in nutrient cycling and organic matter turnover (Dick, 1994). Being the early responders to the soil management changes, they may provide an early indication of grazing effects on soils processes in grasslands, especially in short-term studies. Due to complex high-level interactions we examined enzyme responses individually by site, which helped simplify the patterns as most responses were happening at only one or few sites. Overall, we found that EEA were resistant to treatments (Table 4). At three of our seven sites (Sangudo, Twin River and Onefour) there was no treatment effect on any of the enzyme activities, while all enzymes were affected at Kinsella as well as enzymes related to carbon cycling at Oyen and Mattheis. With the exception of Kinsella, BG, AP and NAG did not vary with treatments at any site.

Table 4: ANOVA results (F and P values) for extracellular enzyme activity across seven sites. Significant p-values are in bold at α = 0.05.

	Kinsella											
	CEEA		BG		CELLO		XYLO		AP		NAG	
	F	P	F	P	F	P	F	P	F	P	F	P
(Intercept)	963.4	0.00	722.9	0.00	484.4	0.00	934.3	0.00	411.1	0.00	469.5	0.00
Drought	41.6	**0.00**	21.5	**0.00**	9.3	**0.00**	7.3	**0.01**	8.4	**0.01**	6.5	**0.01**
Defoliation	9.2	**0.00**	8.3	**0.00**	4.1	**0.01**	0.4	0.82	9.9	**0.00**	7.7	**0.00**
Drought:Defoliation	2.6	**0.05**	3.0	**0.03**	0.6	0.69	0.3	0.90	2.3	0.08	0.9	0.49
	Sangudo											
(Intercept)	599.2	0.00	383.5	0.00	402.9	0.00	341.7	0.00	140.4	0.00	368.2	0.00
Drought	0.1	0.74	1.8	0.20	0.0	0.84	0.7	0.40	3.4	0.07	1.4	0.24
Defoliation	2.3	0.08	2.3	0.08	1.1	0.39	0.4	0.78	0.8	0.53	2.2	0.09
Drought:Defoliation	1.9	0.14	2.1	0.10	1.1	0.38	0.3	0.85	1.3	0.30	1.5	0.24
	Stavely											

(Intercept)	312.5	0.00	224.7	0.00	211.9	0.00	334.3	0.00	87.8	0.00	244.0	0.00
Drought	0.2	0.67	0.7	0.40	4.0	0.053	2.1	0.15	0.3	0.58	2.9	0.10
Defoliation	0.7	0.61	0.3	0.89	0.6	0.69	0.4	0.83	1.6	0.20	1.2	0.31
Drought:Defoliation	2.0	0.11	0.9	0.47	1.4	0.26	0.4	0.78	1.3	0.30	0.7	0.63
Oyen												
(Intercept)	549.0	0.00	259.1	0.00	411.7	0.00	400.6	0.00	159.4	0.00	365.6	0.00
Drought	4.6	**0.04**	2.0	0.17	7.5	**0.01**	1.3	0.27	2.1	0.16	1.8	0.19
Defoliation	0.3	0.87	0.4	0.82	0.4	0.81	0.9	0.49	0.4	0.79	0.8	0.52
Drought:Defoliation	0.9	0.48	0.1	0.99	0.7	0.61	1.9	0.14	0.4	0.81	0.8	0.53
Twinriver												
(Intercept)	457.3	0.00	197.0	0.00	269.8	0.00	312.7	0.00	87.9	0.00	234.7	0.00
Drought	0.8	0.39	0.5	0.48	1.2	0.28	0.0	0.93	1.5	0.23	0.4	0.53
Defoliation	0.5	0.76	0.8	0.51	1.1	0.38	0.4	0.78	0.1	0.99	0.4	0.83
Drought:Defoliation	0.9	0.50	0.7	0.63	1.3	0.31	1.1	0.39	0.6	0.66	0.6	0.65
Mattheis												
(Intercept)	382.7	0.00	186.4	0.00	177.4	0.00	456.0	0.00	92.7	0.00	155.6	0.00
Drought	8.9	**0.00**	1.9	0.18	4.2	**0.05**	4.3	**0.04**	1.6	0.21	1.0	0.33
Defoliation	0.7	0.62	1.5	0.23	0.2	0.93	0.8	0.55	0.6	0.66	0.6	0.68
Drought:Defoliation	0.5	0.73	1.0	0.41	0.1	0.97	0.4	0.80	0.3	0.88	0.7	0.63
Onefour												
(Intercept)	398.2	0.00	249.9	0.00	161.7	0.00	146.3	0.00	156.3	0.00	159.9	0.00
Drought	0.1	0.77	1.4	0.25	0.0	0.99	1.4	0.24	0.0	0.90	0.6	0.46
Defoliation	1.7	0.17	2.2	0.10	1.2	0.35	0.5	0.71	0.1	0.99	0.1	0.97
Drought:Defoliation	0.4	0.80	1.0	0.44	0.2	0.96	0.5	0.75	0.5	0.77	0.2	0.96

Cellobiosiodase activity, an enzyme responsible for the breakdown of plant matter, was significantly reduced by drought treatments at 4 of the 7 sites, and tended to be lower at 2 additional sites (Table 4, Figure 18). Additionally, only at Kinsella, defoliation affected Cello activity such that the twice clipped treatments (heavy-heavy and light-heavy) had greater activity than the treatments that were only clipped once (Heavy none, and none-heavy), and the unclipped treatment was intermediary (Figure 21). The activity of XYLO, an enzyme also responsible for the breakdown of plant matter, was reduced by drought at Kinsella and Mattheis, with no response to treatments at the other sites. At both sites, the activity was greater under ambient conditions (1.65 nmol g^{-1} h^{-1} ±0.048; 1.20 nmol g^{-1} h^{-1} ±0.026) than rainout-shelter (1.57 nmol g^{-1} h^{-1} ±0.048; 1.06 nmol g^{-1} h^{-1} ±0.026) respectively. These reductions in enzyme activity may indicate slowdown of nutrient cycling in these systems that could limit plant growth and slow down the breakdown of soil carbon.

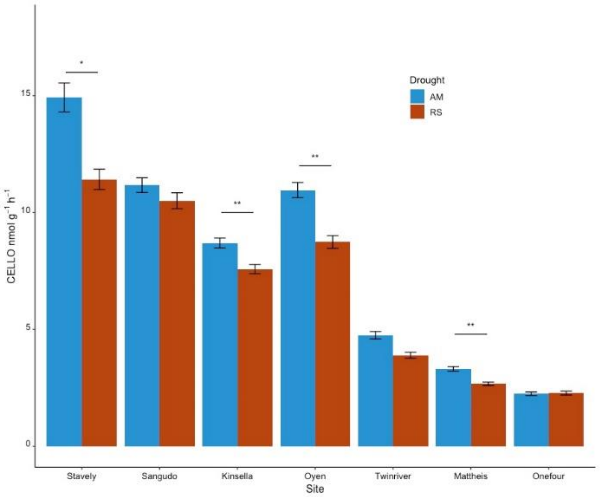


Figure 18: Main(±SE) effect of drought treatment on the activity of CELLO across sites. Asterisks denote the significant differences between the two (AM= ambient, RS = rainout shelters) groups at α = 0.05. Significance is shown between the two treatments at each site individually. denotes significance at α = 0.05, * denotes the value extremely near to significance level (p = 0.053).**

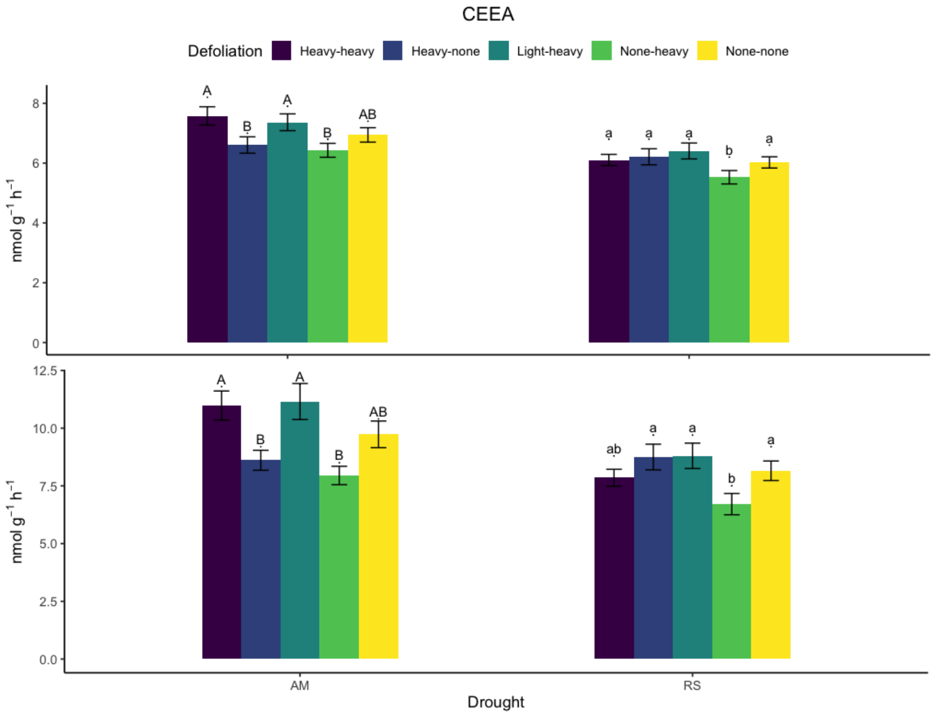


Figure 19: Interaction effect of drought and defoliation on CEEA (top) and BG at Kinsella. Means (±SE) with different letters differed significantly at α = 0.05. Significance is shown between the defoliation treatments at AM and RS separately.

The overall mix of extra-cellular enzymes appears to be largely driven by site specific conditions, and not defoliation or treatments. Non-metric multidimensional scaling (NMDS) resulted in an ordination with a stress value of 0.085 with two dimensions (k = 2) and revealed soil moisture

content, temperature, pH and C and N content as primary environmental factors controlling EEA (Figure 20,

Table 5). A strong positive correlation of C and N content with EEA was observed. A biplot of the environmental factors on the NMDS suggests a negative association of XYLO with soil moisture content (MC), soil C and N content, while the activity of BG and CELLO was associated with soil moisture content (MC), soil C and N (Figure 20 and 21). NAG and AP appear to be negatively associated with soil temperature and pH (Figure 22). Three distinct groups of sites can be visualized in the NMDS biplot; Sangudo, Stavely, Kinsella and Oyen on the left, Twin River in the middle and Mattheis and Onefour on the right. Suggesting that XYLO dominates processes at drier sites, why CELLO is more abundant at wetter sites. Whenever there was a difference due to the drought treatment, drought treatment plots had lower EEA compared to ambient treatments. This is expected as the relationship between EEA and soil moisture content, temperature and substrate availability is well established (Henry, 2012, Brockett et al., 2012, Allison et al., 2008; Kreyling et al., 2008; Sardans and Penuelas, 2010; Zhou et al., 2013). And we found an overall positive relationship between the soil moisture and activity of B-glucosidase and Cellobiosidase (Figure 22).

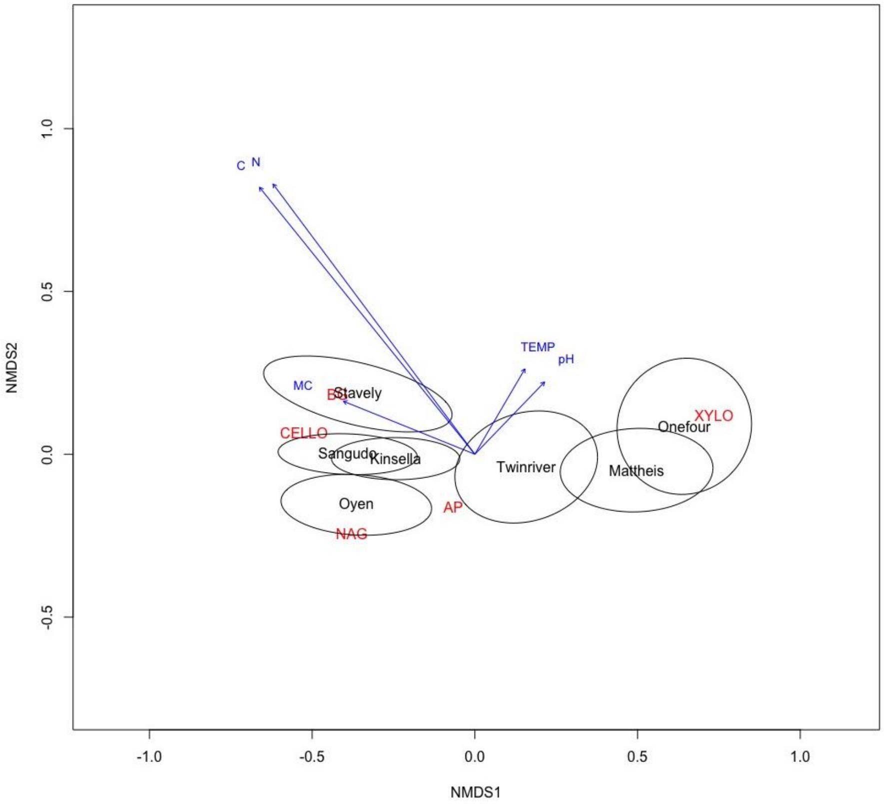


Figure 20: Non-metric multidimensional scaling (NMDS) ordination biplot showing the relationship of extracellular enzyme activity with environmental factors (MC: soil moisture content, TEMP: soil temperature, pH, C: soil carbon content and N: soil nitrogen content) and association with the seven sites included in the study. Ellipses represent 95% of confidence limits.

Table 5: Results of NMDS analysis showing the relationship between EEAs and environmental factors (MC: soil moisture content, TEMP: soil temperature, pH, C: soil carbon content and N: soil nitrogen content), analyzed using envfit function (R-package: vegan). Significant values are in bold at α = 0.05.

Variable	NMDS1	NMDS2	r2	*P*
Temperature	0.51	0.86	0.05	**0.001**
Soil Moisture	-0.93	0.38	0.11	**0.001**
pH	0.70	0.72	0.05	**0.002**
Nitrogen	-0.60	0.80	0.61	**0.001**
Carbon	-0.63	0.78	0.63	**0.001**

Both of the enzymes also showed a strong positive linear relationship with soil carbon and water. content (Figure 22Figure 23, Figure 24). Simple linear regression for both enzymes showed a signification relationship MC and soil C ($p < 0.0001$). Linear regression also showed a significant relationship of both (B-glucosidase and cellobiosidase) enzymes with soil pH ($p < 0.0001$) with a very weak negative correlation (r = -0.14 and -0.20), respectively. Acid phosphatase (AP) and NAG showed a weak negative relationship with soil pH (Figure 8). Simple linear regression for AP and NAG showed a signification relationship with soil pH ($p < 0.001$), MC ($p < 0.001$), and C($p < 0.001$).This study suggests that the EEA and their relationship with environmental variables are site-specific and mesic sites (Sangudo, Stavely, Kinsella and Oyen) showed similar associations between EEAs and environmental variables as opposed to xeric sites (Mattheis and Onefour). The activity of AP and NAG was greater when the pH was more acidic and temperature was lower, while XYLO's activity had association with higher pH and higher temperature. This relationship was further confirmed by linear regression and correlation of coefficients (r); however, the correlation was weak with pH and there was no significant linear relationship of temperature with both enzymes. BG and CELLO activity were positively associated and correlated with soil moisture content. Plotting sites on ordination plot produced distinct groups, where BG, CELLO and NAG activity was closely associated to Kinsella, Sangudo, Stavely and Oyen while XYLO's activity was closely associated with Onefour and Mattheis

The limited effect of drought treatment could be attributed to the climate factors such as MAP, MAT and variability in seasonal rainfall and temperatures, soil characteristics and vegetation type. A meta-analysis by Xiao et al. (2018) observed the effects of climate change (alteration in precipitation and warming) on soil EEA. They found that overall, warming had no effect on the activity of enzymes, while altered precipitation had very little effect on the EEA. Generally, they found that a decrease in precipitation tends to reduce soil EEAs while an increase in precipitation (water addition) tends to increase soil EEA. Despite the generally positive relationship of EEAs with soil moisture content, they could not find significant differences, possibly due to various underlying mechanisms such as substrate diffusion, drying and rewetting cycles (Henry, 2012). Similarly, multiple studies (McDaniel et al., 2013; Gutknecht et al., 2010; Sardans and Penuelas, 2005) have observed inconsistent effects of altered precipitation on EEA due to the fact EEA was driven by multiple factors such as environmental conditions, underlying soil microbial community, resource availability and soil properties. This could explain the inconsistency of the main effect of drought treatment in our study across the sites as these sites are situated in different ecoregions with varying soil and environmental properties.

The effect of defoliation on AP and NAG was somewhat similar at Kinsella. For both enzymes, activity increased with the defoliation (e.g. greater at heavy-heavy and light-heavy). This could be due to the increase in rhizodeposition, readily available substrate for the plants and possibly an increase in root death because of defoliation (Bahn et al., 2006; Hewins et al., 2015). The increased activity of NAG with defoliation supports that the defoliation may increase the flow of C into the soil, thus an increased demand of N mineralization by microbes (McNaughton et al., 1997). In natural grassland ecosystems, defoliation is known to stimulate root exudation, which in turn increased the growth of soil microbial communities leading to greater microbial activity and N mineralization (Hamilton et al., 2008), suggesting an increase in EEA since the majority of the extracellular enzymes in natural ecosystems are derived from microorganisms.

Overall, we found that EEA were resilient to treatment effects, except for Kinsella and the enzyme CELLO (except Kinsella), defoliation did not have any effect on EEAs and did not support our hypothesis, partially in agreement with the previous study of defoliation by Hewins et al. (2015). Defoliation effects were observable at only one site was possibly due to the sensitivity of enzymes to many other environmental factors such as soil type and vegetation as microbial community responses can vary depending on the plant species (Hokka et al., 2004; Jones et al., 2004). An explanation of the patterns specific to Kinsella is in the appendix (Figures 18 and 19), but because of the lack of generality we have excluded the details here. Why Kinsella is particularly sensitive is not clear. That EEA were highly responsive to treatments at only one site may be due to site-specific characteristics such as soil texture, pH, substrate availability and vegetation. The detectable differences of defoliation on EEAs at Kinsella may be attributed to Kentucky bluegrass (*Poa pratensis* L.) an introduced grass species which is known to increase due to grazing and is common at the study site Kinsella which is in the central parkland ecoregion (Chuan et al., 2020; Willms et al., 1985). Hamilton et al. (2001) found that the defoliation of *poa pratensis* in a growth chamber experiment increased the rhizospheric processes and photosynthetic activity. Results in our study at Kinsella are in accordance with previous findings as this grass species is known to be grazing tolerant and has stimulated growth as grazing intensity increases. Furthermore, changes in soil moisture content can have a direct impact on soil microbial communities and microbial biomass, which in turn may affect EEA (Schimel, 2018). The larger response of Kinsella to drought treatment could also be attributed to the location of site as it remains more in the mid-range in terms of environmental properties such as annual precipitation (Table 1) and more likely had more rapid dry-rewetting cycles due to the greater clay content (39%) and bulk density (inverse-texture effect) which could lead to an observable effect of drought on underlying biogeochemical properties of soil.

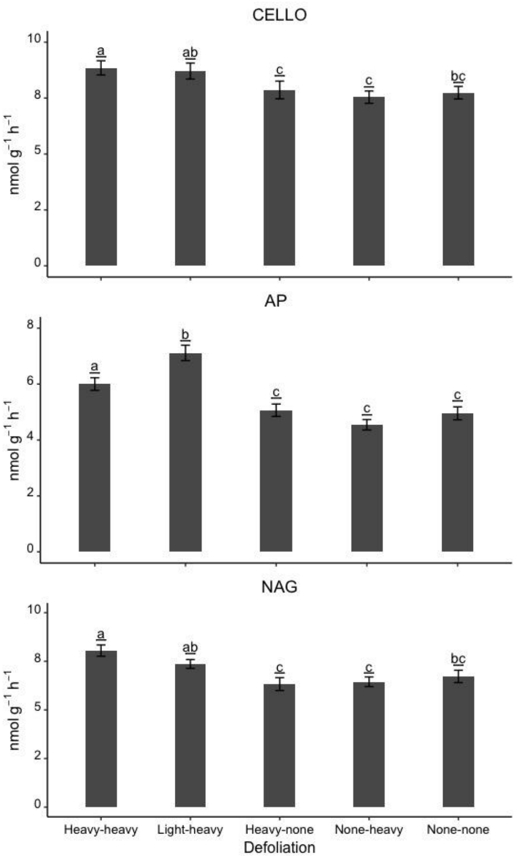


Figure 21: Mean (±SE) activity of CELLO at Kinsella. Means with different letters differed significantly ($p < 0.05$).

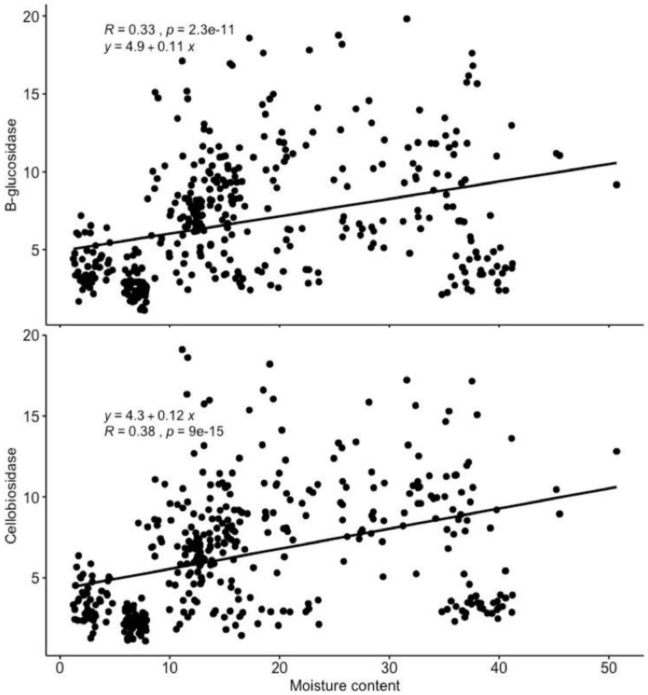


Figure 22: Simple linear regression and correlation coefficients (r) graphs of b-glucosidase and cellobiosidase with soil moisture content.

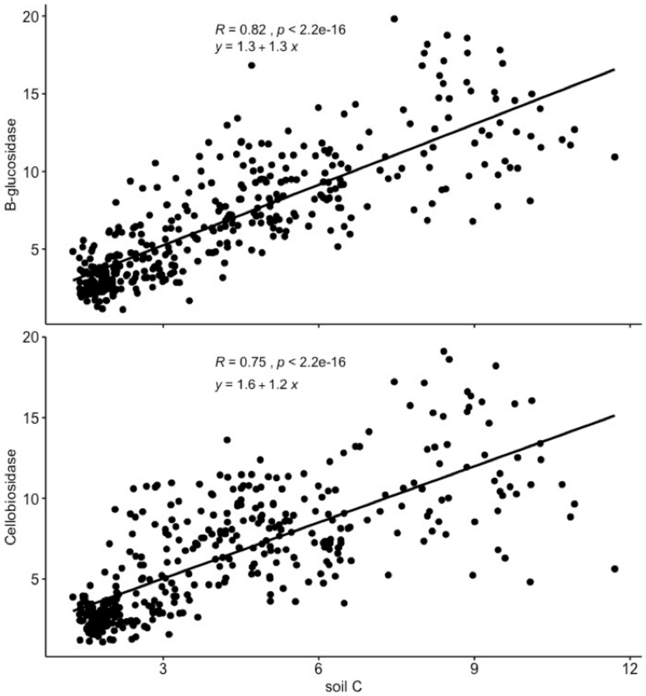


Figure 23: Simple linear regression and correlation coefficients (r) graphs of b-glucosidase and cellobiosidase with soil C content (%).

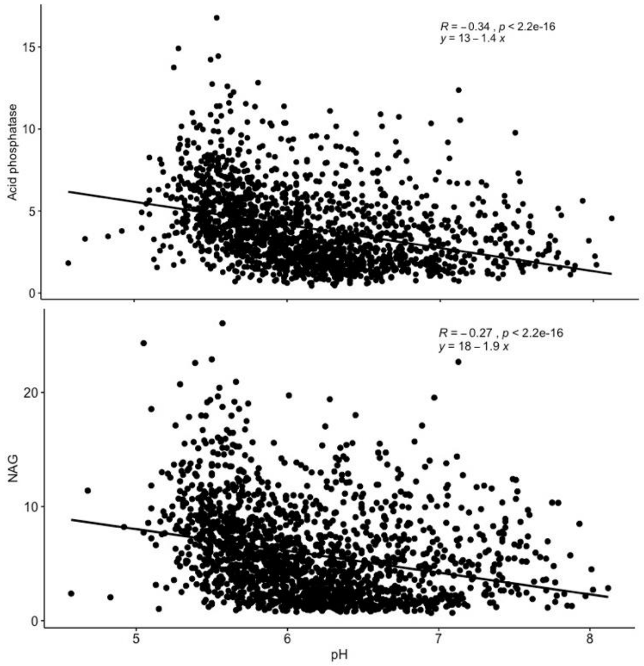


Figure 24: Simple linear regression and correlation coefficients (r) graphs of Acid phosphatase and N-acetyl-B-D-glucosaminidase (NAG) with soil pH.

Greenhouse Gases: Grasslands are known to have the potential to reduce GHG emissions (Reid et al., 2004), hence could contribute to the mitigation of climate change (Allard et al., 2007). In this study, CO_2, N_2O, and CH_4 fluxes were measured from grasslands across a climate gradient from April to September in 2017 and 2018. Results will be used to determine the differences in GHG emission rates among variable grazing intensities and frequencies in grasslands to evaluate the GHG mitigation potential of various grazing systems across a climate gradient.

We calculated cumulative emissions of soil GHG flux in order to focus on the net effect of treatment effects, rather than the variation between sampling times, which is likely to be driven by localized weather. Cumulative CO2 emissions were affected by interaction of drought and defoliation treatments at three sites (Kinsella, Mattheis and Onefour) out of seven sites in 2017 (Table 6). There was an effect of drought treatment at Twinriver where emissions were greater under ambient (3060 ±47.5) condition than rainout shelter (2918 ±47.5). At Kinsella, emissions in AM-none-heavy and none-none were greater than RS-none-heavy, heavy-none, light-heavy and none-none. AM-heavy-heavy had greater emissions than RS-none-heavy, heavy-none and light-heavy. And AM-light-heavy had greater emissions than RS-none-heavy and RS-heavy-none. In 2018 there was main effect of drought and defoliation treatment at Kinsella, but no interaction was observed. Cumulative emissions of CO2 were greater under ambient condition (5571 ±147) than rainout shelter (4817 ±147). Defoliation treatments heavy-heavy, none-heavy and none-none were greater than light-heavy treatment. At Mattheis, AM-heavy-heavy, none-none and none-heavy had greater emissions of CO2 than RS-heavy-heavy, none-none, none-heavy, heavy-none and AM-heavy-none in 2017 (Table 3). Furthermore, treatment RS-light-heavy was greater than RS-heavy-heavy. In 2018, significant effect of drought treatment was observed where emissions were greater under ambient conditions (1857 ±54.8) than rainout shelter (1495 ±58.9). At Onefour, treatment AM-none-none, heavy-none, heavy-heavy and RS-light-heavy had greater CO2 emissions than RS-heavy-none and none-heavy in 2017.

Furthermore, treatment AM-light-heavy was greater than RS-heavy-none. In 2018, significant effect of drought treatment was observed where the emissions were greater under ambient conditions (2367 ±90.6) than rainout shelter (1973 ±90.6). There was a significant effect of drought treatment at Sangudo in 2018 where the CO2 emissions were greater under ambient conditions than rainout shelter.

Table 6: Effects of drought and defoliation treatments on soil carbon dioxide flux at each of the seven study sites.

Kinsella				
	2017		2018	
	F-value	p-value	F-value	p-value
(Intercept)	12066.179	0.0000	2480.840	0.0000
Defoliation	3.780	**0.0190**	7.086	**0.0010**
Drought	67.503	**0.0000**	13.069	**0.0017**
Defoliation:Drought	3.007	**0.0429**	2.343	0.0898
Stavely				
(Intercept)	3030.049	0.0000	1143.543	0.0000
Defoliation	0.326	0.8569	0.381	0.8198
Drought	2.066	0.1661	0.144	0.7084
Defoliation:Drought	1.273	0.3134	0.486	0.7455
Sangudo				
(Intercept)	3773.495	0.0000	1674.420	0.0000
Defoliation	1.551	0.2260	1.613	0.2100
Drought	1.687	0.2088	4.601	**0.0444**
Defoliation:Drought	0.735	0.5788	0.667	0.6223
Oyen				
(Intercept)	3773.574	0.0000	1411.297	0.0000
Defoliation	1.550	0.2262	0.671	0.6197
Drought	1.687	0.2088	2.083	0.1644
Defoliation:Drought	0.735	0.5787	0.890	0.4880
Twinriver				
(Intercept)	7924.365	0.0000	1658.973	0.0000
Defoliation	0.115	0.9757	0.212	0.9284
Drought	4.510	**0.0464**	0.007	0.9319
Defoliation:Drought	0.987	0.4370	0.068	0.9908
Mattheis				
(Intercept)	5421.360	0.0000	1736.682	0.0000
Defoliation	2.446	0.0798	1.961	0.1395
Drought	41.351	**0.0000**	20.347	**0.0002**
Defoliation:Drought	9.065	**0.0002**	1.984	0.1359
Onefour				
(Intercept)	4141.472	0.0000	1147.930	0.0000
Defoliation	4.977	**0.0060**	1.265	0.3165
Drought	22.533	**0.0001**	9.448	**0.0060**
Defoliation:Drought	3.766	**0.0193**	1.582	0.2179

Table 7:ANOVA table of the linear mixed model of drought and defoliation effects on greenhouse gas emissions

	2017				2018			
					CO2			
	F	Df	Df.res	p-value	F	Df	Df.res	p-value
(Intercept)	36.51	1	6	0.001	36.077	1	6	0.001
Drought	2.246	1	194	0.136	1.935	1	194	0.166
Defoliation	1.498	4	194	0.204	2.37	4	194	**0.054**
Drought:Defoliation	2.335	4	194	**0.057**	2.169	4	194	0.074
					N2O			
(Intercept)	1.541	1	7	0.255	6.688	1	13	0.023
Drought	1.503	1	194	0.222	0.013	1	194	0.911
Defoliation	0.447	4	194	0.775	0.976	4	194	0.422
Drought:Defoliation	0.405	4	194	0.805	0.907	4	194	0.461
					CH4			
(Intercept)	2.947	1	13	0.110	2.740	1	17	0.116
Drought	0.232	1	194	0.631	0.099	1	194	0.753
Defoliation	0.251	4	194	0.909	0.265	4	194	0.900
Drought:Defoliation	0.207	4	194	0.934	0.784	4	194	0.537

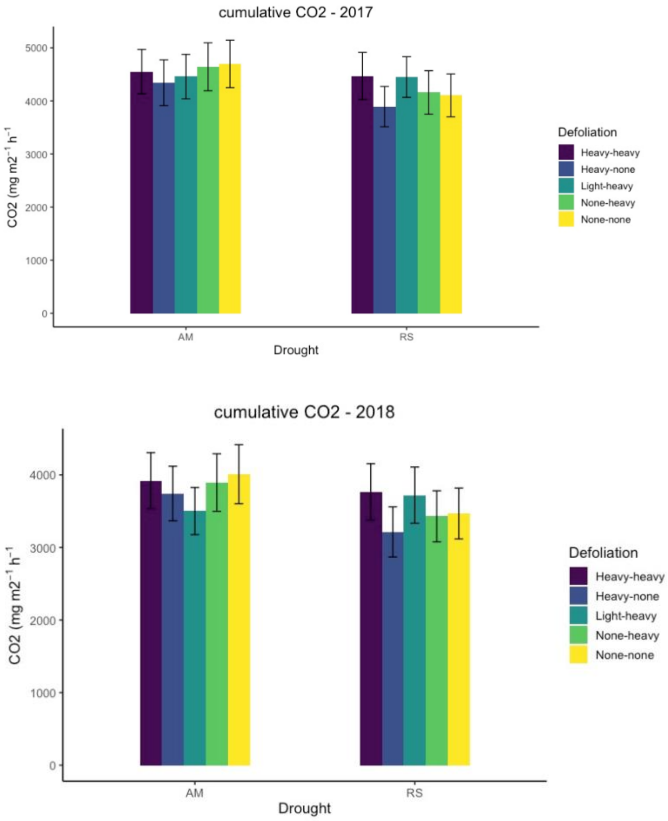


Figure 25: Drought and defoliation effects on cumulative CO2 emissions in 2018 and 2017, average across all sites.

When we examined the response ratio of CO2 flux across all sites, a promising pattern emerged. The amount of CO_2 flux was lower under some treatments compared to others but the effect depended on the rainfall treatment (Figure 25,Table 8) and it appears that multiple defoliations (Heavy-heavy, and light-heavy) have greater CO2 flux. However, the size of this effect was greatest at drier sites and diminished as sites became more mesic (Figure 26). The explanation as to why the light-heavy clipping treatment is exhibiting the trend that it is not yet fully clear and needs to be examined in light of plant and microbial community responses. This is a promising result, as it suggests that most grazing regimes won't lead to a n increase in CO_2 flux under future climate scenarios that include more frequent drought, but it may be concerning that this particular treatment does if it corresponds to management practices. The risks associated here might include loss of soil carbon leading to reduced water holding capacity, nutrient turnover and ultimately plant production. We also measured the flux of methane and nitrous oxide (Table 11,Table 12), but they were not affected by treatments.

Table 8:ANOVA table of linear model performed on natural log-response ratio of drought treatment on CO2 emissions

	2017			
	Sum Sq	Df	F value	Pr(>F)
MAP	0.045	1	3.060	0.083
Defoliation	0.250	4	4.281	**0.003**
MAP:Defoliation	0.159	4	2.718	**0.034**
Residuals	1.388	95		
	2018			
	Sum Sq	Df	F value	Pr(>F)
MAP	0.142	1	5.656	**0.019**
Defoliation	0.359	4	3.592	**0.009**
MAP:Defoliation	0.182	4	1.815	0.132
Residuals	2.377	95		

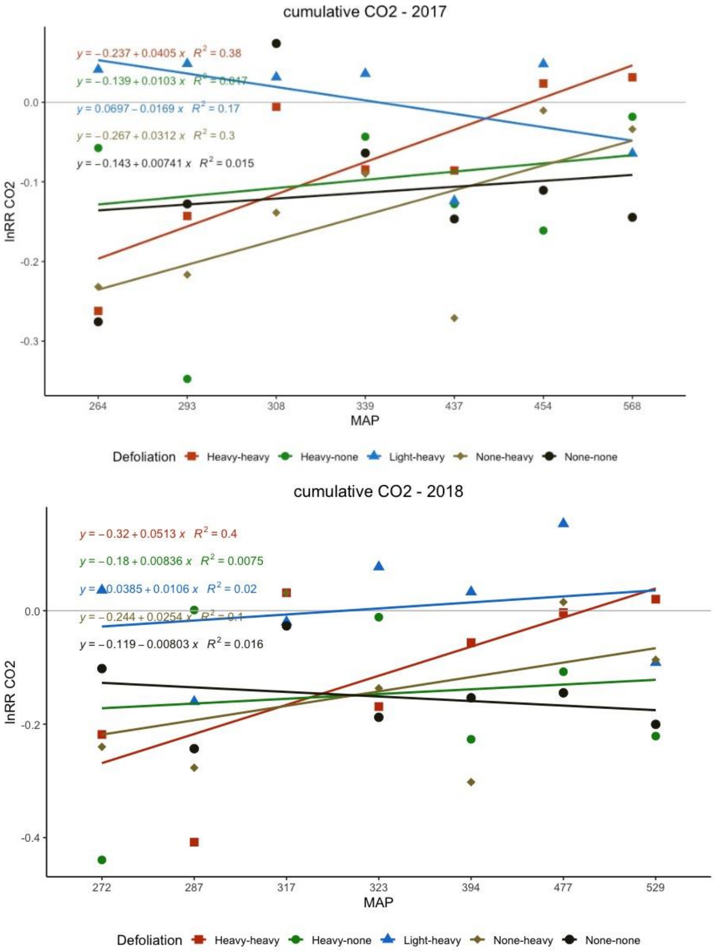


Figure 26: Simple linear regression and correlation between log-Response ratio of drought treatment CO2 emissions and mean annual precipitation (MAP) in 2017 and 2018

Table 9:Standardized beta coefficient estimates of mixed effects linear regression model performed on monthly CO2 flux

	Estimate	Standard Error	df	t-value	p-values
(Intercept)	6.390	0.387	11.469	16.503	0.000
PC1	0.168	0.011	1252.168	15.444	**0.000**
TEMP	0.0140	0.003	1251.284	4.930	**0.000**
MC	0.000	0.002	1251.011	0.236	0.814
pH	-0.0375	0.032	1251.753	-1.181	0.238

Table 10:Standardized beta coefficient estimates of mixed effects linear regression model performed on monthly CO2 flux

	Standardized coefficient estimate	Standardized standard error
(Intercept)	0	0
PC1	0.412	0.027
TEMP	0.130	0.026
MC	0.006	0.026
pH	-0.028	0.023

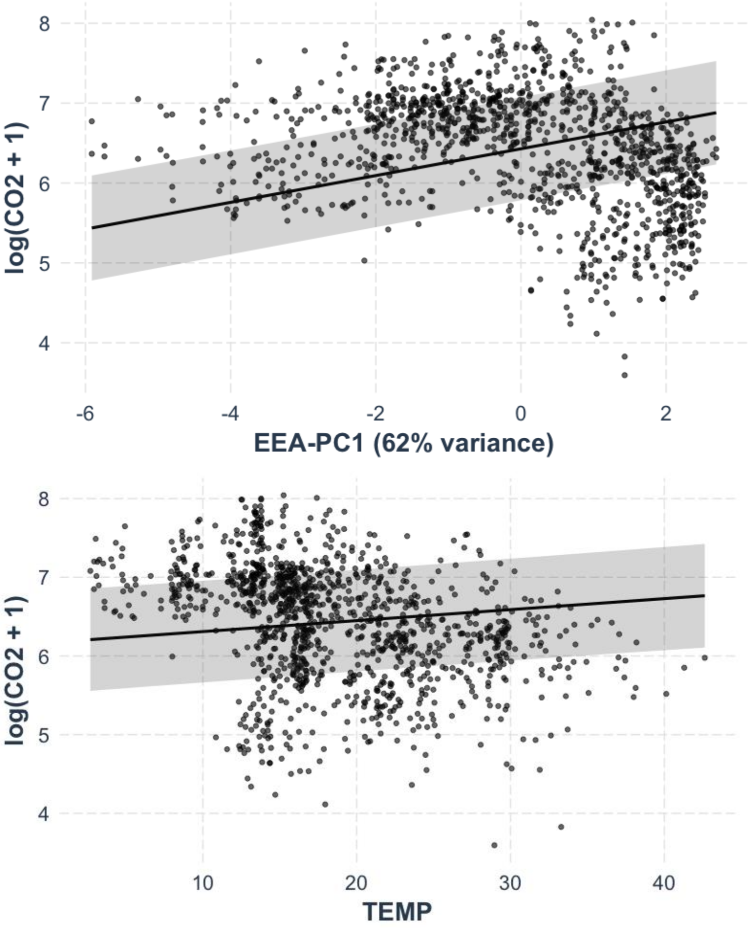


Figure 27: Effect plot of PC1 (62% variance explained) from principal component analysis of soil extracellular enzymes (β-glucosidase, β-Cellobiosidase, β-xylosidase, acid phosphatase and N-acetyl- β-glucosaminidase), and soil temperature on carbon dioxide flux.

Table 11: Effects of drought and defoliation treatments on soil nitrous oxide flux at each of the seven study sites.

	Kinsella					
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	12.07	4	0.999	0.431	0.946	0.458
Drought	0.73	1	0.241	0.629	1.824	0.192
Defoliation:Drought	18.55	4	1.536	0.230	0.922	0.471

	Stavely					
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	2.42	4	1.233	0.329	1.221	0.333
Drought	0.15	1	0.312	0.583	0.106	0.748
Defoliation:Drought	0.85	4	0.431	0.784	0.673	0.619

	Sangudo					
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	0.24	4	0.158	0.957	0.264	0.898
Drought	0.60	1	1.591	0.222	2.335	0.142

Defoliation:Drought	0.22	4	0.147	0.962	0.658	0.628

Oyen

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	20.58	4	0.652	0.632	1.979	0.137
Drought	1.59	1	0.201	0.659	1.772	0.198
Defoliation:Drought	61.85	4	1.958	0.140	0.398	0.808

Mattheis

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	0.13	4	0.340	0.848	0.241	0.912
Drought	0.07	1	0.779	0.388	0.101	0.754
Defoliation:Drought	0.67	4	1.750	0.179	1.319	0.297

Twinriver

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	5.18	4	0.750	0.570	1.210	0.338
Drought	0.04	1	0.023	0.880	0.081	0.778
Defoliation:Drought	5.94	4	0.860	0.505	1.377	0.277

Onefour

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	0.32	4	0.977	0.442	0.688	0.609
Drought	0.07	1	0.896	0.355	0.984	0.333
Defoliation:Drought	0.47	4	1.441	0.257	1.317	0.298

Table 12: Effects of drought and defoliation treatments on soil methane soil flux at each of the seven study sites.

Kinsella

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	17.51	4	1.068	0.398	1.771	0.174
Drought	4.84	1	1.180	0.290	2.290	0.146
Defoliation:Drought	16.27	4	0.993	0.434	2.789	0.054
Residuals	81.96	20				

Stavely

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	6.01	4	0.325	0.858	0.633	0.645
Drought	2.84	1	0.614	0.443	0.666	0.424
Defoliation:Drought	13.79	4	0.746	0.572	0.650	0.634
Residuals	92.42	20				

Sangudo

	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	1.06	4	0.187	0.942	0.099	0.981
Drought	1.35	1	0.957	0.340	1.463	0.241
Defoliation:Drought	6.19	4	1.094	0.386	1.048	0.408
Residuals	28.29	20				

Oyen

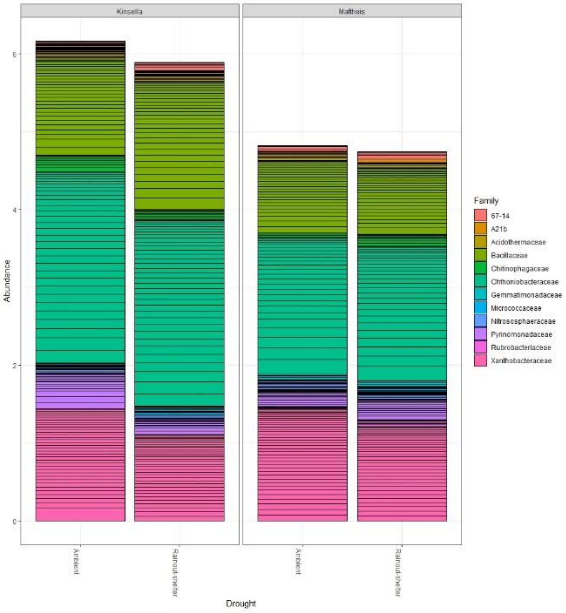
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	1.71	4	0.423	0.790	0.083	0.987
Drought	3.68	1	3.654	0.070	0.275	0.606
Defoliation:Drought	1.74	4	0.430	0.785	0.328	0.856
Residuals	20.17	20				

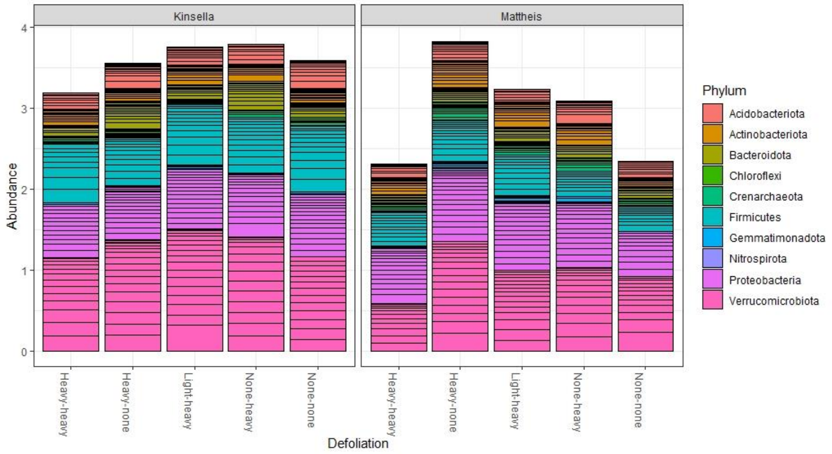
Mattheis						
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	14.89	4	2.473	0.077	2.025	0.130
Drought	0.21	1	0.141	0.711	0.048	0.830
Defoliation:Drought	3.72	4	0.617	0.655	0.442	0.777
Residuals	30.12	20				

Twinriver						
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	1.47	4	1.276	0.313	0.476	0.753
Drought	0.13	1	0.435	0.517	0.001	0.977
Defoliation:Drought	1.02	4	0.888	0.489	0.927	0.468
Residuals	5.75	20				

Onefour						
	Sum Sq	Df	F value	Pr(>F)	F value	Pr(>F)
Defoliation	1.09	4	0.588	0.675	0.726	0.585
Drought	0.01	1	0.015	0.903	0.000	0.997
Defoliation:Drought	2.12	4	1.141	0.366	0.598	0.668
Residuals	9.29	20				

Microbial Community Response: The abundance of bacteria and fungi were affected by defoliation and rainout shelters. The heavy-heavy defoliation treatment reduced the abundance of bacteria at both sites (Figure 28) and drought reduced (Figure 29).





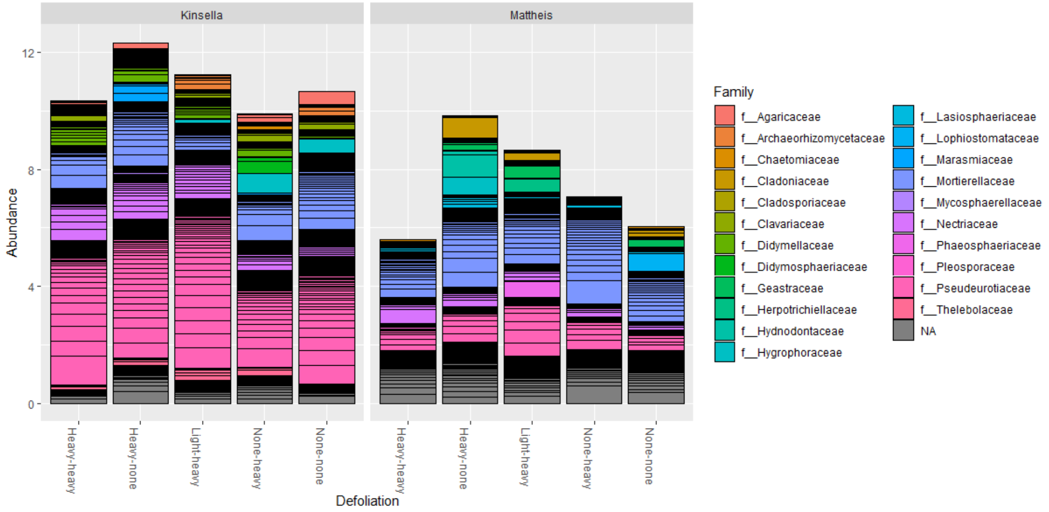
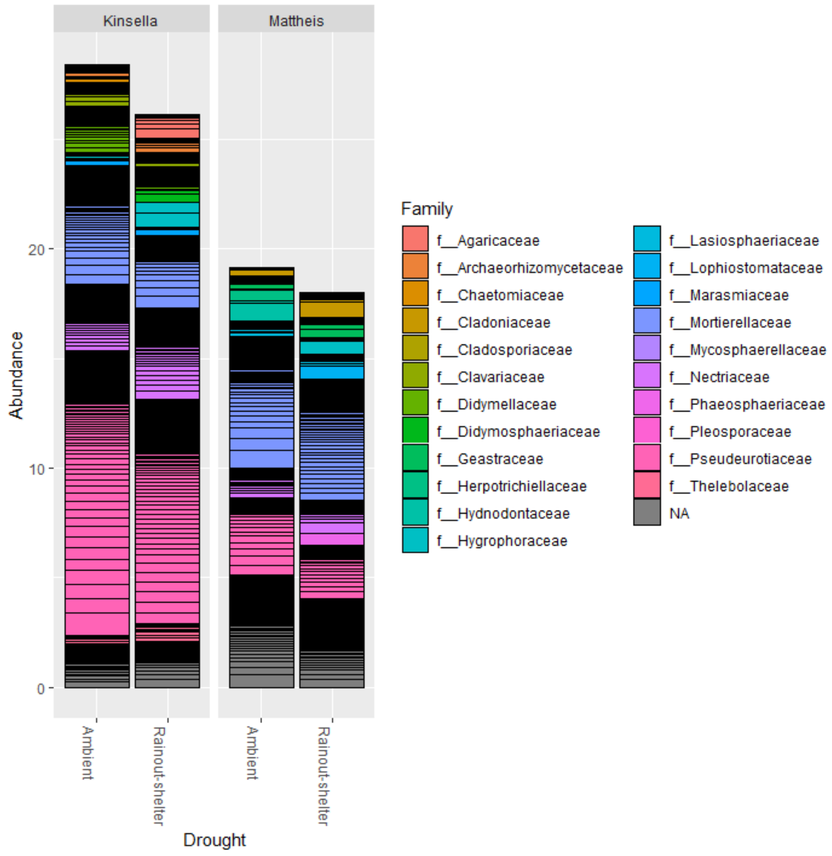


Figure 28: Abundance of bacterial and fungal (bottom) OTUs under different



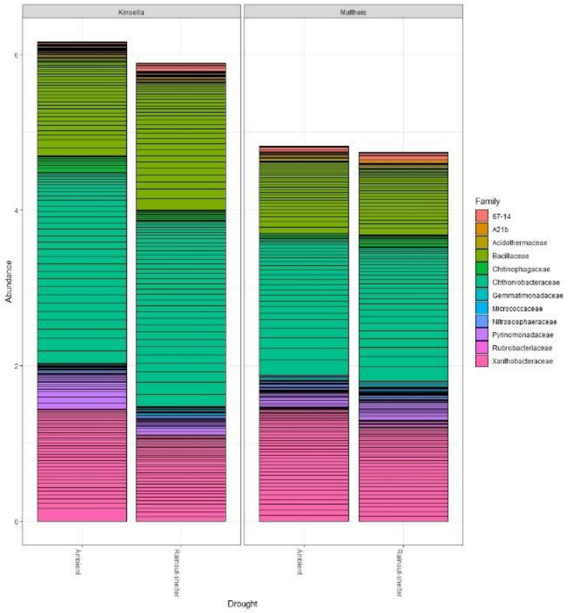


Figure 29: Fungal and bacterial OUT abundance under ambient and drought conditions at Kinsella and Mattheis.

Overall community diversity metrics were unaffected by treatments and diversity measures did not change ($p>0.05$) and compositon did not change in response to treatments either, although there were differences at the site level between Kinsella and Mattheis (Figure 30).

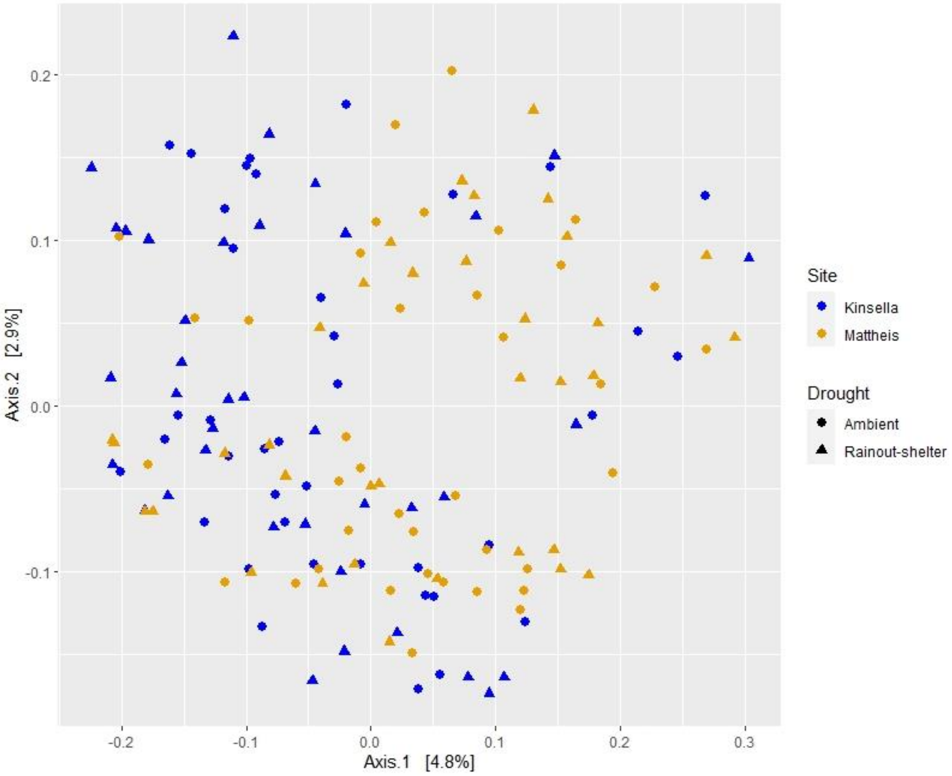


Figure 30: Non-metric multi-dimensional scaling ordination of bacterial communities under ambient and drought conditions at Kinsella and Mattheis.

NDVI: NDVI was intended to serve as a potential indicator of plant stress or productivity. While we found that NDVI did predict production across sites, we did not find that it was a good predictor of production within sites or in response to treatments (Figure 31), as it was only correlated with ANPP at 2 of the 7 locations. Hence, while we may return to examine this measurement in more depth later on, we have left its analysis for the time being.

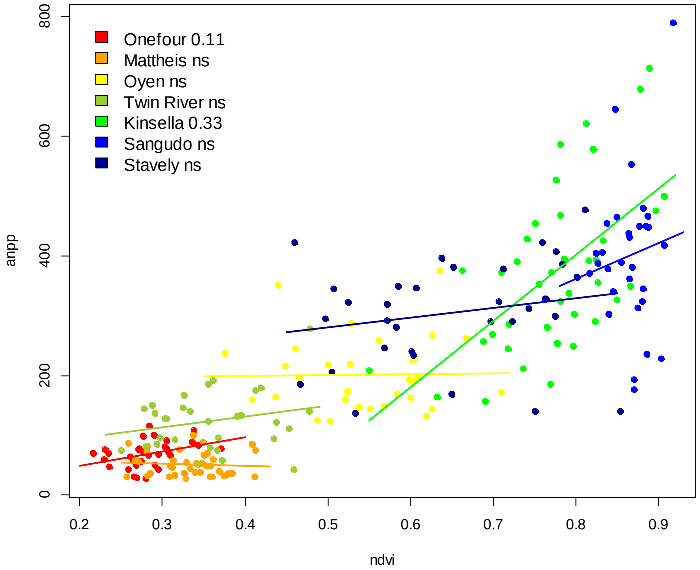


Figure 31: Relationship between NDVI (normalized difference vegetation index) and above ground net primary production (ANPP, g/ m2). Each site is represented by a different colour given in the legend, following the site is the R2 value for generalized linear model describing the relationship for each site, ns = "not significant", where the value is provided p<0.05.

Effect of grazing on forage sensitivity to changes in precipitation. 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 precipitation (Sloat et al., 2018, Diffenbaugh et al., 2015). Yet, the sensitivity of key ecosystem functions to these co-occurring stressors is often overlooked productivity (Godde et al., 2019, Sloat et al., 2018, Smith et al., 2009, Maurer et al., 2020). As expected, we found a direct, positive relationship between precipitation and ANPP (Figure 32). Additionally, we found that grazing increased ANPP sensitivity to interannual changes in precipitation (Figure 33). 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 (Figure 34). 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 (Figure 33). Similarly, grazing reduced ANPP during dry years but had no effect during wet years (Figure 35). 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.

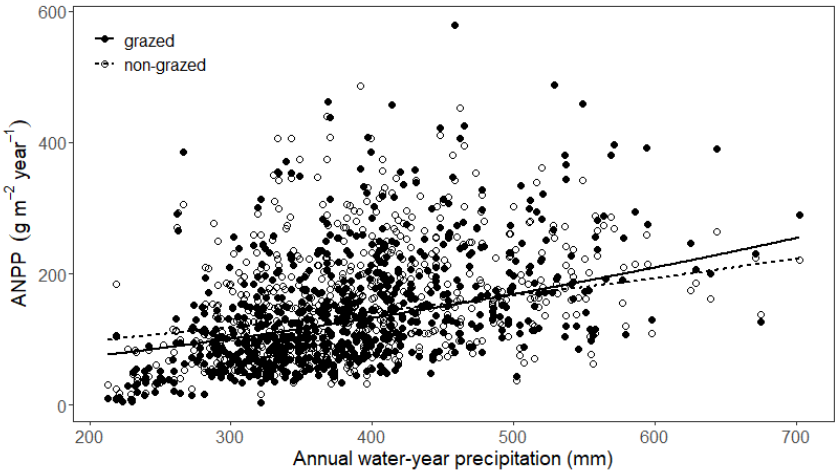


Figure 32: The relationship between annual water-year precipitation (September 1-August 31) and aboveground net primary production (ANPP) across 31 sites with pairs of long-term grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation (Table S1). Lines are linear mixed-effect model fits for grazed and non-grazed locations.

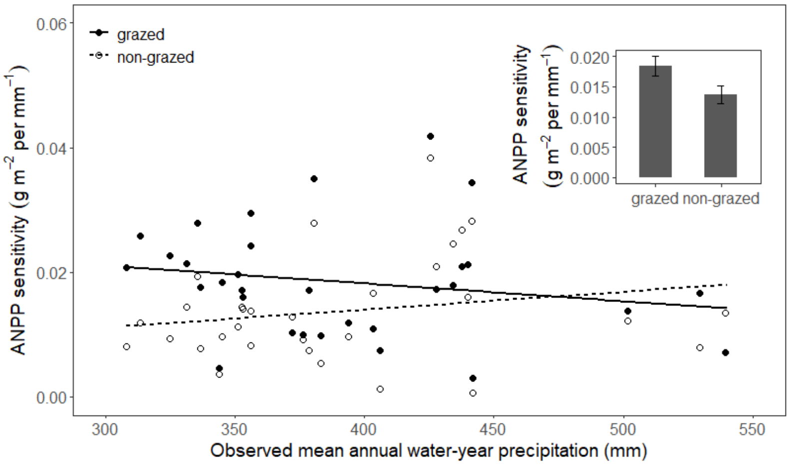


Figure 33: The relationship between observed mean annual water-year precipitation (September 1-August 31) and ANPP sensitivity. Data points represent 31 sites with pairs of grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation. Lines 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.

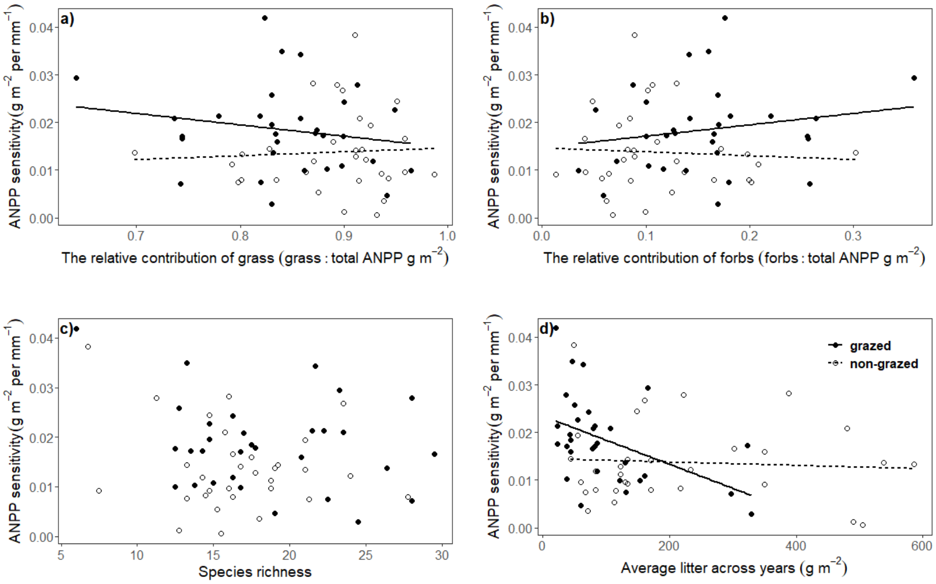


Figure 34: The relationships of ANPP sensitivity with the relative contribution of grass (a) and forbs (b) biomass to total ANPP, species richness (c), and litter (d), respectively. Data points represent 31 sites with pairs of grazed and non-grazed locations, which experienced substantial variability of precipitation during the period of observation.

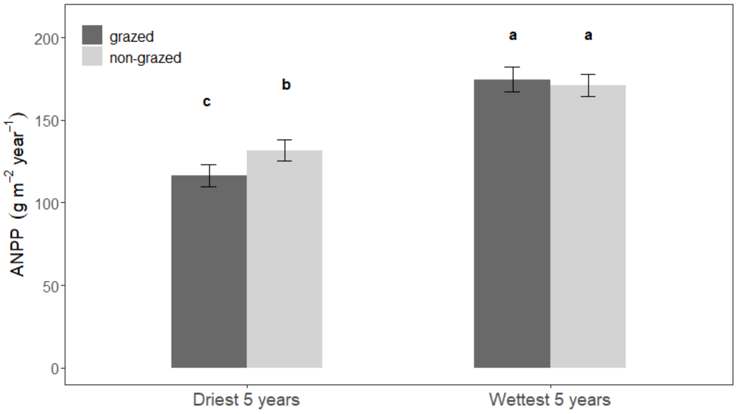


Figure 35: The effects of grazing on ANPP (means ± SE) during the five driest and five wettest years on record for 31 sites that experienced substantial variability of precipitation during the period of observation (see Figure S2). Bars sharing a letter are not statistically different ($p > 0.05$).

Response of biological soil crusts to drought and grazing. Biological soil crusts (BSCs) are communities on the soil surface composed primarily of lichens, bryophytes and cyanobacteria (cite a source for definition). Members of BSC communities often are tolerant to conditions to which vascular plants are sensitive, and can occupy up to 75% of cover in drylands like grasslands (Ferrenberg, Tucker, & Reed, 2017). BSCs are critical contributors to ecosystem function in drylands, however, projected changes in climate and land use intensification may alter these communities (Ferrenberg, Reed, & Belnap, 2015), threatening the sustainability of current grassland ecosystem function. BSCs play important roles in soil stabilization and formation (Belnap & Büdel, 2016), regulation of the hydrologic cycle (Chamizo, Belnap, Eldridge, Cantón, & Malam Issa, 2016), regulating seed establishment and germination (Li, Jia, Long, & Zerbe, 2005), and global carbon and nitrogen cycling (Porada, Weber, Elbert, Pöschl, & Kleidon, 2014; Poulter et al., 2014). Projected climate conditions for the next 50 years in the Canadian Prairies are anticipated to increase drying (Tam et al., 2019) and increase climatic variability, with concomitant increases in extreme wet and dry years, and an increase in the frequency of extreme weather events (Kharin & Zwiers, 2000; Kharin, Zwiers, Zhang, & Hegerl, 2007; Mladjic et al., 2010). Given the important role of BSCs in grassland ecosystem function, it is important to understand their response to drought under different management practices. Thus, we examined the response of BSC (namely Selaginella densa, lichens and bryophytes to the treatments). Generally, the effects of treatments were site specific, possibly owing to the site-specific communities at each site (Figure 36), and the general shift in communities from being lichen dominated to moss dominated as sites became more mesic. Through the duration of the experiment, defoliation treatments had little effect on the BSC community, but drought limited BSC growth, particularly Selaginella Densa, and increase moss cover which may be an indirect effect mediated by reductions in vascular plants (Figure 37). None the less, results indicated general sensitivity to drought, but fewer effects of defoliation.

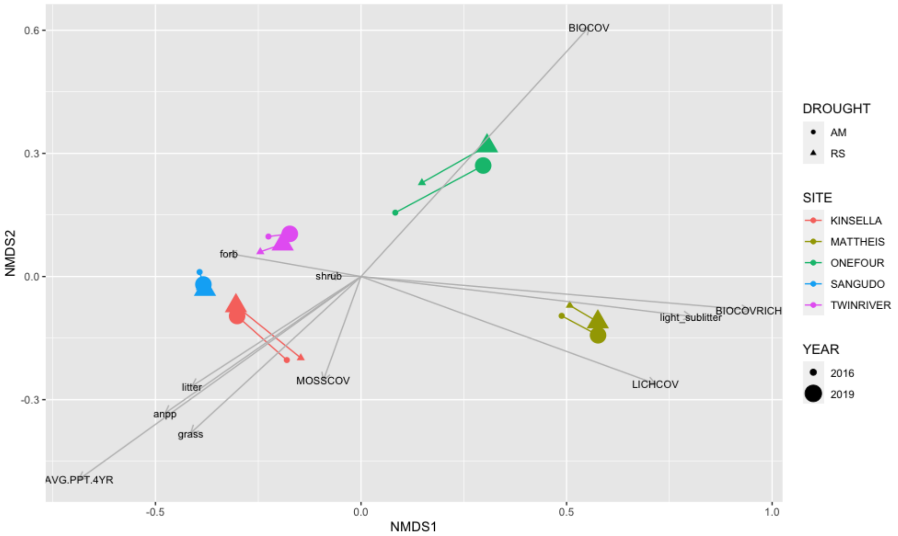


Figure 36: NMDS ordination of biological soil crust communities at five locations in Alberta's grasslands over a three year period. The results suggest that communities varied in their sensitivity to drought treatments.

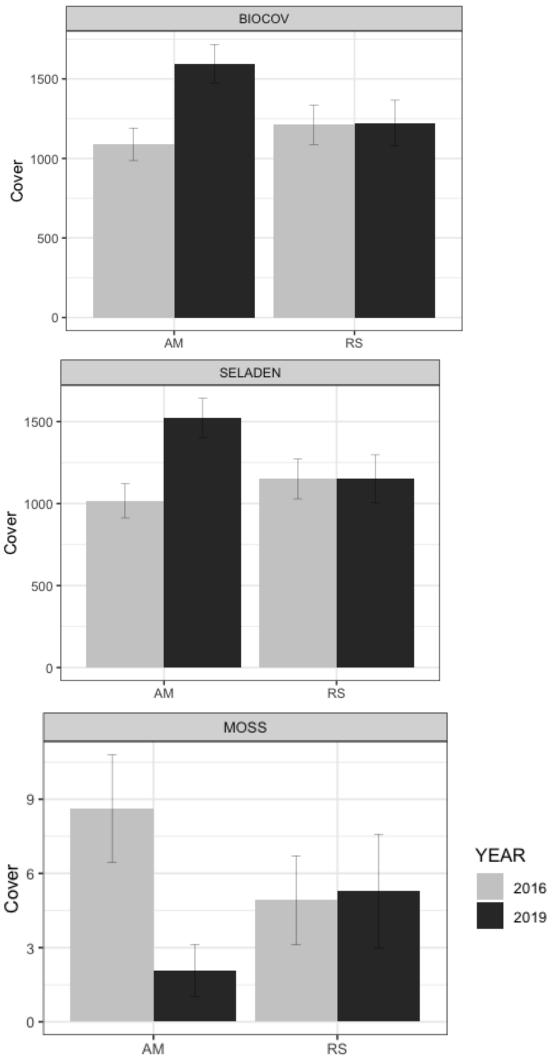


Figure 37: Change in total BSC, Selaginella densa and moss over three years of drought treatment. Results suggest that under ambient conditions total BSC response was driven largely by Selaginella, and that moss cover increased with rainout shelters, which is unexpected, but may be due to changes in the vascular plant community.

Effect of drought and grazing on forage quality: These results are still incomplete although all samples have been analyzed. Our preliminary analyses suggest that both drought and grazing treatments altered the concentration on nitrogen in plant tissue. However, effects varied between sites (**Error! Reference source not found.**, Figure 38: The interaction effect of drought and site on mean plot protein concentration. The different clipping treatments affected protein content at Kinsella but not Matheis. Rain-out treatment increases the mean plot protein concentration at Kinsella but decreases mean plot protein concentration at Mattheis.). At Matthies, clipping had no large effect on plant tissue nitrogen concentration, but at Kinsella the protein equivalent ranged from 8.5 to 12.5 percent, with plots that were more heavily clipped in the spring having greater protein content. Rainout shelters increase protein content at Kinsella, while they reduced protein content at Mattheis. The pattern at Kinsella is contrary to prior work from the site that indicated quality decreased with reduced rainfall (White et al. 2014). The reason for the pattern is not yet clear, but may be due to differences in the vegetation types between the two study locations within the ranch.

Regardless, this highlights the ongoing theme of our research that site responses are highly individual. Such changes in nitrogen concentration can affect long-term soil carbon storage, if nitrogen availability limits the activity of microorganisms breaking down organic material (Le and Carlyle, 2018).

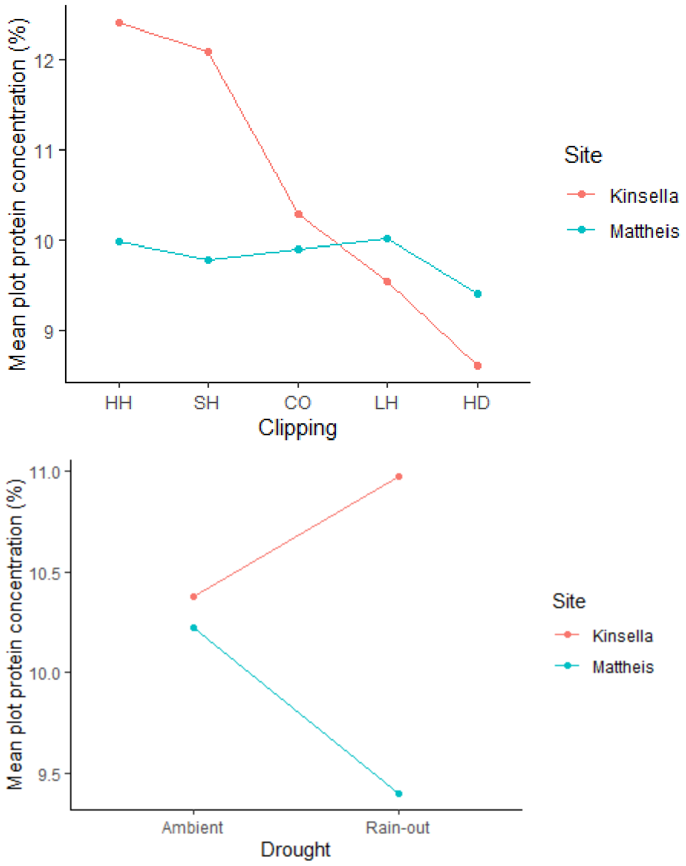


Figure 38: The interaction effect of drought and site on mean plot protein concentration. The different clipping treatments affected protein content at Kinsella but not Matheis. Rain-out treatment increases the mean plot protein concentration at Kinsella but decreases mean plot protein concentration at Mattheis.

ADVANCEMENTS MADE TOWARD COMMERCIALIZATION, COMMERCIAL DEPLOYMENT OR MARKET ADOPTION

There is limited commercialization opportunity directly associated with this project. However, the project will contribute to a growing body of literature around grazing management in Alberta's (and the rest of the Canadian Prairie) grasslands as a means to reduce GHG. Since this project began there have been significant steps made to implement carbon offset protocols related to grassland conservation and grazing management.

Project Challenges and Delays

The project's end date was moved back for three reasons. First, due to project savings we were able to include an additional field season of sampling to the project – sampling had been planned to conclude in 2018, but 2019 was the final year. The addition of another year of data is valuable in order to get a better picture of ecosystem response to the experimental manipulations. Secondly, one of the PhD students on the project experienced a number of personal challenges including medical an family circumstances that delayed completion of some parts of the project. Thirdly, COVID had a significant impact because our ability to access University labs was reduced, and even prohibited at times, and there were delays in the arrival of critical supplies needed for lab work. Additionally, COVID led to the cancellation of a number of conference opportunities that we had planned to present results from this project at. However, despite these challenges, all project objectives were met, although the PhD students' theses are not yet complete, but both are expected to finish in December 2021.

GREENHOUSE GAS BENEFITS

We have demonstrated variation in greenhouse gas flux and indicators of carbon change in response to grazing treatments and rainfall conditions. Using these indicator values we can suggest potential grazing scenarios that could be adopted by cow-calf producers in Alberta's grasslands. These indicators are important because offset programs are in development for the conservation and sequestration of soil carbon. The current carbon tax in Canada will soon be $50/tonne and will rise to $170 by 2030, but pricing for carbon offsets has less certainty and there may be costs associated with registration and validation. None-the-less there will likely be opportunities for cow-calf producers to receive payment for carbon offsets in the future. The implementation of such programs will not only benefit cow-calf producers, the Canadian Prairie and especially grasslands are threatened by conversion to other landuses which leads to loss of biodiversity. Canada is committed to protection of biodiversity and these scenarios may prevent the conversion of privately owned grasslands and the consequent loss of biodiversity.

To review, we found no change in soil carbon values, but there were scenarios where indicators of carbon change responded to different grazing scenarios. In general, plant biomass (shoots and roots) increased under fall grazing, compared to other grazing scenarios. Furthermore, root growth and turnover increased under more frequent grazing, which may release more exudates that contain carbon and fuel microbial activity. Indeed, this pattern was reflected in soil enzyme activity, where carbon cycling enzymes, microbial abundance and carbon dioxide flux increased under treatments that were clipped twice.

Furthermore, we demonstrated that the effects can change under drought, meaning that these scenarios may be sensitive to a future affected by climate change where drought is more frequent. However, the responses to drought among response variables were not correlated meaning that predicting overall response in the future is challenging. Additionally, there is an important note of caution on interpreting these values, as reported above soil gas flux is more responsive to treatments than soil carbon thus, we are presenting an incomplete picture of the overall carbon balance and the estimates presented here are the GHG released from the system without accounting for the GHG sequestered by the system. Greater soil GHG flux is often the result of a more productive ecosystem and it is the balance of carbon fixed through photosynthesis and carbon lost through plant and microbial respiration that ultimately determine net sequestration.

OVERALL CONCLUSIONS

The results from this study paint a complex picture of grassland ecosystem goods and service response to different defoliation treatments under drought conditions. Overall, our results suggest that responses are highly idiosyncratic, likely the result of specific weather, climate, soil or vegetation properties at a particular site. Consequently, it is challenging to make consistent recommendations for grazing management that will lead to enhanced resistance of forage to drought or mitigation strategy for GHG reductions. Our results did indicate that the combination of a light early season defoliation, followed by a fall defoliation led to an increase in soil CO2 flux from soils, while all other defoliation treatments led to reductions. In line with a recent and evolving line of thought is the observation that the responses of various components of these ecosystems are decoupled from each other and are not correlated or equally sensitive to these changes. Importantly, we found that grazing increased the sensitivity of plant production to reduced rainfall and that these effects were exaggerated in drier years and at drier sites. Increased levels of litter mitigated these effects to some degree, suggesting that grazing management that reduces the impacts on plant production and allows the development of a litter layer may be an important strategy in the future which is expected to have higher frequency of drought.

NEXT STEPS:

We have identified that more arid grasslands in Alberta are more sensitive to the combined effects of drought and grazing, and that generally some grazing regimes alter indicators of carbon sequestration more than others. Future work should focus on working with cow-calf producers to enact and test grazing management scenarios identified here that may both increase carbon storage and provide more resilient forages under drought. This could be accomplished in two ways, 1) by working with producers who have been practicing different scenarios and 2) preferably, by instituting new grazing management on existing farms that would enable a long-term tracking of C change. Some opportunity is available to do this work, such as through the Agriculture and Agri-Food Canada Living Labs initiatives, which we are currently involved in developing. Furthermore, since implementation of this project carbon offset protocols have made some progress, future work should engage with organizations involved in these endeavours to ensure that not only are beneficial management practices recognized but that measurements taken in research projects align with verification protocols.

COMMUNICATIONS & SCIENTIFIC ACHIEVEMENTS:

Communications on this project has been extensive: 4 presentations have been made to industry groups, 4 presentations were made at scientific conferences and there have been in two articles related to the project in trade publications. Furthermore, one scientific paper has been published, one more is in revisions and an additional 5 that are part of student theses are in preparation for submission for publication. Our team will continue to communicate the results from this project as journal papers are published and opportunities arise to present at conferences and events.

Scientific publications:

1. Batbaatar A, Bork EW, Broadbent T, Alexander M, Cahill JF, Carlyle CN. 2020. Grazing alters the sensitivity of plant productivity to precipitation in northern temperate grasslands. Journal of Vegetation Science.
2. Batbaatar A, Carlyle CN, Chang SX, Bork EW, Cahill JF. In Revision. Multi-year drought altered plant species composition more than productivity across northern temperate grasslands. Journal of Ecology.

3. Batbaatar A, Carlyle CN, Chang SX, Bork EW, Cahill JF. In prep. Interactive effects of drought and defoliation on above and below ground plant biomass and growth.
4. Batbaatar A, Carlyle CN, Chang SX, Bork EW, Cahill JF. In prep. Growing season drought and defoliation interact to affect aboveground biomass more than root biomass across northern temperate grasslands
5. Rajper A, Chang SX, Bork EW, Willing B, Cahill JF, Carlyle CN. In Prep. Defoliation pattern effects on soil GHG flux under drought conditions.
6. Rajper A, Chang SX, Bork EW, Willing B, Cahill JF, Carlyle CN. In Prep. Defoliation pattern effects on soil extra cellular enzyme activity under drought conditions.
7. Rajper A, Chang SX, Bork EW, Willing B, Cahill JF, Carlyle CN. In Prep. Microbial community response to defoliation pattern effects on soil GHG flux under drought conditions.

Scientific presentations:

1. Carlyle CN. May 2017. Climate and grazing management contribute to uncertainty about carbon storage and sequestration in the Canadian prairies. Canadian Society for Ecology and Evolution.
2. Carlyle, CN. March 2018. Cattle grazing effects on grassland ecosystem goods and services: forage production, carbon cycling and biodiversity. University of Toronto, Mississauga, ON.
3. Carlyle, CN. July 2018. Grazing management provides an opportunity to conserve the Canadian prairies through carbon storage. North American Congress for Conservation Biology. Toronto, ON. ~30 attendees
4. Rajper AM, Amgaa B, Bork EW, Willing BP, Chang SX, Cahill JF, Carlyle CN. 2018. The interactive effects of manipulated drought and grazing on extracellular enzyme activity in grassland soils across Alberta, Canada. American Geophysical Union, Fall Meeting 2018, Washington, DC.

Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.):

1. Hewins DB, Carlyle CN, Bork EW. 2016. Rangeland Ecosystem Goods and Services: a review of current research efforts in Alberta. Canadian Forage and Grassland Association. Winnipeg MB. November.
2. Carlyle CN. 2016. Ecosystem goods and services in Alberta's grasslands. Saskatchewan Strategic Advisory Council on Forage and Crops. Saskatoon SK. November.
3. Carlyle CN. 2016. Field presentation to ranchers and general public at UofA Mattheis Research Ranch Field Day. July.
4. Carlyle CN. Alberta's rangelands and ecosystem goods and services. Rancher's Rangeland Management Workshop, Nature Conservancy Canada. Twinn Butte, AB. June.
5. Carlyle CN. 2017. Grassland management to reduce greenhouse gasses. SPARK (ERA, Alberta Innovates) Edmonton AB. November.
6. Cahill JC. 2017. What plants talk about: A behavioral understanding of plants and soil. Alberta Grazing and Soil Health Conference. December.
7. Carlyle CN. 2017. Rangeland EG&S: A review of current UA research efforts supporting beef sustainability. Canadian Roundtable on Sustainable Beef. Edmonton, AB. December.
8. Bork EB, Carlyle CN. Rangeland Ecosystem Goods and Services: a review of current research efforts in Alberta. Canadian Forage and Grassland Association: Carbon Protocol Workshop. Guelph, ON. Remote presentation.
9. Bork, EB. June 2018. Field presentation to teachers at the Mattheis Research Ranch.
10. Carlyle, CN. November 2018. Cattle can increase carbon sequestration and biodiversity. Canadian Forage and Grasslands Association Annual Meeting. Calgary, AB. ~100 + attendees
11. Carlyle, CN. November 2018. Opportunities for grassland conservation and carbon offsets. Alberta Biodiversity Monitoring Institute: Ecosystem Services Symposium. Edmonton, AB. ~ 60 attendees

12. Carlyle CN. June 2018. Ecosystem goods and services from rangelands in the Canadian Prairie: carbon storage and biodiversity. Saskatchewan Stockgrowers Association, Annual General Meeting. Swiftcurrent, SK. ~80 attendees.
13. Various speakers. July 2018. Three field days at the study sites near Oyen, Onefour and Stavely, AB. Events organized by AAFC. ~15 attendees at each event
14. Carlyle, CN. February 2019. The benefits of cattle for carbon storage and biodiversity in the Canadian Prairie. Canadian Agri-food Policy Institute. Calgary, AB. ~100 attendees

Media activities (*e.g.*, radio, television, internet, etc.):

The project was mentioned in an article that ran in 2 different trade publications:
Melchior J. March 2016. Making hay of environmental goods and services. Canadian Cattlemen: the beef magazine and Country Guide (Western Edition). http://www.agcanada.com/canadiancattlemen/wp-content/uploads/2016/02/CCT160307.pdf#_ga=1.268948081.1634208799.1454959483

Abstract and Keywords

The Canadian prairies store large amounts of carbon in grassland soils and have the potential to sequester more carbon and reduce atmospheric greenhouse gases (GHG). These ecosystems are also used for cattle grazing, which has the potential to alter the ecosystem in a way that stores more carbon. However, climate change threatens the economic stability of cow-calf production through increased drought risk that reduces forage availability and ultimately profitability, and conversion of grasslands to cropland will release large amounts of stored carbon to the atmosphere. Thus, cattle management that manipulates grassland ecosystems in a way that increases soil carbon will be beneficial for reduction of GHG, but new management must also increase the stability of forage production to ensure cow-calf operations are profitable. Thus, the purpose of this project was to examine whether different grazing systems, tested as different timing and intensity of plant defoliation, are beneficial for GHG reduction and forage production under both ambient and drought conditions at different locations within Alberta's grasslands.

We conducted an experiment (2016-2019) at seven grassland locations in Alberta that represented different grassland types, in which we altered rainfall, and simulated grazing by clipping the vegetation. We created an extreme drought using rainout shelters and had five clipping treatments including no-clipping, clipped in fall only, clipped heavily in spring and fall, clipped lightly in spring and heavily in fall and a spring only clipping. Many components of the ecosystem were measured including: plant biomass, plant community composition, root growth and mass, greenhouse gas flux at the soil surface, soil microbial community activity, soil carbon content and normalized difference vegetation index (NDVI, a measure of plant stress).

We found that most responses were site specific although a few general trends emerged. As expected, drought and more intense clipping reduced plant biomass. Wetter grasslands had a larger reduction in plant biomass production due to drought than dry sites; however, dryer sites were more sensitive to drought when defoliated. When examining patterns among response variable there were few correlations, meaning that assumptions about matched response (e.g. plant biomass and soil carbon responding similarly) need to be made with caution. We found that GHG emissions from soils were differentially affected by one clipping treatment, the treatment where vegetation was clipped lightly in spring and heavily in fall increased carbon dioxide emission under drought conditions while all other clipping treatments reduced emissions, this effect was more pronounced at drier sites compared to wetter sites. Similar to the plant responses, we found that microbial activity was idiosyncratic across sites but was largely resistant to change. One site, located in the aspen parkland, was very responsive to the treatments and the underlying reason for this has not yet been determined. Enzymes that did respond tended to be associated with the breakdown of carbon compounds and generally were reduced by the drought treatment, and had a positive relationship with soil moisture.

This project has demonstrated that Alberta's grasslands are largely resilient to change, but that under some circumstances may be susceptible to variation in grazing management, particularly under drought that will lead to the potential loss or slower accumulation of soil carbon. In particular, further investigation of grazing management in the drier regions of the province, in collaboration with producers using a variety of grazing management, could identify specific management practices to improve carbon sequestration and enhance cow-calf production.

Key words: Drought, Grasslands, Grazing, Soil Organic Carbon, Vegetation,

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