THE IMPACT OF ALTERED PRECIPITATION PATTERNS ON PLANT PRODUCTIVITY, SOIL RESPIRATION AND WATER-USE EFFICIENCY IN A NORTHERN GREAT PLAINS GRASSLAND

LAVINIA HAASE
Bachelor of Science, University of Applied Science Eberswalde, 2012

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LAVINIA HAASE

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Dr. Lawrence B. Flanagan Supervisor	Professor	Ph.D.
Dr. Stewart B. Rood Thesis Examination Committee Member	Professor	Ph.D.
Dr. Robert Laird Thesis Examination Committee Member	Associate Professor	Ph.D.
Dr. Dan Johnson Chair, Thesis Examination Committee	Professor	Ph.D.

ABSTRACT

Precipitation patterns are expected to shift towards larger but fewer rain events, with longer intermittent dry periods, associated with climate change. The larger rain events may compensate for and help to mitigate climate change effects on key ecosystem functions in semi-arid grasslands. I experimentally manipulated the amount and frequency of simulated precipitation added to treatment plots that were covered by rain shelters, and measured the response in plant productivity, soil respiration and water-use efficiency in a native grassland near Lethbridge, Alberta. The observed responses were compared to the predictions of a conceptual ecosystem response model developed by Knapp et al. 2008. Two experiments were conducted during 14 weeks of the growing season from May-August. The first experiment applied total growing season precipitation of 180 mm (climate normal), and the second experiment applied total precipitation of 90 mm (reduced amount). In both experiments, precipitation was applied at two frequencies, one rain event every week (normal frequency) and one rain event every two weeks (reduced frequency). Plant productivity decreased in response to larger but fewer rain events in the first experiment, but was not significantly different in the second experiment. Soil respiration rate was significantly higher for the larger but fewer rain events in the second experiment, as well as for the normal compared to the reduced amount treatments. Stable carbon isotope composition of plant tissue was largely insensitive to precipitation alterations, but showed significantly lower $\delta^{13}C$ values for the normal compared to the reduced amount treatments. The results of this study have implications for understanding the mechanisms underlying ecosystem responses to anticipated precipitation change in the Great Plains.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANPP	Aboveground net primary production
MAP	mean annual precipitation
MAT	Mean Annual Temperature
GSP	Growing Season Precipitation
NPP	Net primary productivity
BNPP	Belowground net primary productivity
NEE	Net ecosystem exchange
WUE	Water-use efficiency
CO_2	Carbon dioxide
$\delta^{13}C$	Stable carbon isotope composition of plant tissue
^{13}C	Carbon 13
VPD	Vapor pressure difference
NN	Normal – Normal
NR	Normal – Reduced
RN	Reduced – Normal
RR	Reduced – Reduced
PAR	Photosynthetically active radiation
Gcc	The green chromatic coordinate
RGB	Red, green and blue
DOY	Day of year
ETP	Experimental time period
STP	Plant senescence time period
LAI	Leaf area index
R_h	Heterotrophic respiration
PPT	Precipitation

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction

Climate change is impacting all ecosystems globally leading to alterations of ecosystem services at an alarming scale and pace (Smith et al. 2015). Global change encompasses simultaneous changes in multiple environmental factors that impact ecosystem processes. As all components of an ecosystem are interconnected, changes in climatic drivers have the potential to modify the structures and functions of our ecosystems with huge implications for all organisms dependent on them (Fuhrer 2003). Ecosystem responses to these climatic changes are often nonlinear, which makes them hard to predict and emphasizes the need for further research (Zhou et al. 2008).

Grasslands make up one third of the Earth's terrestrial surface and vary greatly in productivity on a spatial as well as on a temporal scale (Knapp et al. 2001). Rising greenhouse gas concentrations are expected to increase the mean global surface temperature between 1.1 and 6.4 °C by 2100 (IPCC. 2007). Elevated global temperatures will result in drier conditions in semi-arid grassland regions, which are particularly sensitive to changes in precipitation patterns and water availability, significantly impacting their ecosystem processes (Knapp and Smith 2001, Huxman et al. 2004).

This research intends to examine the effects of changing precipitation patterns that are caused by global climate change and their effect on multiple aspects of ecosystem function, including plant productivity, soil respiration and water-use efficiency in a southern Alberta native grassland.

For the purpose of this research I am going to introduce concepts that are important for the context of my experiment in the following paragraphs. This includes contrasting

the effects of precipitation and temperature on plant productivity in grasslands, introducing expected climate change-induced alterations of precipitation patterns and the intensification of droughts, and their anticipated impact on ecosystem processes including plant productivity, soil respiration and water-use efficiency.

1.2. Contrasting precipitation and temperature effects on grassland productivity

Precipitation has been identified as the primary limiting factor of ecosystem services (Sala et al. 1988) with aboveground net primary production (ANPP) showing the highest sensitivity to changes in water availability (Knapp et al. 2001, Huxman et al. 2004). Previous research has shown that 90% of the variation in ANPP responses are caused by changes in mean annual precipitation (MAP), which makes precipitation a stronger driver than temperature for changes in grassland productivity (Mowll et al. 2015). Temperatures are expected to have an indirect effect on grasslands by negatively influencing the water balance (Penuelas et al. 2007, Xu et al. 2012, Dulamsuren et al. 2013), making temperature - ANPP relationships more complex than precipitation - ANPP relationships (Vicente-Serrano et al. 2013). Therefore, explaining sensitivities of ecosystems to temperature changes can be challenging. Overall precipitation has been proven to be the stronger driver for ANPP variations (Sala et al. 1981, Heitschmidt et al. 2005, Knapp et al. 2008) but growing season temperature has been shown to also play a role in the sensitivities of ecosystems, although it is a secondary factor. This is a result of inter-annual coefficient of variation in temperature being lower than in precipitation, which also makes the effects on ANPP harder to detect. The indirect effects of temperature on ANPP become apparent through the strong correlation of mean annual temperature

(MAT) with the distribution of species with C_3 and C_4 photosynthetic pathways (Wittmer et al. 2010). Previous research has shown that the responses of ANPP to temperature changes are lagging the more immediate responses to precipitation changes due to the slower reactions of community composition and species distribution to changes in temperature (Smith et al. 2009, Vermeire et al. 2009). Warming experiments can also advance the onset of the growing season, thus lengthening the time frame for plants to grow and reproduce, as long as water availability is not limiting (Cleland et al. 2006, Hovenden et al. 2008).

1.3. Changes in precipitation patterns and drought occurrences – sensitivities and resiliencies of grassland ecosystems

Extreme precipitation years are expected to occur more frequently in the future according to climate models (IPCC 2007, Singh et al. 2013). One major challenge in defining extreme years is that they are based on historic precipitation variabilities that are associated with high spatial variability (Jentsch 2006, Knapp et al. 2017). Previous research has shown that there are other factors that define an extreme year besides precipitation amount (Gilgen and Buchmann 2009). These factors include, (i) rain event size, (ii) rain event frequency, as well as the impact of previous year's conditions (legacies) (Oesterheld et al. 2001, Wiegand et al. 2004).

An important predicted trend for precipitation patterns is larger rainfall events with longer dry periods between rain events (IPCC 2007, Knapp et al. 2008). This is associated with an intensification of the global hydrological cycle as caused by global warming (Huntington 2006, IPCC 2007). Elevated temperatures have increased annual

precipitation by 10% over the last century in the contiguous United States and this is correlated with an intensification of large rain events (Karl and Knight 1998). Alterations of intra-annual precipitation patterns have already been reported globally, including increased frequencies of wet days in parts of North America, Europe and Southern Africa (New et al. 2001, Groisman et al. 2005).

Changes in precipitation patterns in combination with earlier onset of snowmelt and higher temperatures will lead to shifts in the timing of ecological processes that may increase the intensity of summer droughts (Polley et al. 2013). Previous research has shown that the responses to drought in terrestrial ecosystems vary immensely across biomes (Byrne et al. 2013), depending on the magnitude of the drought and the specific drought sensitivity of each ecosystem (Smith et al. 2009, Fraser et al. 2013). A directional response to drought has been shown to be the most common response pattern for herbaceous ecosystems. This is described as continuous change of species interactions, community dynamics, and ecosystem processes in a specific direction over time in response to chronically altered climate conditions. This response pattern has been observed for long-term rain manipulations while short-term manipulations often show 'no effect' (Smith et al. 2015). Time, as a factor, greatly impacts whether ecosystem sensitivities to drought can be detected, because resource manipulations do not immediately lead to extreme changes in resource availabilities that exceed species tolerances and resource thresholds (Aber et al. 1998). Grasses are known to be water-wasters that rapidly deplete soil water and consequently wilt faster than shrubs or trees under drought stress. However, grasslands can regain functioning faster than shrublands and forests, making grasslands more resistant against drought (Albert et al. 2011).

It has also been shown that ecosystem responses to drought can be buffered by species diversity, because asynchrony among species activity patterns can compensate or reduce losses in overall ecosystem metabolism to environmental stresses (Diaz and Cabido 2001, Hautier et al. 2014). The basic idea is, the more asynchronously the species develop in a community, the more stable the ecosystem is. These stabilizing mechanisms vary across precipitation gradients, with a higher magnitude of stability expected in arid grasslands (Hallett et al. 2014). At sites with higher MAP dominant species play a more important role in compensating for plant productivity deficits (Hallett et al. 2014).

Jones et al. (2016) reported that changes in plant community composition in a native tallgrass prairie in northeast Kansas, USA were not detectable until ten years after the initiation of the rain manipulation experiment. A stable species composition, and therefore a plant community resistant to altered precipitation patterns, was observed in the years previous to that. Possible reasons explaining high community stability and minimal changes in community species composition may be mitigation processes through genetic diversity and diversity in functional traits of the dominant grasses. The effect of dominant species in a plant community, along with changes in species diversity and composition are important factors when assessing the sensitivities and resiliencies of grassland ecosystems to chronic alterations of precipitation patterns on a long-term scale.

1.4. The effect of changing precipitation patterns on ecosystem processes

Change of precipitation patterns towards more extreme rainfall events and more frequent occurrences of intense droughts will significantly impact ecosystem processes (IPCC 2007, Knapp et al. 2008). Increased frequency of large precipitation events is

particularly important in arid ecosystems because it may lead to an increase in soil water storage and a decline in soil water evaporation losses (Knapp et al. 2008). Furthermore, larger rain events appear to have no negative impact on surface runoff and soil water availability (Loik et al. 2004); instead they are expected to promote the growth of deep-rooted plants (Kulmatiski and Beard 2013), increase the importance of hydraulic redistribution (Yu and D'Odorico 2014), counteract the loss of ecosystem functions like ANPP (Cherwin and Knapp 2012) and possibly alter water-use efficiency (Knapp et al. 2008). Overall these changes in frequency and intensity of precipitation will alter the supply of water to terrestrial ecosystems significantly, even if no net change in the annual precipitation occurs (Knapp et al. 2008). Shifts in precipitation frequencies and intensities are expected to impact the abundance of within-season drought, evapotranspiration and the amount of runoff from soils (Fay et al. 2003, MacCracken et al. 2003) and have a direct effect on how water moves through the soil (McAuliffe 2003). Altogether these changes will have a significant influence on the amount of water available for plants and soil biogeochemical processes, thereby altering ecosystem productivity, soil respiration and water-use efficiency of plants (Weltzin et al. 2003). Changes in precipitation patterns are going to be highly spatially and temporally variable and the response of ecosystems affected depends on the magnitude of change in precipitation as well as the ability of the ecosystem to buffer and/or adapt to the new climatic conditions (Smith et al. 2009, Beier et al. 2012).

1.4.1. Plant productivity

Less frequent rain events with higher rainfall intensity have been found to lead to significantly higher levels of ANPP in xeric (dry) ecosystems (Sala et al. 1992, Heisler and Knapp 2008). Therefore, larger rain events might be able to partially compensate for ecosystem productivity losses caused by low precipitation in dry years. Semi-arid grasslands in particular show little response to drought due to this compensation mechanism (Cherwin and Knapp 2012). In mesic (moist) ecosystems, ANPP is expected to decline in response to larger, less frequent rain events due to the increased time periods of water stress in between rain events (Heisler and Knapp 2008). For instance, larger but less frequent rain events led to an 18% decline in ANPP in a tallgrass prairie in Kansas, whereas ANPP was increased by 30% in a semi-arid grassland in Colorado by the same treatment. The mixed grass prairie in Kansas showed the highest ANPP sensitivity to larger, but less frequent rain events with a 70% increase in ANPP (Heisler-White et al. 2009). Previous responses of primary productivity to drought and altered precipitation patterns have been highly irregular, displaying asymmetric responses ranging from negative to positive (Fay et al. 2000, Penuelas et al. 2004, Swemmer et al. 2007, Jentsch et al. 2011). It is therefore of particular importance to investigate how sensitivities of ANPP in response to extreme events vary, depending on grassland type and regional climatic conditions. This will aid in the identification of new response patterns and increase the accuracy in forecasting the impact of precipitation changes on plant productivity.

1.4.2. Soil respiration and ecosystem carbon budgets

Changes in precipitation patterns and extended droughts will also have a significant impact on carbon cycling processes, as precipitation is recognized as a major driver of ecosystem photosynthesis and respiration (Sala et al. 1988, Del Grosso et al. 2008). The response of net primary productivity (NPP) is particularly important for understanding the consequences precipitation changes will have on carbon cycling processes, because NPP represents the carbon available for harvest or secondary growth of consumer organisms (Easterling et al. 2000, Fraser et al. 2013). NPP is described as the amount of biomass of living photosynthetic organisms in an ecosystem, and is made up of aboveground net primary production (ANPP) and belowground net primary production (BNPP). In this study I primarily focus on the response of ANPP, but it is essential to mention the importance of BNPP, for making proper assessments of total NPP (Wilcox et al. 2015). BNPP is known to often exceed ANPP in grassland ecosystems (Milchunas and Lauenroth 2001), especially under drier conditions, because of the high allocation of carbon to roots during water shortages (McCarthy and Enquist 2007). Experimental warming has been shown to significantly increase NPP due to the earlier onset and extended length of the growing season, which in turn enhances the mineralization of nutrients in the soil and acts to increase photosynthetic activity (Rustad et al. 2001, Wan et al. 2005, Wu et al. 2011).

I investigated the impact of altered precipitation patterns on soil respiration, as it is the largest source of CO_2 in terrestrial ecosystems and even minor variations in soil respiration will impact the ecosystem carbon budget. A general consensus is that climate warming will lead to an increase in soil respiration, contributing to higher total ecosystem

respiration rates and resulting in a net loss of CO_2 to the atmosphere. This will act as a positive feedback on atmospheric CO_2, further enhancing warming and climate change (Heimann and Reichstein 2008, Weaver 2008). Long-term effects of reoccurring droughts also influence soil respiration through changes in soil structure and soil microbial communities (Sowerby et al. 2008). Fluctuations in soil respiration rates are caused by biotic and abiotic factors that act directly and indirectly on respiration processes. Two main factors are the activity of plant roots and soil microbes (Flanagan and Johnson 2005, Fontaine et al. 2007, Bardgett 2011). Carbohydrates released from plant roots (exudates), stimulate the microbial activity in the rhizosphere, which is described as the area of highest soil respiratory activity (Fontaine et al. 2007, Bardgett 2011). Soil respiration is therefore influenced directly by increased soil microbial activity, which is stimulated by increases in temperature. Previous studies have shown that relatively small increases in temperature stimulate soil microbial activity directly, when soil moisture, carbon availability and enzyme activity are not limiting. These effects can result in a CO_2 loss from the ecosystem to the atmosphere, if photosynthesis is not stimulated enough to compensate for the loss of carbon through soil respiration (Davidson and Janssens 2006, Conant et al. 2011, Flanagan 2013). Particularly in northern climate zones, warmer temperatures in combination with elevated atmospheric CO_2 can stimulate photosynthesis rates, increasing the amount of carbon available in the soil for microbes (through root exudates and plant litter), which has an indirect effect on soil respiration (Wu et al. 2011, Drake et al. 2011, Phillips et al. 2011). However, soil moisture availability has been shown to be the primary factor controlling variations in soil respiration in semi-arid grasslands (Chen 2008, Chou 2008, Flanagan and Johnson 2005). This is due to soil moisture being the major driver of plant productivity in grasslands, resulting in

temperature effects being secondary when water availability is limiting (Sala et al. 1988, Knapp et al. 2001, Weltzin et al. 2003). Particularly in semi-arid ecosystems, the wetting of dry soils can increase the availability of microbial substrate (Huxman et al. 2004) and lead to rapid responses of microbes, stimulating soil respiration (Liu et al. 2002, Huxman et al. 2004, Chou et al. 2008). Soil respiration has also been shown to be particularly sensitive to changes in the timing of rainfall, independent of changes in rainfall amount (Harper et al. 2005). As moisture availability and temperature both influence ecosystem processes that impact soil respiration rates, their interactive effects need to be considered. The interactive effects of moisture availability and temperature have been shown to control grassland productivity and the net uptake of CO_2 at the grassland in Lethbridge (Flanagan and Adkinson 2011). Plant productivity and net carbon sequestration was stimulated by warmer temperatures during years of average soil moisture content. Furthermore, Flanagan and Johnson (2005) found that the temperature sensitivity of ecosystem respiration varied with changes in soil water availability, highlighting the interactive effects of temperature and moisture that affect plant productivity and ecosystem CO_2 exchange. Therefore, it is necessary to assess the effects of moisture and temperature on all processes that influence soil respiration directly and indirectly, including plant productivity, to make informed implications on its impact on ecosystem carbon budgets.

1.4.3. Water-use efficiency

Variations in the water-use efficiency (WUE) of plants also have an impact on ecosystem function through the alteration of plant physiological processes, such as

photosynthetic activity and stomatal conductance (Ponton et al. 2006). At the leaf scale, water-use efficiency (WUE) is defined as the ratio of carbon gain in net photosynthesis to water loss during transpiration and varies depending on variations in leaf gas exchange characteristics of plants and the ambient environmental conditions (Farquhar et al. 1989). Therefore, alterations in any of the two components, photosynthetic activity and/or stomatal conductance, lead to changes in WUE (Farquhar et al. 1989). Higher WUE at the leaf scale is achieved through a reduction of stomatal conductance, as it limits water loss to transpiration more than CO_2 assimilation (Farquhar & Sharkey 1982). At the ecosystem scale, evapotranspiration can be increased despite stomatal closure due to the interacting effects of varying leaf temperature, stomatal and aerodynamic conductance and dry air in the planetary boundary layer (Baldocchi et al. 2001). The boundary layer conductance, stomatal conductance and capacity for photosynthesis of an ecosystem dictate to what extend a change in stomatal conductance will influence photosynthesis and transpiration that control WUE (Cowan 1988, Farquhar et al. 1988). These interactions become less important if variations in WUE are due to changes in photosynthetic capacity (Cowan 1988). Stable isotope measurements can be utilized to study variations in WUE and the physiological processes causing these variations (Farquhar et al. 1989).

Stable carbon isotope measurements have been shown to provide information about WUE, because both the carbon isotope composition of plant tissue and WUE are influenced by leaf intercellular CO_2 concentration (Farquhar et al. 1989). Carbon uptake during photosynthesis and the water balance of plants are controlled by plant guard cells that adjust the opening of stomata, to regulate the amount of carbon dioxide diffusing into the leaf during photosynthesis, and the diffusion of water vapour out of the leaf during

transpiration (Farquhar and Sharkey 1982). The diffusion of CO_2 into the leaf is driven by the CO_2 concentration gradient from ambient air to the intercellular air spaces. Isotope effects alter the stable carbon isotope composition ($^{13}C/^{12}C$) of plant tissue during photosynthetic gas exchange, and give an indication of the relative amount of carbon taken up by the plant and the relative amount of water lost during transpiration (Farquhar et al. 1989). The carbon isotope composition of plant tissue becomes depleted in ^{13}C, when stomatal conductance is high in relation to photosynthetic capacity. Conversely, the carbon isotope composition of plant tissue becomes enriched in ^{13}C when stomatal conductance is low relative to photosynthetic capacity (Flanagan 2009). Previous studies have shown a strong negative correlation between leaf $\delta^{13}C$ values and ANPP. This is due to low water availability causing reduced stomatal conductance, which decreases photosynthetic activity and results in lower biomass production and higher $\delta^{13}C$ values (Flanagan 2009). Grasses have been found to have higher ci/ca (ratio of intercellular to ambient CO_2) values and lower $\delta^{13}C$ values compared to other plant functional types, as they are short-lived species that take advantage of soil moisture when it is available and go into dormancy during times of water stress (Smedley et al. 1991, Ehleringer 1993, Ponton et al. 2006). Based on these findings we would expect that WUE is reduced during times of sufficient moisture availability and is increased when plants are under water stress. Farquhar and Sharkey (1982) have suggested that WUE is higher when stomatal conductance is reduced because it limits water loss through transpiration more than CO_2 assimilation. This pattern was previously observed at the ecosystem scale in a comparison of WUE during years of high and low growing season precipitation at the Lethbridge grassland (Wever et al. 2002).

1.5. Challenges quantifying ecosystem responses to changing climate conditions

Currently there is no comprehensive understanding of the impacts of changing climate conditions on grasslands and the challenges associated with quantifying these impacts due to the diversity of grassland ecosystems (Weltzin et al. 2003, Heisler and Weltzin 2006, Williams and Jackson 2007, Williams et al. 2007). Simultaneous shifts in multiple drivers further complicate the analysis of ecosystem sensitivities because they exhibit strong interactions, but the effects are not necessarily additive (Beierkuhnlein et al. 2011, Larsen et al. 2011). A lack of understanding of the factors that influence ecosystem sensitivity to precipitation changes remains, as these differ immensely on an inter-annual scale as a result of different plant communities, traits of dominant species, and biogeochemistry (Paruelo et al. 1999, Knapp et al. 2001, McCulley et al. 2005). The response patterns of ecosystems are being triggered either directly or indirectly by climate change drivers that alter resource levels (Smith et al. 2009). These patterns have been shown to persist once they occur in form of continuous directional responses, especially in herbaceous ecosystems and in some cases, implicate shifts in community composition over time that are driven by higher species turnover rates in combination with faster physiological responses (Gross et al. 2000, Collins et al. 2008, Smith et al. 2015). These shifts could be an important indicator in identifying patterns of sensitivities to climate change in terrestrial ecosystems.

Detecting these sensitivities remains a challenge due to mechanisms of resistance to resource alterations that have been found particularly in grasslands (Vittoz et al. 2009, Hallett et al. 2014). This resistance is driven by species diversity and plant species functional composition which have been shown to stabilize productivity through

asynchronous development of the plant community (Diaz and Cabido 2001, Hautier et al. 2014). A lag in the response time between the resource alteration and the crossing of resource thresholds and species tolerances adds to the complication of detecting ecosystem sensitivities to resource alterations (Aber et al. 1998).

1.6. Rationale and significance of my research

A conceptual ecosystem response model developed by Knapp et al. (2008) predicts that more extreme rainfall regimes, characterized by larger but fewer events, will decrease soil water stress in xeric (dry) systems and increase soil water stress in mesic (moist) ecosystems. This is caused by soil water levels in mesic ecosystems usually being above the drought stress levels. Therefore, larger but fewer rain events lead to extended dry periods between rain events, resulting in more frequent occurrences of drought stress in mesic ecosystems. In contrast, xeric ecosystems are normally confronted with chronic soil water stress, therefore larger rain events allow soil water levels to increase above drought levels and remain there for longer periods of time compared to the control scenarios (Knapp et al. 2008). This research was conducted in a tallgrass prairie in Kansas and a semi-arid shortgrass steppe in northeast Colorado, climatically similar to the semi-arid mixed grass grassland in Lethbridge. The major difference between the sites in Colorado and Lethbridge is the average maximum rain event size that is significantly higher in Lethbridge with 50.1 mm in comparison to the 18.2 mm in Colorado (Appendix A). This significant difference in maximum rain event size emphasizes the importance of investigating whether the ecosystem response to larger, but fewer rain events (increased plant productivity) is consistent with responses in semi-arid grasslands of the northern

Great Plains, that naturally show higher maximum rain event sizes. Additionally I am interested in investigating whether this ecosystem response will also occur under drier conditions that have been predicted for the future in semi-arid grasslands. A treatment with 50% reduced precipitation has been selected, as it represents the lowest average precipitation recorded in the historic climate analysis (Appendix A). Experiments under extreme dry conditions are gaining importance, as increasing surface temperatures are causing overall drier conditions, due to higher evaporative demand, and droughts occur more frequently (Knapp et al. 2008).

Understanding the processes of ecosystem responses to changing climatic conditions will improve our ability to accurately predict the impact of future precipitation changes on the dynamics of grassland ecosystems and their key ecosystem functions. Overall these findings will be useful to predict future changes in important ecosystem services that have i) economic implications for the Agricultural sector (forage production) as well as ii) ecological implications through improving the general understanding of the mechanisms underlying ecosystem response patterns to climate change and improving the accuracy of ecosystem response modelling for the Great Plains region.

CHAPTER 2. THE IMPACT OF ALTERED PRECIPITATION PATTERNS ON PLANT PRODUCTIVITY, SOIL RESPIRATION AND WATER-USE EFFICIENCY IN SOUTHERN ALBERTA GRASSLANDS

2.1. Introduction

Grasslands make up one third of the Earth's terrestrial surface and vary greatly in productivity on a spatial as well as on a temporal scale (Knapp et al. 2001). Elevated greenhouse gas concentrations are expected to increase the mean global surface temperature between 1.1 and 6.4 °C by 2100 (IPCC 2007). Rising global temperatures will result in drier conditions in semi-arid grassland regions by increasing evapotranspiration rates. However, previous research has shown that 90% of the variation in aboveground net primary production (ANPP) responses in grasslands are caused by changes in mean annual precipitation (MAP), which makes precipitation a stronger driver than temperature for changes in grassland productivity (Mowll et al. 2015). Therefore, water is the primary limiting factor for aboveground net primary production (Lehouerou et al. 1988, Churkina and Running 1998) and it will be impacted directly by warmer and drier conditions associated with climate change, particularly in semi-arid grasslands.

Precipitation responses to climate change are uncertain and vary spatially and temporally. An analysis of precipitation trends in the Canadian Prairies reports a significant increase in the amount of precipitation received over the previous 75 years (Akinremi et al. 1999). Akinremi et al. also report an increase in the number of low-intensity rain events, suggesting that rain events are not getting more intense in this particular region. This contrasts with the intensification hypothesis, which suggests that

greenhouse warming will cause an intensification of the hydrological cycle of the Earth (Idso and Balling 1991), resulting in more intense climatic events. Therefore, globally precipitation changes caused by climate change are expected in the form of larger rainfall events with longer dry periods between rain events (IPCC 2007, Easterling et al. 2000). Larger but less frequent precipitation events are particularly important in arid ecosystems because they may lead to an increase in soil water storage and a decline in soil water evaporation losses (Knapp et al. 2008). It has been shown that large rain events can partially compensate for ecosystem productivity losses in semi-arid grasslands caused by low precipitation in dry years (Knapp et al. 2008). Semi-arid grasslands in particular show little response to drought due to this compensation mechanism (Cherwin and Knapp 2012). Overall shifts in precipitation frequencies and intensities are expected to impact the abundance of within-season drought, evapotranspiration and the amount of runoff (Fay et al. 2003, MacCracken et al. 2003), and have a direct effect on how water moves through the soil (McAuliffe 2003). Larger rain events are also expected to promote the growth of deep rooted woody plants (Kulmatiski and Beard 2013), increase the importance of hydraulic redistribution (Yu and D'Odorico 2014), and impact water-use efficiency (Knapp et al. 2008). Overall these changes in frequency and intensity of precipitation will alter the supply of water to terrestrial ecosystems significantly, even if no net change in the annual precipitation occurs (Knapp et al. 2008). Shifts in precipitation regimes have a significant influence on the amount of water available for plants and soil biogeochemical processes, thereby altering ecosystem productivity, soil respiration and water-use efficiency (Knapp et al. 2008).

A conceptual ecosystem response model developed by Knapp et al. (2008) predicts that more extreme rainfall regimes, characterized by larger but fewer events, will

decrease soil water stress in xeric (dry) systems and increase soil water stress in mesic (moist) ecosystems (Fig. 1). This is caused by soil water levels in mesic ecosystems usually being above the drought stress levels. Therefore, larger but fewer rain events lead to extended dry periods between rain events, resulting in more frequent occurrences of drought stress in mesic ecosystems. In contrast, xeric ecosystems are normally confronted with chronic soil water stress, therefore larger rain events allow soil water levels to increase above drought levels and remain there for longer periods of time compared to the control scenarios (Knapp et al. 2008) (Fig. 1).

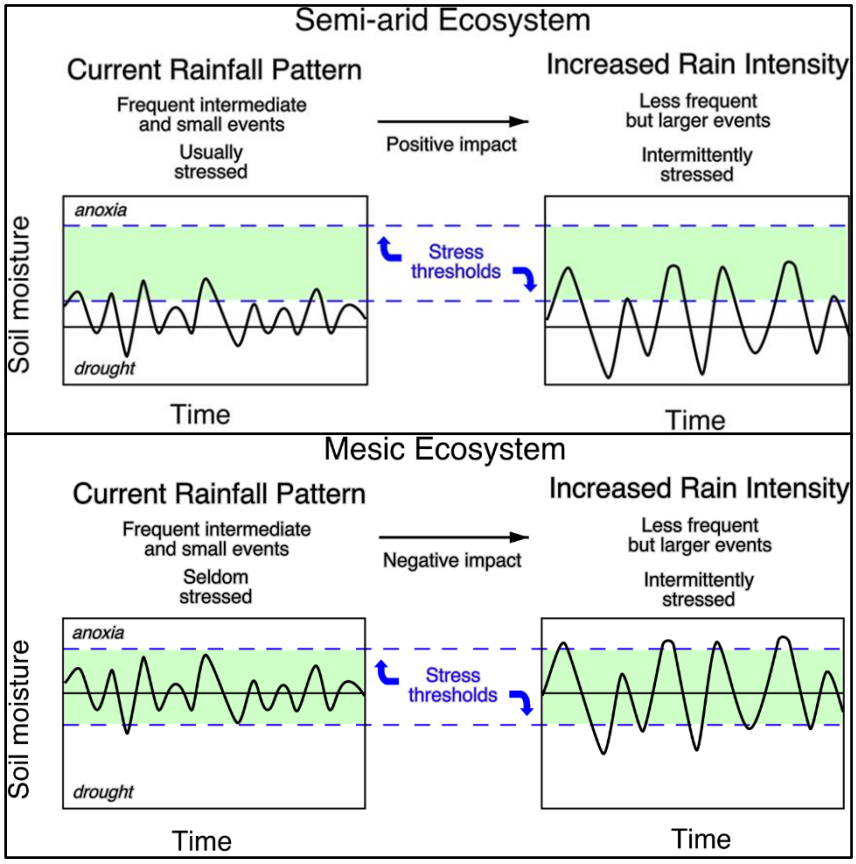

Fig. 1 Diagram of conceptual ecosystem response model as described by Knapp et al. (2008).

Changes in water availability and temperature also have an impact on soil respiration through several mechanisms. For instance, increases in soil water content and temperature can stimulate photosynthetic activity and increase the amount of carbohydrates released by plant roots that stimulate the metabolic activity of microorganisms in the rhizosphere and lead to an increase in soil respiration (Bardgett 2011, Flanagan et al. 2013). Changes in soil water content can also shift the allocation of carbon to the roots, increasing root growth and biomass production, which influences soil respiration rate (Shaver et al. 2000). Even though soil moisture is the major driver of grassland productivity, elevated soil temperatures can strongly stimulate soil respiration if soil moisture levels are held constant at a relatively high level (Sala et al. 1988, Knapp et al. 2001, Weltzin et al. 2003).

Water-use efficiency is defined as the ratio of carbon gain in net photosynthesis to water loss during transpiration and varies depending on differences in leaf gas exchange characteristics of plants and ambient environmental conditions (Farquhar et al. 1989). Measurements of the stable carbon ($^{13}C/^{12}C$) isotope composition of plant tissue provides information on the water-use efficiency of plants through the isotope effects that get expressed during photosynthetic gas exchange (Farquhar et al. 1989, Flanagan and Farquhar 2014). Previous studies have shown a strong negative correlation between leaf $\delta^{13}C$ values and ANPP (Flanagan 2009). This is due to low water availability causing reduced stomatal conductance, which decreases photosynthetic activity and results in lower biomass production and enrichment of ^{13}C in plant biomass (Flanagan 2009).

My research project was designed to experimentally manipulate the amount and frequency of simulated precipitation added to trenched treatment plots that were covered

by rain shelters in a semi-arid short/mixed-grass grassland near Lethbridge, Alberta, Canada. The response of multiple ecosystem functions including plant productivity, soil respiration and plant water-use efficiency to these altered precipitation patterns were measured. In addition, the green chromatic coordinate (gcc) was determined, which can be used as a non-destructive proxy for plant biomass and provides a temporal pattern for changes in plant biomass throughout the growing season (Richardson et al. 2007).

The primary objective of this study was to test whether the response to the altered rainfall patterns based on long-term average precipitation was consistent with the conceptual ecosystem response mechanism developed by Knapp et al. (2008). Secondly, I experimentally decreased the total amount of precipitation received by 50% to assess whether fewer but larger rainfall events were able to compensate for productivity losses associated with drier conditions predicted for the future. For both experiments, I hypothesized that in response to larger but fewer rain events, plant productivity would increase due to extended periods of soil water levels above the drought stress threshold, leading to elevated soil respiration rates due to stimulated soil microbial activity and root growth, and decreased water-use efficiency due to increased photosynthetic activity and higher rates of transpiration.

2.2. Methods and Materials

2.2.1. *Field site description*

The experimental site is a 64 ha semi-arid short/mixed grass grassland located west of Lethbridge, Alberta, Canada (Lat. 49.470919 N; Long. 112.94025 W; 951 masl) in the Great Plains biome of North America (Wever et al. 2002). The dominant plant species at the site were the grasses *Agropyron dasystachyum* and *Agropyron smithii* (Carlson 2000, Flanagan and Johnson 2005). Other major plant species were: *Vicia americana, Artemesia frigida, Koeleria cristata, Carex filifolia, Stipa comata* and *Stipa viridula.* The climate of the Great Plains in the study area is semi-arid continental, with a mean annual precipitation (1971 - 2000) of 386.3 mm and mean annual temperature of 5.9 °C (Flanagan and Johnson 2005). The growing season (May - August) shows a mean temperature (1951 - 2015) of 14.9 °C with the highest temperatures occurring in July with an average of 18.5 °C. The mean precipitation (1951-2015) for the growing season is 199.5 mm, much of which is received in June with an average of 82.8 mm. The frequencies of rain events (1951 - 2015) averaged at 14 events during the growing season, with an average event size of 14.1 mm. These measures were recorded at the Lethbridge Regional Airport, located 14 km east of the study site (Environment Canada). The experimental site is flat and the soil is characterized as orthic dark-brown chernozem (Agriculture Canada, 1987; Flanagan and Johnson, 2005). Underneath the soil is a thick glacial till with low water permeability and no water table (Scracek 1993, Berg 1997). The soil profile is made up of an A horizon of clay loam (28.8% sand, 40 % silt, 31.2% clay) which is 9 cm thick, followed by a 16 cm thick B horizon of a clay texture (27.4% sand, 29.6% silt, 40% clay; Carlson, 2000). The top 10 cm of the surface soil horizon

contains 5.2% organic matter and has a density of 1.2 g cm^3. The study area has not been grazed by livestock for approximately 45 years.

2.2.2. *Experimental treatments and protocol*

Experimental treatments were developed by analyzing the historic climate data from 1951 – 2015, as recorded at the Lethbridge Regional Airport (Environment Canada). Two experiments were conducted during the 15 weeks of the growing season from May 1st until August 14th. This time frame was chosen because it is characterized as the peak growing time period after which the amount of green plant biomass produced is expected to decline.

The first experiment applied total growing season precipitation of 180 mm (climate normal), which represents the historic average for the time frame of 1 May – 14 August (Appendix A). The second experiment (reduced amount) applied total precipitation of 90 mm, which was at the extreme low end of the historic distribution (Appendix A). In both experiments, precipitation was applied at two frequencies, one rain event every week (normal frequency) and one rain event every two weeks (reduced frequency).

In the climate normal experiment, the average rain event was 12.8 mm for the normal frequency treatment. This average rain event size occurred in 8 years during 1951-2015, and was near the middle of the historic distribution (Appendix A). The average rain event size for the reduced frequency treatment was 25.7 mm, and this was typical of the extreme high end in the historic distribution (Appendix A).

In the reduced amount experiment, the average rain event size was 6.4 mm for the normal frequency treatment, which was found at the extreme low end in the historic distribution (Appendix A). The average rain event size of the reduced frequency treatment was at 12.8 mm, the same as for the climate normal and normal frequency treatment (Appendix A). The treatments were given the following two-word names based on, first the total amount of rain applied, and second, the frequency at which the rain was applied: Normal – Normal (NN), Normal – Reduced (NR), Reduced – Normal (RN) and Reduced – Reduced (RR) (Table 1).

Table 1. Details of the treatments for the 15 week rain manipulation experiment (1 May – 14 August 2017) including total amount of rain added, rain event frequency, total number of rain events during the experiment and rain event size were calculated based on an analysis of historic climate data for Lethbridge, AB, Canada. The Normal – Normal treatment represents a pattern developed by analyzing precipitation data collected for the 1951 – 2015 time period.

Climate stats	**Treatments**			
	Normal - Normal	***Normal - Reduced***	***Reduced - Normal***	***Reduced - Reduced***
Total rain	180 mm	180 mm	90 mm	90 mm
Rain event frequency	1/week	1 every 2 weeks	1/week	1 every 2 weeks
Total number rain events	14	7	14	7
Rain event size	~12.8 mm	~25.7 mm	~6.4 mm	~12.8 mm

The precipitation amounts for each treatment were manually applied to the experimental plots using an 11.4 L watering can according to seasonal distribution patterns based on historic data (1951 - 2015). The seasonal distribution patterns were developed for the experimental protocol by calculating average weekly (normal frequency) and biweekly (reduced frequency) precipitation amounts for each rain application throughout the 15 week experiment to follow the seasonal pattern.

2.2.3. Experimental design

The experimental design is a randomized complete block design with a 2 x 2 factorial treatment structure and consisted of five blocks that were made up of four plots each. The plots were randomly assigned to one of the four experimental treatments and spaced out 3.7 m apart within the block (Fig. 2). During May 2016 each plot was trenched to approximately 60 cm below the surface and lined with 6 mm plastic to minimize sub-surface lateral water flow into the experimental plots. The dimensions of each plot were 2.13 x 2.13 m (4.54 m^2) with a core plot of 1 x 1 m surrounded by 0.57 m buffer. Rain shelters were installed over the plots from May 1st 2017 until August 14th 2017 (15 weeks) to prevent ambient rainfall input on the experimental plots. The shelter structures consisted of four wooden posts anchored into the soil with a detachable roof. The roofs were constructed with a wooden frame and clear corrugated polycarbonate sheeting (Suntuf, Palram) installed 1 m above the ground at a slight angle towards the north side of the plots to allow drainage of ambient rainfall (Fig. 3). Five additional plots, not covered by rain shelters, were included as a control to record ecosystem characteristics during 2017 in plots exposed to normal ambient conditions of precipitation and other environmental factors. The effects of the shelter roofs on the microclimate, including transmitted photosynthetically active radiation (PAR) and air temperature, were assessed in June 2016 (Appendix B).

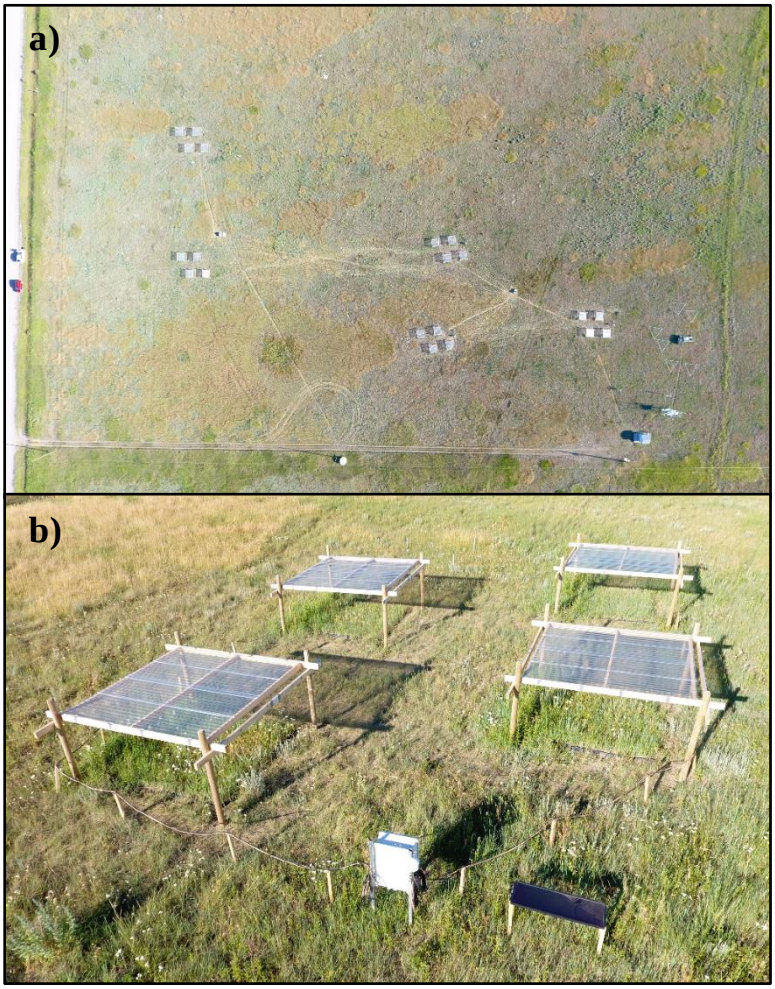

Fig. 2 Pictures of **a)** the experimental site, **b)** one experimental block.

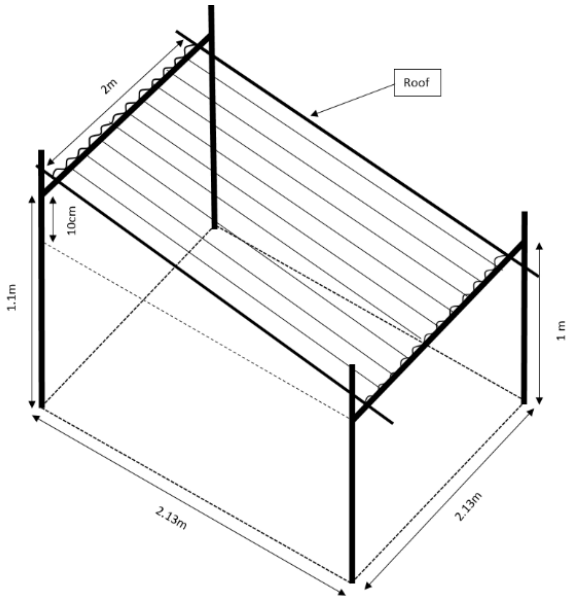

Fig. 3 Diagram of the Rainout shelter.

2.2.4. Environmental measurements

The four treatment plots in three of the five blocks were instrumented to monitor microclimatic conditions throughout the experiment. The sensors were installed in the 50 x 50 cm instrumentation quadrat within the core plots (Fig. 4). Soil moisture was measured using soil water reflectometers (CS-616, Campbell Scientific Ltd.) that were inserted into the ground at a 63.4° angle so that they integrated measurements of soil volumetric water content over 0-15 cm depth. Calibration of the soil moisture probes followed the procedure as described in Flanagan et al. (2013). Additionally thermocouple probes (105-T, Campbell Scientific Ltd.) were buried horizontally at 7.5 cm depth next to the water reflectometers to measure soil temperature. Each block was equipped with one air temperature probe (T-107, Campbell Scientific Ltd.) that was installed at 1 m above the ground and covered with a radiation shield (41303-5A, R. M. Young Company). The sensors were installed in early April 2017. All sensor signals were scanned at 5 s intervals and recorded on a data logger (CR23X, Campbell Scientific Ltd.) as 30 min averages. These measurements were then averaged for each treatment to obtain daily means. No sensors were installed in the ambient plots due to logistical reasons, but data for soil moisture in plots not exposed to experimental treatments was available from another meteorological station at the same site.

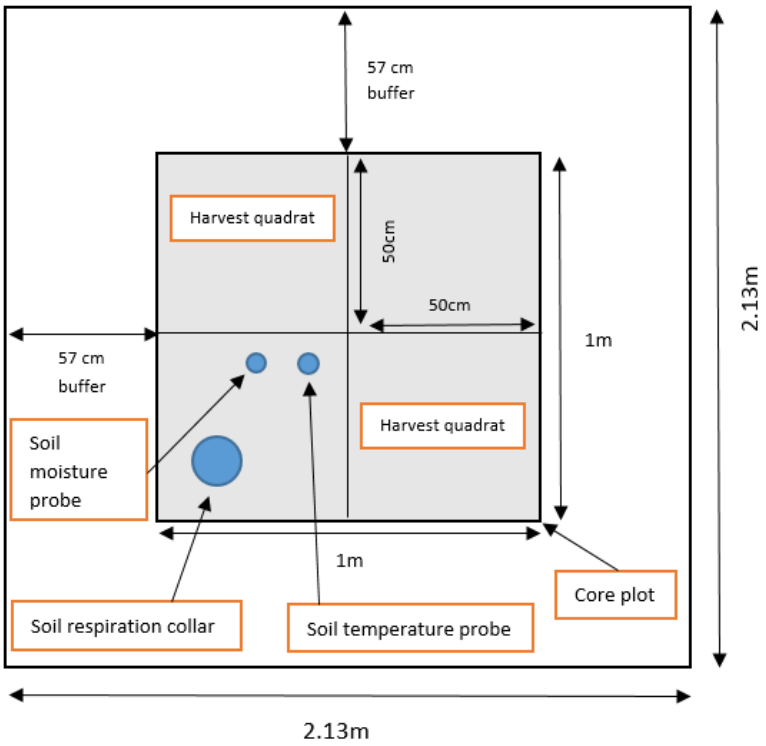

Fig. 4 Diagram of the Experimental plot set up and Instrumentation**.**

2.2.5. *Plant biomass and greenness measurements*

Aboveground plant biomass was measured on August 14th by harvesting (clipping) all plant material from two 50 x 50 cm harvest plots within the 1 x 1 m core plot of each experimental plot (Fig. 4). All previous year's dead plant biomass had been removed in late March 2017 before the start of the growing season to simplify the harvest of the focal year's plant biomass at the end of the experiment. The harvested biomass was oven-dried at 60 °C for 48 h, and weighed to the nearest 0.1 g (Mettler PJ400, Greifensee, Switzerland).

The green chromatic coordinate (gcc) is a proxy for biomass, a non-destructive estimate of seasonal changes in green biomass and leaf area (Richardson et al. 2007, Sonnentag et al. 2012). Digital images of the vegetation plots were taken weekly on 15

sampling days during the growing season (1 May – 15 August) in 2017. Location and settings of the camera were kept constant throughout the experiment. The camera was mounted on a metal frame, specifically constructed to fit under the rain shelters that allowed the camera to face straight down (Fig. 5). The digital vegetation images were analyzed for the gcc using an Image Analysis script written for MATLAB (see Appendix D). This script calculated the gcc by extracting the red, green and blue (RGB) color channel information as digital numbers from a defined area of each vegetation image. The green chromatic coordinate (gcc) was then calculated (Gillespie et al. 1987):

$$gcc = \frac{total\ green}{(total\ red + total\ green + total\ blue)} \quad (1)$$

Fig. 5 Metal frame used to stabilize camera for taking weekly greenness pictures under the shelter roofs.

2.2.6. Soil respiration measurements

Soil respiration rates were measured weekly using a dynamic closed chamber measurement technique. The technique made use of an Ultra-Portable Greenhouse Gas Analyzer (UGGA, Los Gatos Research, Mountain View, CA, USA), a soil respiration chamber (LI-6000-09 Soil respiration chamber, LI-COR, Lincoln, Nebraska) and tubing. The tubing connects the gas analyzer with the respiration chamber which was attached to a respiration collar mounted in ground (Fig. 6). The respiration collar (10 cm tall) was inserted about 8 cm into the ground in the instrumentation quadrat within the core area of each experimental plot. The gas analyzer measured the change in concentration of CO_2. Based on those measurements the soil respiration rates were calculated. The ground area of the soil chamber was 71.6 cm^2 and had a volume of 962 ml. The volume of the soil collar was calculated for each plot based on how far it was inserted into the ground. The effective volume of the Gas analyzer varied with changing pressure inside the analysis cell of the Gas Analyzer as well as the internal temperature of the analysis cell and was therefore adjusted for each measurement. Soil respiration rates were calculated as:

$$Rate = \frac{[\Delta CO_2 * System\ Vol * Density\ of\ air]}{Soil\ area} \quad (2)$$

where $Rate$ is the soil respiration rate (μmol CO_2 m^{-2} s^{-1}), ΔCO_2 is the rate of change in CO_2 concentration over time (μmol mol^{-1} s^{-1}), $System\ Vol$ is the volume (m^3) of the entire system including the soil chamber minus the overlap of the soil collar plus the volume of the collar above ground and the effective volume of the Gas analyzer cell, $Density\ of\ air$ (mol m^{-3}) is calculated from the Ideal Gas Law using values of air temperature (K) and atmospheric pressure (Pa), $Soil\ area$ (m^2) is the area enclosed by the soil collar.

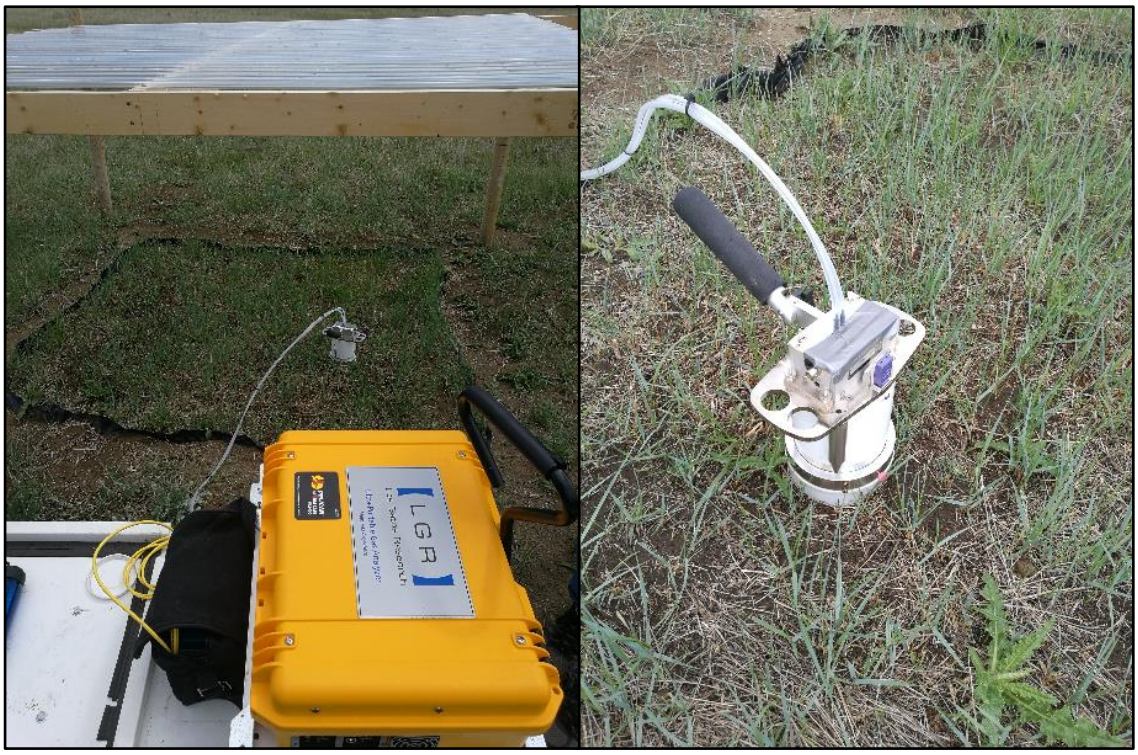

Fig. 6 Soil respiration measurement via a dynamic closed chamber technique utilizing a Portable Gas Analyzer (UGGA) and a soil respiration chamber.

2.2.7. Stable carbon isotopes ($\delta^{13}C$)

Subsamples of the biomass were ground to a fine powder using a coffee grinder and a ball mill (Retsch MM200, Haan, Germany) and sent to an analytical Lab at the University of Calgary where they were analyzed for stable carbon isotope composition ($^{13}C/^{12}C$, ‰) of plant tissue to make estimates of plant water-use efficiency (Flanagan and Farquhar 2014). The analysis of the stable isotope composition included measurements of total nitrogen content (mg N g^{-1} biomass) of the dried biomass which provided insight into the maximum photosynthetic capacity of the plant tissue.

2.2.8. Statistical Analyses

One-way analysis of variance (ANOVA) was used to test for significant differences between the precipitation treatment pairs (NN vs. NR, RN vs. RR) for ANPP and $\delta^{13}C$ measurements. A separate one-way ANOVA was run on the ambient versus NN treatment and a combination of normal (NN + NR) and reduced (RN + RR) precipitation treatments.

A repeated-measures analysis of variance (RM-ANOVA) was used to test for significant differences among treatments in absolute and relative values of soil respiration, gcc, soil temperature and soil moisture measurements, specifically testing for effects of treatment, time (sample date) and their interaction for the ambient versus NN, NN versus NR, RN versus RR and the normal (NN + NR) versus reduced (RN + RR) precipitation treatments combined.

The approximate peak of the plant biomass production was observed around 23 June (day 174), and I therefore ran an analysis on the entire 15 weeks of the experiment (days 121-226), as well as on the period of plant senescence (days 174-226) to more clearly identify possible treatment effects that could be apparent only during later time periods of the experiment.

Lastly a 3-way ANOVA was run on all measurements to test for possible Block effects next to the effects of rain amount and frequency treatment. The repeated measurements of gcc, soil respiration, soil moisture and soil temperature were averaged over the season and then analyzed via the 3-way ANOVA.

Statistical analyses done using either MATLAB (MathWorks, Version R2016b), SPSS (IBM SPSS Statistics, Version 21) or Excel (Microsoft Office 2013).

2.3. Results

2.3.1. Historical Context for Environmental Conditions in 2017

Monthly average air temperature in 2017 was higher than the 64-year average for every month (May – August) of the experiment with a maximum difference of almost 4°C in July (Fig. 7b). Total precipitation for May 2017 was equal to the 64-year historic average. For the months of June, July and August in 2017 total precipitation was lower than the historic average (Fig. 7a). The above average air temperatures and low amount of precipitation received during the months of May to August 2017 indicated the occurrence of an extremely dry summer.

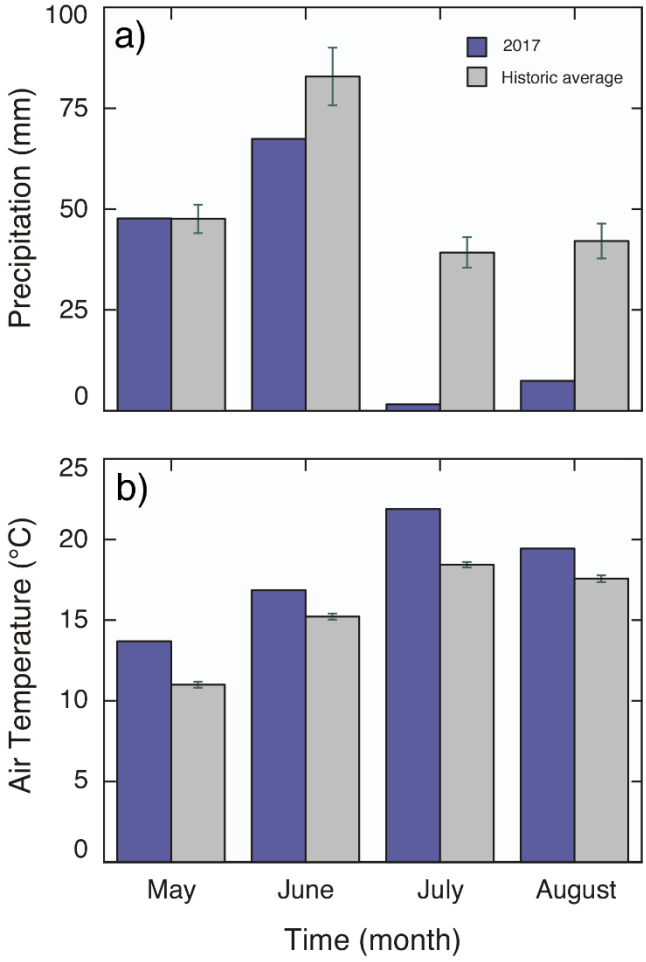

Fig. 7 Comparison of **a)** monthly total precipitation, **b)** monthly average air temperature for May – August 2017 (n=3) to the historic average ± SE (1951-2015, Environment Canada) at the experimental site in Lethbridge, Alberta, Canada.

2.3.2. *Environmental Measurements - Treatment Effects on Soil Moisture and Temperature*

Significant time and interaction effects (between time and treatment) were found for average soil moisture content for all four treatment comparisons, confirming a distinct seasonal pattern of high average soil moisture in May, with a small peak in June and a gradual decline until August (Fig. 8a, b, c, d, Table 2). Average soil moisture for the NR treatment was slightly higher than NN during the second half of the experiment (Fig. 8a). However, average soil moisture for the RN treatment showed slightly higher values than RR during the first half of the experiment (Fig. 8b). Surprisingly, average soil moisture for the normal (NN and NR combined) and the reduced (RN and RR combined) precipitation treatments were identical throughout the experiment (Fig. 8c). Soil moisture measurements under ambient conditions were provided by another meteorological station on site, as the ambient plots were not instrumented. Average soil moisture was higher under ambient conditions in comparison to the NN treatment plots for May and June after which soil moisture declined for both treatments (Fig. 8d). A significant treatment effect on average soil moisture for the Ambient and NN treatment was found based on relative soil moisture values for the plant senescence time period, showing higher soil moisture content for NN (Table 3, Fig. 9).

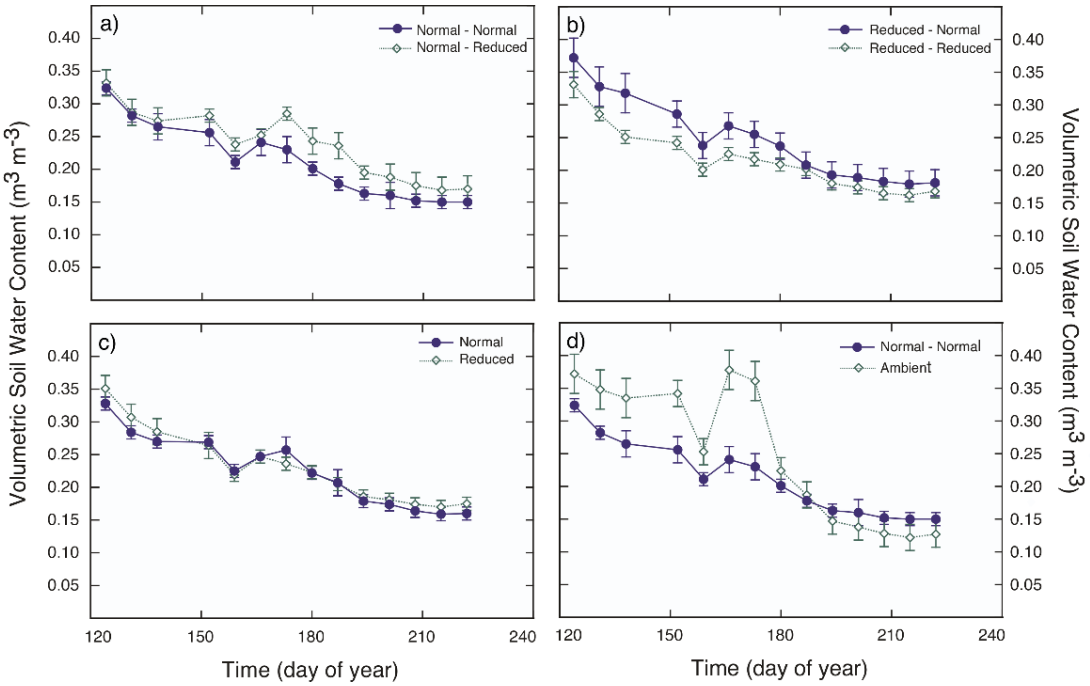

Fig. 8 Pairwise comparison of seasonal variation in average (±SE) soil moisture content (0–15 cm depth) for treatments **a)** NN and NR (n=3), **b)** RN and RR (n=3), **c)** Normal and Reduced (n=6), **d)** NN and Ambient (n=3). Measurements of ambient soil moisture were supplied by another meteorological station at the same site.

Table 2. Results for the repeated-measures analysis of variance for treatment (between subjects effect), time (within subjects effect) and their interaction on ***soil moisture content (0-15 cm depth)*** between **a)** NN and NR, **b)** RN and RR, **c)** N and R, **d)** Ambient and NN with time (DOY) as the repeated factor. Results are shown for the 15-week experimental time period (ETP) and the plant senescence time period (STP). Soil moisture was only measured in 3 (of 5) replicate plots for each treatment. Significant effects are marked with asterisks.

		Treatment		**Time**		**Time x Treat.**	
		ETP	*STP*	*ETP*	*STP*	*ETP*	*STP*
a) NN vs. NR	*F*	1.58	2.44	121.32*	98.15*	2.45*	4.38*
	$df_{n,,d}$	1, 94	1, 52	15, 80	8, 45	15, 80	8, 45
	P	0.2771	0.1936	0.000	0.000	0.0073	0.0012
b) RN vs. RR	*F*	1.41	0.51	229.87*	53.5*	6.06*	2.31*
	$df_{n,,d}$	1, 94	1, 52	15, 80	8, 45	15, 80	8, 45
	P	0.3009	0.5129	0.000	0.000	0.000	0.0443
c) N vs. R	*F*	0.11	0.08	204.07*	100.5*	2.43*	3.84*
	$df_{n,,d}$	1, 190	1, 106	15, 176	8, 99	15, 176	8, 99
	P	0.7483	0.7823	0.000	0.000	0.0033	0.0007
d) NN vs. Ambient	*F*	1.6	0.02	306.01*	370.86*	34.96*	73.35*
	$df_{n,,d}$	1, 110	1, 61	15, 96	8, 54	15, 96	8, 54
	P	0.2622	0.8967	0.000	0.000	0.000	0.000

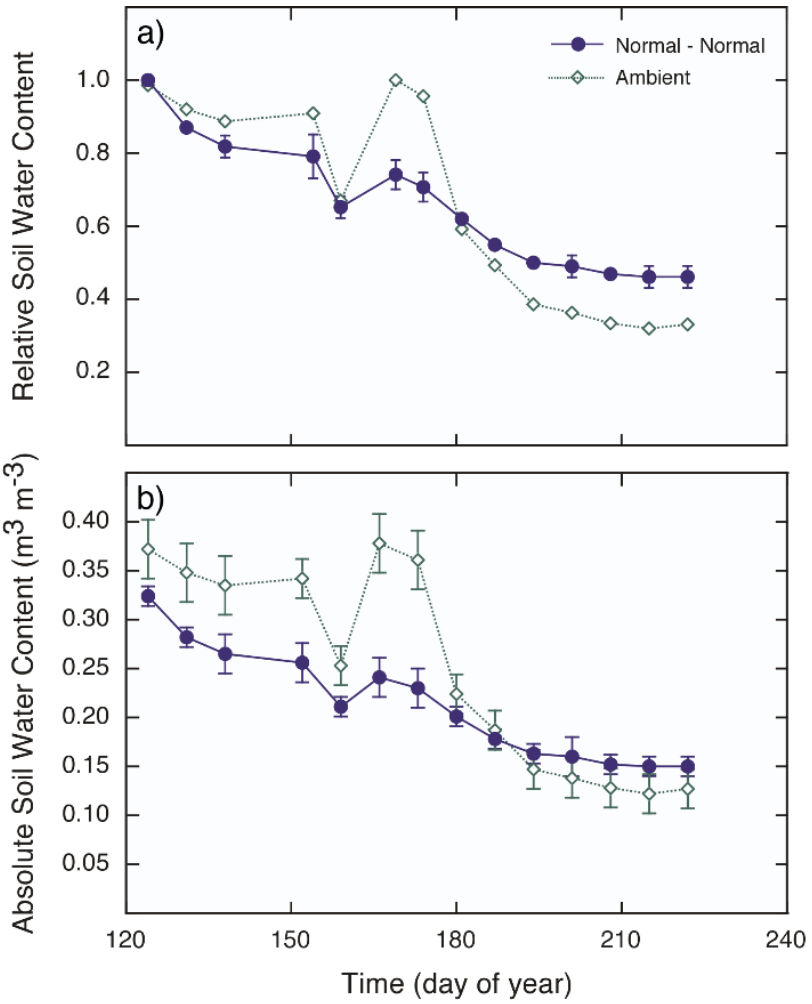

Fig. 9 Comparison of **a)** Relative, and **b)** absolute soil moisture for NN and Ambient plots. Average ± SE, n = 3.

Table 3. Significant results for the repeated-measures analysis of variance ***based on relative values*** for **a)** Treatment effect on soil moisture (NN vs. Ambient) and **b)** Time x Treatment effect on soil temperature (NN vs. NR) for the plant senescence time period (STP).

		Treatment	**Time x Treat.**
		STP	*STP*
Soil moisture ***(NN vs. Ambient)***	*F*	264.65	
	$df_{n,,d}$	1, 61	
	P	0.000	
Soil temperature ***(NN vs. NR)***	*F*		2.53
	$df_{n,,d}$		8, 45
	P		0.0296

Average soil temperature showed a significant time effect for all three treatment comparisons following the same distinct seasonal pattern of lower average soil temperatures in May that gradually increased through June and July and reached a peak in August (Fig. 10a, b, c, Table 4). The RM-ANOVA also reported a significant interaction effect of time and treatment for the normal and reduced precipitation treatments (Fig. 10c, Table 4). However, a significant interaction effect on average soil temperature was also reported for the NN and NR treatment based on relative soil temperature values for the plant senescence time-period (Table 3).

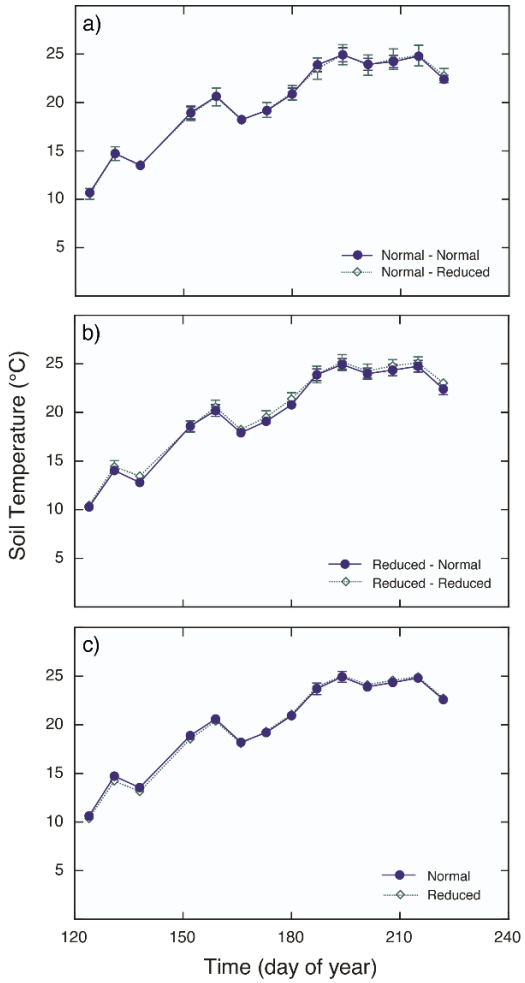

Fig. 10 Pairwise comparison of seasonal variation in average (±SE) soil temperature (7.5 cm depth) for treatments **a)** NN and NR (n=3), **b)** RN and RR (n=3), **c)** Normal and Reduced (n=3). The comparison for treatment NN and Ambient is not available as ambient plots were not instrumented.

Table 4. Results for the repeated-measures analysis of variance for treatment (between subjects effect), time (within subjects effect) and their interaction on ***soil temperature (7.5 cm depth)*** between **a)** NN and NR, **b)** RN and RR, **c)** N and R, **d)** Ambient and NN with time (DOY) as the repeated factor. Results are shown for the 15-week experimental time period (ETP) and the plant senescence time period (STP). Soil temperature was only measured in 3 (of 5) replicate plots for each treatment. Significant effects are marked with asterisks.

		Treatment		**Time**		**Time x Treat.**	
		ETP	*STP*	*ETP*	*STP*	*ETP*	*STP*
a) NN vs. NR	*F*	0	0	1032.11*	268.7*	0.7	1.71
	$df_{n,,d}$	1, 94	1, 52	15, 80	8, 45	15, 80	8, 45
	P	0.9786	0.9874	0.000	0.000	0.7775	0.1341
b) RN vs. RR	*F*	0.2	0.14	1279.32*	410.15*	0.83	1.64
	$df_{n,,d}$	1, 94	1, 52	15, 80	8, 45	15, 80	8, 45
	P	0.6776	0.7231	0.000	0.000	0.6388	0.1538
c) N vs. R	*F*	0.01	0.06	2410.44*	572.73*	2*	0.08
	$df_{n,,d}$	1, 190	1, 106	15, 176	8, 99	15, 176	8, 99
	P	0.9219	0.8092	0.000	0.000	0.0184	0.9996

2.3.3. *Plant biomass production and seasonal variation in plant greenness (gcc)*

Contrary to expectations, the average aboveground biomass of the NN treatment was significantly higher than the NR treatment (One-way ANOVA, $F_{(1, 8)} = 10.58$, $P = 0.012$; Fig. 11a). However, there was no significant difference in average aboveground biomass between the RN and RR treatments, although there was a trend towards slightly higher biomass for RN (One-way ANOVA, $F_{(1, 8)} = 1.85$, $P = 0.211$; Fig. 11b). Average aboveground biomass for the normal precipitation treatments was significantly higher than for the reduced precipitation treatments (One-way ANOVA, $F_{(1, 18)} = 18.29$, $P = 0.000$; Fig. 11c). Biomass of NN plots was also significantly higher than the ambient plots (One-way ANOVA, $F_{(1, 8)} = 21.39$, $P = 0.002$; Fig. 11d).

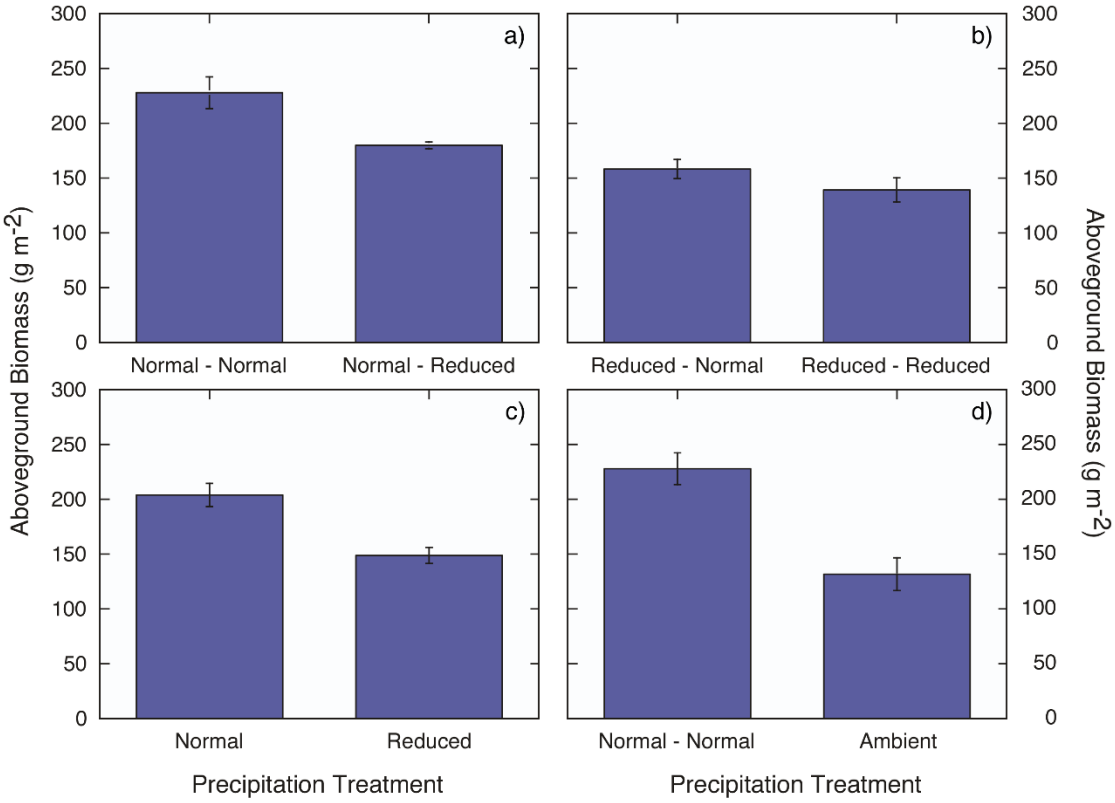

Fig. 11 Pairwise comparison of average (±SE) aboveground biomass for treatment **a)** NN and NR (n=5), **b)** RN and RR (n=5), **c)** Normal and Reduced (n=10), **d)** NN and Ambient (n=5). Biomass was harvested on 14 August 2017.

Average gcc values for all four of the pairwise treatment comparisons started out low at the beginning of May, gradually increased until the end of June and then declined throughout July and August, showing a significant time effect for the 15-week and the plant senescence time periods (Fig. 12, Table 5). After day 180, average gcc values of NN were slightly higher than of NR (Fig. 12a). Average gcc values for treatments RN and RR were not significantly different throughout the entire experiment (Fig. 12b).

A significant treatment effect on average gcc values was shown for the normal and reduced precipitation treatments (Fig. 12c) and the NN and ambient treatments (Fig. 12d) for the plant senescence time period (Table 5). Average gcc values for the normal precipitation treatments were significantly higher than for the reduced treatments (Fig.

12c) and the average gcc values of the NN treatment were significantly higher than for the ambient plots (Fig. 12d) for the second half of the experiment. In addition, significant interaction effects of treatment and time were found for the normal and reduced precipitation treatments (Fig. 12c) and the NN and ambient treatments (Fig. 12d), indicating a temporal effect with distinct seasonal patterns for the contrasting treatments.

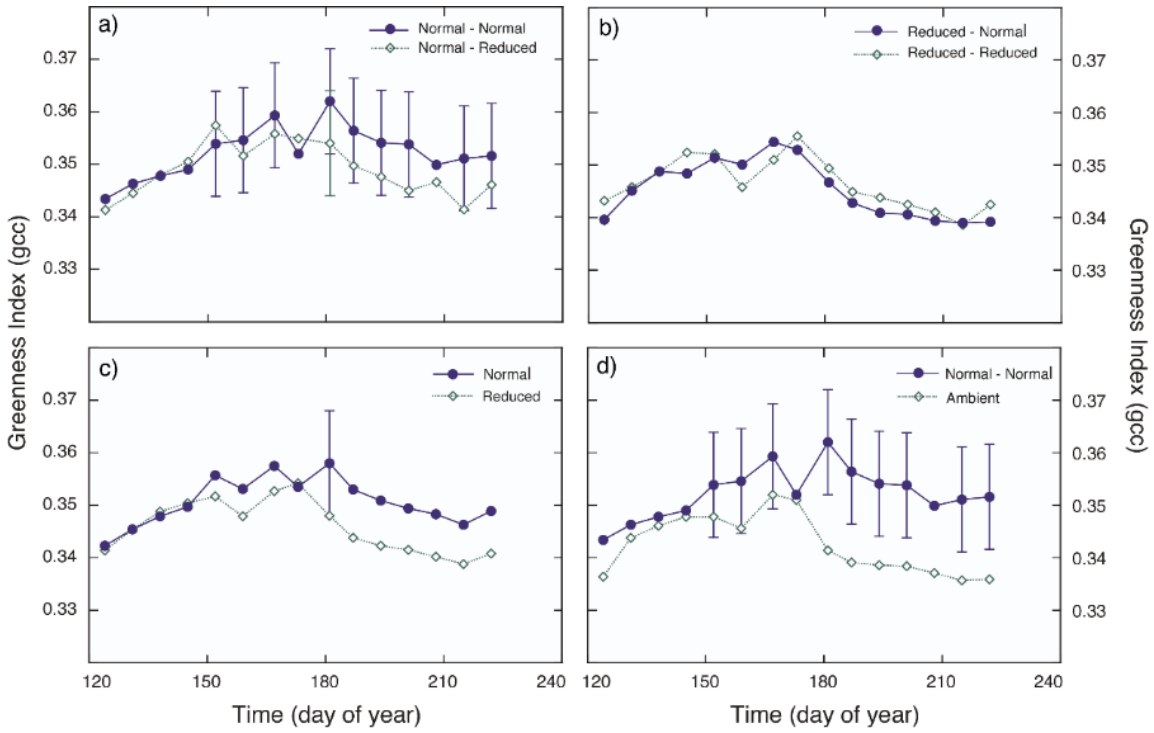

Fig. 12 Pairwise comparison of seasonal variation in the average (±SE) Greenness Index (absolute gcc) for treatments **a)** NN and NR (n=5), **b)** RN and RR (n=5), **c)** Normal and Reduced (n=10), **d)** NN and Ambient (n=5).

Table 5. Results for the repeated-measures analysis of variance for treatment (between subjects effect), time (within subjects effect) and their interaction on the ***Greenness Index (gcc)*** between **a)** NN and NR, **b)** RN and RR, **c)** N and R, **d)** Ambient and NN with time (DOY) as the repeated factor. Results are shown for the 15-week experimental time period (ETP) and the plant senescence time period (STP). Significant effects are marked with asterisks.

		Treatment		**Time**		**Time x Treat.**	
		ETP	*STP*	*ETP*	*STP*	*ETP*	*STP*
a) NN vs. NR	*F*	0.38	0.77	6.3*	6.61*	1.37	1.94
	$df_{n,,d}$	1, 148	1, 78	14, 135	7, 72	14, 135	7, 72
	P	0.5541	0.4049	0.000	0.000	0.1783	0.0802
b) RN vs. RR	*F*	0.35	2.5	23.84*	41.14*	1.38	0.5
	$df_{n,,d}$	1, 148	1, 78	14, 135	7, 72	14, 135	7, 72
	P	0.5708	0.1527	0.000	0.000	0.1738	0.8274
c) N vs. R	*F*	2.88	4.97*	16.77*	23.56*	3.73*	3.91*
	$df_{n,,d}$	1, 298	1, 158	14, 285	7, 152	14, 285	7, 152
	P	0.1072	0.0387	0.000	0.000	0.000	0.0007
d) NN vs. Ambient	*F*	4.37	6.71*	6.61*	6.37*	4.33*	4.66*
	$df_{n,,d}$	1, 148	1, 78	14, 135	7, 72	14, 135	7, 72
	P	0.071	0.0321	0.000	0.000	0.000	0.0004

2.3.4. *Soil respiration fluxes*

Average soil respiration rates for all four treatment comparisons showed a significant time effect indicating a seasonal pattern of respiration (Table 6). Respiration rates started out low in May, increased until June, and steadily declined through July and August (Fig 13a, b, c, d).

Significant treatment and interaction effects on average soil respiration rate for RN and RR, reduced and normal, as well as NN and ambient treatments were shown (Table 6). Indicating that those treatments were significantly different from one another, each following a distinct seasonal pattern over the course of the experiment. Average soil respiration rates for RR were significantly higher than for RN and showed clear peaks that were dependent on the watering days, whilst the seasonal course of RN was smoother and less influenced by water addition (Fig. 13b). However, average soil respiration rate for the normal precipitation treatment was significantly higher than the reduced, with the

respiration rates of the reduced treatments showing the same peaks as for RR indicating a stronger influence by water addition than the normal precipitation treatments (Fig 13c). The average soil respiration rate of the ambient plots was significantly lower than of NN and reached its low point at the end of July (Fig. 13d).

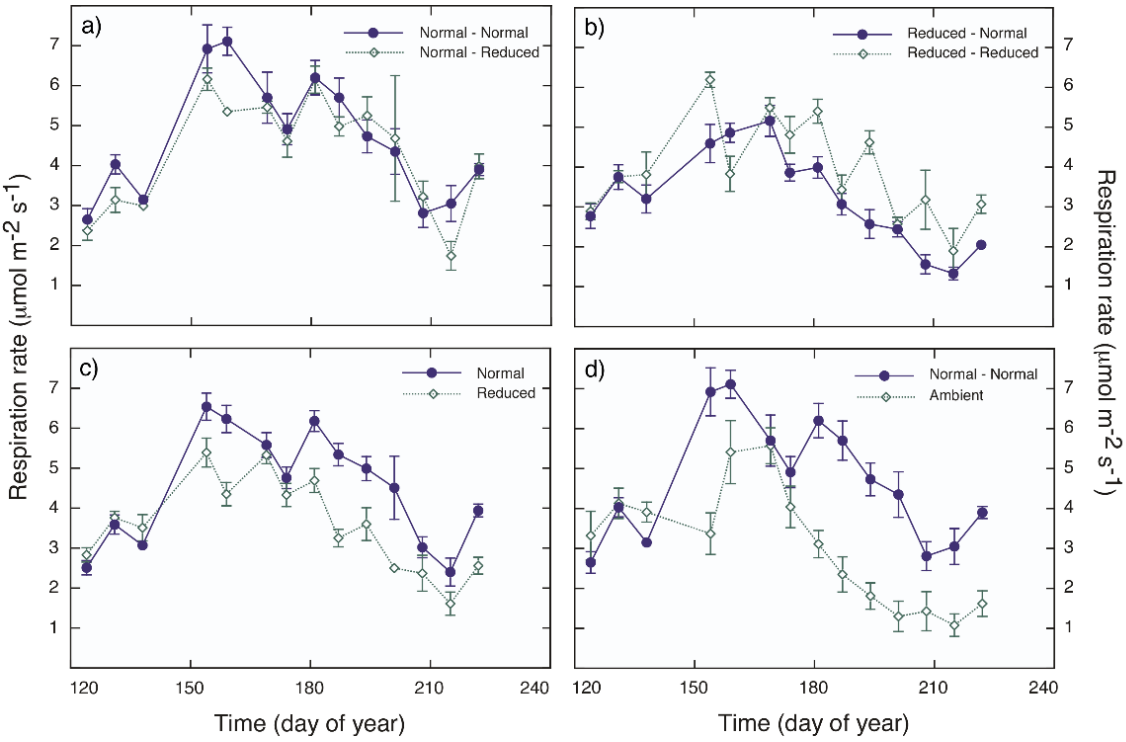

Fig. 13 Pairwise comparison of seasonal variation in average (±SE) soil respiration rate for treatments **a)** NN and NR (n=5), **b)** RN and RR (n=5), **c)** Normal and Reduced (n=10), **d)** NN and Ambient (n=5).

Table 6. Results for the repeated-measures analysis of variance for treatment (between subjects effect), time (within subjects effect) and their interaction on ***Soil respiration*** between **a)** NN and NR, **b)** RN and RR, **c)** N and R, **d)** Ambient and NN with time (DOY) as the repeated factor. Results are shown for the 15-week experimental time period (ETP) and the plant senescence time period (STP). Significant effects are marked with asterisks.

		Treatment		Time		Time x Treat.	
		ETP	*STP*	*ETP*	*STP*	*ETP*	*STP*
a) NN vs. NR	*F*	0.92	0.07	25.41*	14.93*	1.37	0.96
	$df_{n,,d}$	1, 138	1, 78	13, 126	7, 72	13, 126	7, 72
	P	0.3657	0.7973	0.000	0.000	0.1847	0.4666
b) RN vs. RR	*F*	7*	10.4*	28.44*	27.24*	3.57*	2.66*
	$df_{n,,d}$	1, 138	1, 78	13, 126	7, 72	13, 126	7, 72
	P	0.0295	0.0121	0.000	0.000	0.0001	0.0191
c) N vs. R	*F*	12.41*	15.51*	41.63*	32.62*	5.25*	2.5*
	$df_{n,,d}$	1, 278	1, 158	13, 266	7, 152	13, 266	7, 152
	P	0.0024	0.001	0.000	0.000	0.000	0.0195
d) NN vs. Ambient	*F*	20.3*	31.4*	24.04*	22.78*	8.39*	4.45*
	$df_{n,,d}$	1, 138	1, 78	13, 126	7, 72	13, 126	7, 72
	P	0.002	0.0005	0.000	0.000	0.000	0.0005

2.3.5. *Stable carbon isotope composition and total plant nitrogen: Implications for water-use efficiency and photosynthetic capacity*

The analysis of average $\delta^{13}C$ values revealed that only the $\delta^{13}C$ values for the normal (NN+NR) versus reduced (RN+RR) precipitation treatments were significantly different from one another (One-way ANOVA, $F_{(1, 18)} = 21.35$, $P = 0.000$; Fig. 14c). The average $\delta^{13}C$ value of the normal precipitation treatment was significantly lower than the reduced precipitation treatment (Fig. 14c). The differences between the average $\delta^{13}C$ values for the pairwise treatment comparison of NN vs. NR, RN vs. RR and NN vs. ambient were not significant (One-way ANOVA, NN vs. NR: $F_{(1, 8)} = 0.00$, $P = 1.000$, Fig. 14a; RN vs. RR: $F_{(1, 8)} = 0.04$, $P = 0.849$, Fig. 14b; NN vs. Ambient: $F_{(1, 8)} = 2.96$, $P = 0.123$, Fig. 14d).

The total nitrogen content of plant tissue (g m^{-2}) was significantly higher for the normal precipitation treatments (NN+NR) in comparison to the reduced precipitation

treatments (RN+RR) (One-way ANOVA, $F_{(1, 18)} = 26.29$, P = 0.000; Fig. 15c). However, no significant difference in plant nitrogen content was found between NN and NR (One-way ANOVA, $F_{(1, 8)} = 0.72$, P = 0.421; Fig. 15a), and between RN and RR (One-way ANOVA, $F_{(1, 8)} = 0.34$, P = 0.579; Fig. 15b). As expected, the nitrogen concentration of ambient plant tissue was significantly lower than of the NN treatments (One-way ANOVA, $F_{(1, 8)} = 18.90$, P = 0.002; Fig. 15d).

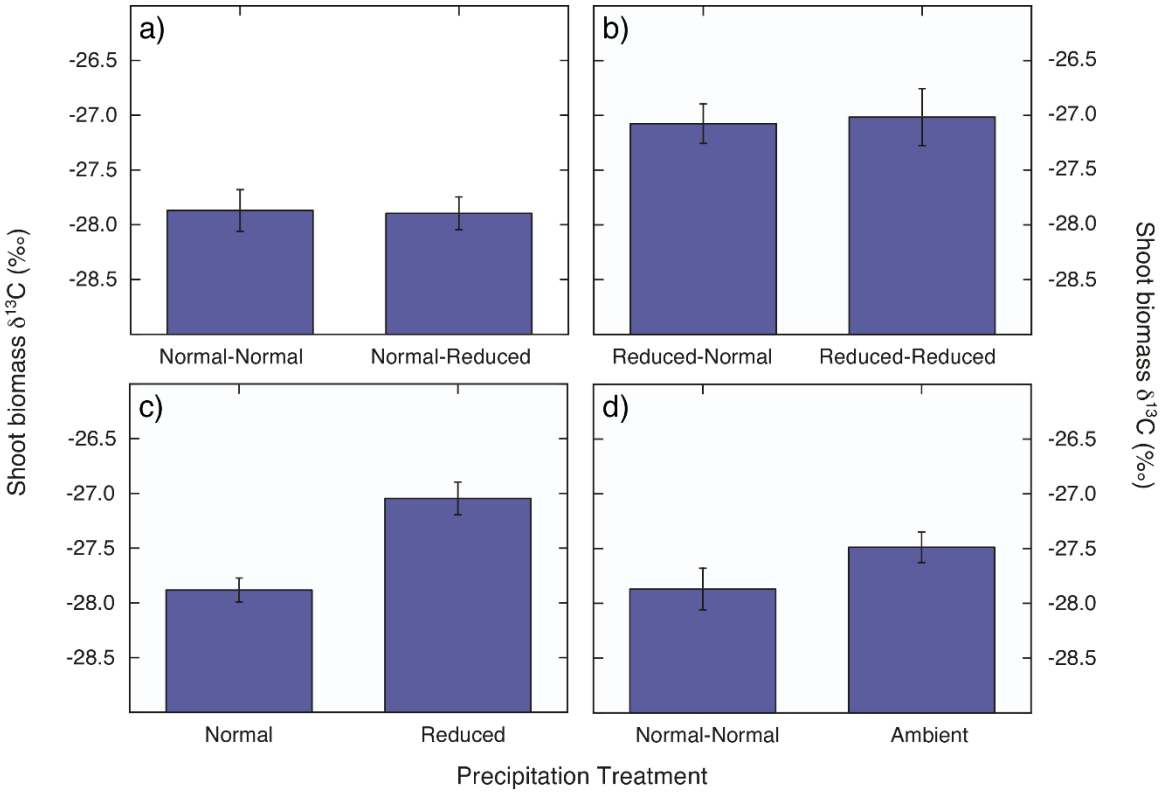

Fig. 14 Pairwise comparison of average (± SE) stable carbon isotope composition ($\delta^{13}C$) of the shoot biomass samples collected August 2017 for treatment **a)** NN and NR (n=5), **b)** RN and RR (n=5), **c)** Normal and Reduced (n=10), **d)** NN and Ambient (n=5).

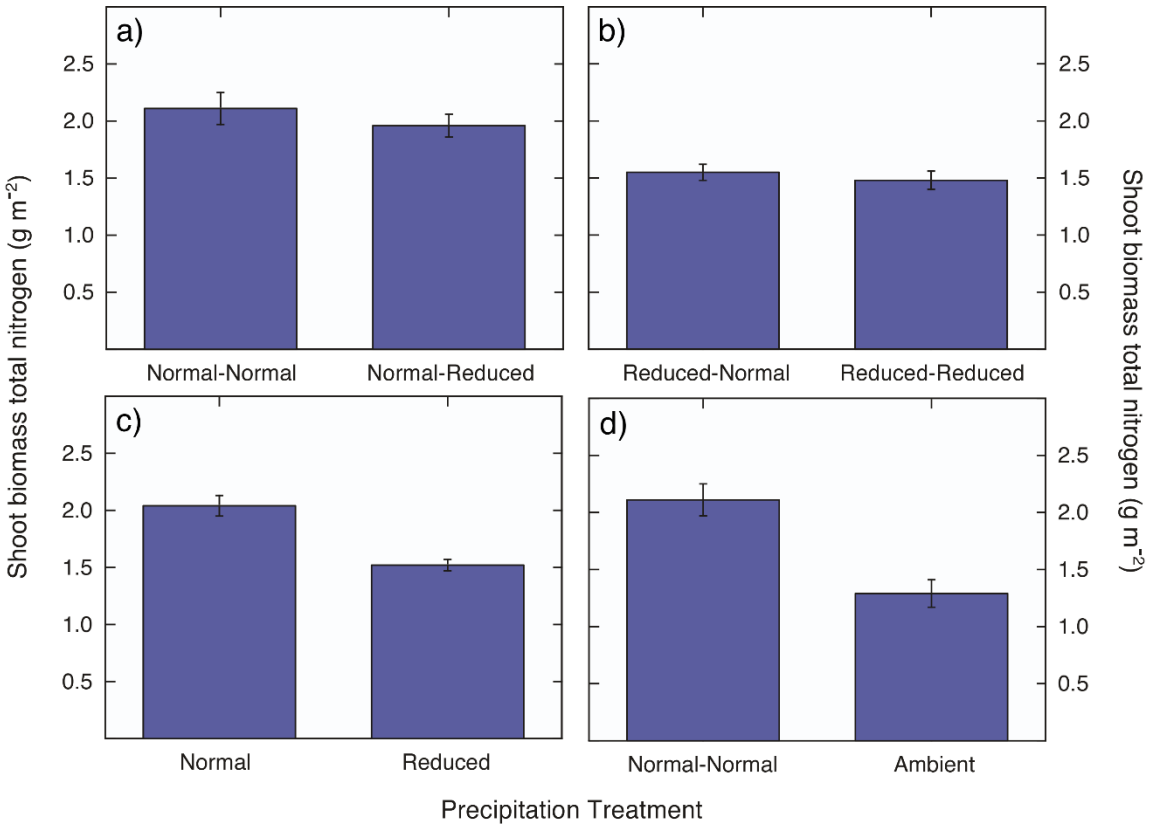

Fig. 15 Pairwise comparison of average (± SE) shoot biomass total nitrogen content for samples collected August 2017 for treatment **a)** NN and NR (n=5), **b)** RN and RR (n=5), **c)** Normal and Reduced (n=10), **d)** NN and Ambient (n=5).

2.3.6 Block effects

The 3-way ANOVA to analyze possible Block effects on the different measurements revealed a sig. Block effect for the $\delta^{13}C$ values ($F_{(4, 15)} = 4.65$, $P = 0.017$), as well as for the soil moisture ($F_{(2, 17)} = 21.43$, $P = 0.002$) and soil temperature ($F_{(2, 17)} = 32.2$, $P = 0.001$) measurements. These effects account for the heterogeneity of the treatment plots, which was not the main focus of this research, but needs to be mentioned.

2.4. Discussion

2.4.1. *Historical Context for Environmental Conditions in 2017*

Measurements of aboveground plant biomass in ambient plots (131.6 ± 15.0 g m^{-2}) were significantly lower than those of the NN treatment plots (227.8 ± 14.4 g m^{-2}), with a total reduction of 42%. Peak aboveground biomass (PAB) records from the same site provide additional perspective to assess plant productivity in 2017. The years of 1999-2001 and 2004 were particularly dry with an average PAB of 116.1 ± 1.9 g m^{-2} in comparison to the years of 2002, 2003, 2005 and 2006 that can be described as average with a PAB of 237.6 ± 3.6 g m^{-2}, placing 2017 just above the average of previous dry years (Flanagan and Adkinson 2011).

Greenness values were also significantly lower in ambient plots in comparison to NN, showing significant treatment effects for the second half of the experiment (Fig. 12d, Table 5). Additional context for this observation was provided by the previous records of greenness values from the PhenoCam (Richardson et al. 2017) installed at the study site. Greenness values in 2017 showed an overall narrower peak with an early, steep incline in May, an above average maximum value at the beginning of June and an early transition into plant senescence at the end of June in comparison to the 2012-2016 average (Fig. 16).

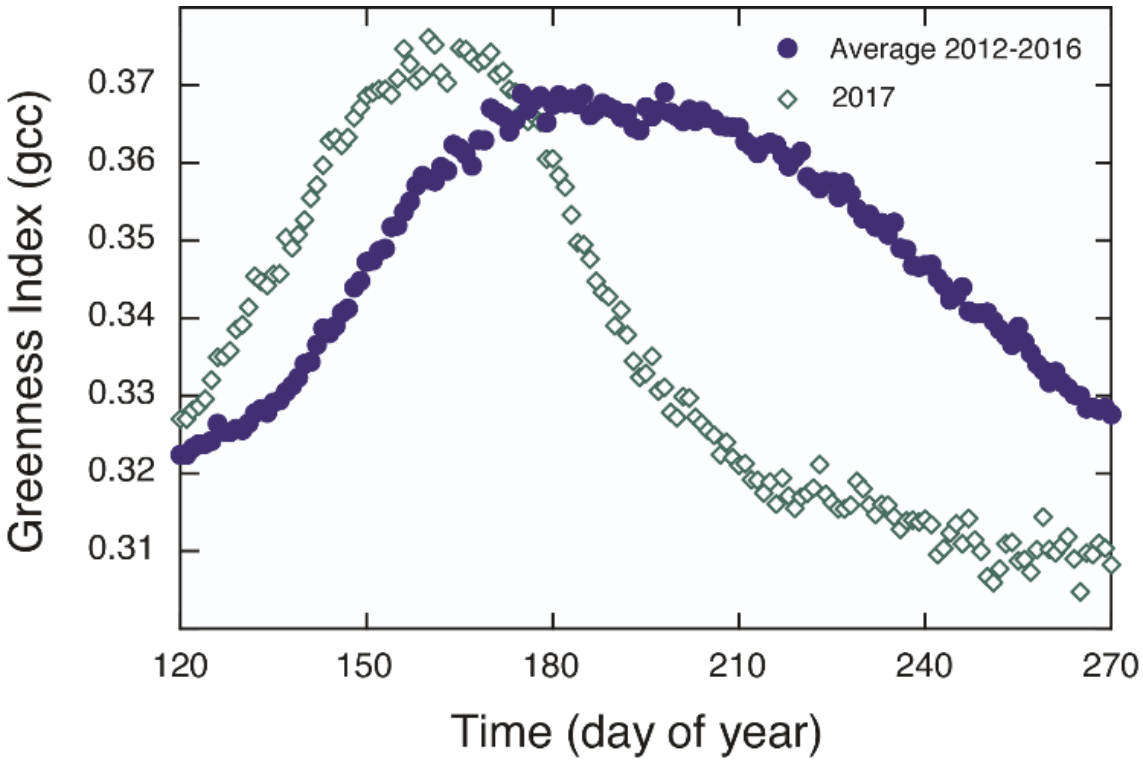

Fig. 16 Comparison of the average gcc values for 2012-2016 to gcc values of 2017 for May - August. Data provided by Phenocam record from the grassland site in Lethbridge, AB, Canada. Data source: https://phenocam.sr.unh.edu/webcam/sites/lethbridge.

The low productivity recorded in the ambient plots for the growing season of 2017 can be attributed to several factors. First, the amount of precipitation for the months of July and August in 2017 was drastically lower than the historic average (Fig. 7a). This was confirmed by the strong reduction in soil moisture content in July and August that was recorded under ambient conditions at the study site (Fig. 8d). Additionally, air temperatures were higher for every month of the growing season in 2017 compared to the historic average (Fig. 7b), likely resulting in higher evaporative demands. Subsequently, the combination of extremely low precipitation and above normal temperatures caused soil moisture to drastically decline during the second half of the experiment, limiting

plant productivity and resulting in extremely low aboveground biomass mid-August in the ambient plots.

2.4.2. *Plant physiological responses to large precipitation events*

Two experiments were conducted to assess the impact of larger but less frequent rain events on several aspects of ecosystem function in a northern Great Plains grassland. The first was based on climate normal growing season precipitation, and its objective was to investigate whether larger but less frequent rain events would alleviate plant water stress and increase aboveground biomass. The second experiment was based on 50% reduced growing season precipitation to simulate drier conditions expected for the future caused by climate warming and increased evaporative demand. The goal of the second experiment was to investigate whether larger, but less frequent rain events can partially compensate for productivity losses associated with low precipitation in dry years.

2.4.2.1. *Experiment one: climate normal precipitation*

Aboveground plant biomass for the reduced frequency treatment (NR: 179.9 ± 3.0 g m^{-2}) was significantly lower than plant biomass measured in the normal frequency treatment plots (NN: 227.8 ± 14.4 g m^{-2}). The greenness index (gcc) steadily increased throughout May and June as the plant community developed, followed by a gradual decline during the second half of the experiment, indicating reduced plant productivity. This was related to the fact that soil moisture levels reached their minimum in July, limiting plant productivity and inducing dormancy in the grasses. Overall gcc values were higher for NN than NR during the second half of the experiment, consistent with the

biomass results. The biomass results contrast with findings reported by Knapp et al. (2008) who showed significantly higher plant biomass in response to larger, less frequent rain events in a semi-arid shortgrass steppe in northeastern Colorado. However, Heisler-White et al. (2009) did report an 18% decrease in ANPP when a mesic tallgrass prairie was exposed to larger less frequent rain events, due to extended periods of plant water stress caused by long periods of below-average soil water content. This provides very good agreement with the ANPP reduction of 20.9 % for the reduced frequency treatment in this experiment, showing the predicted ANPP response of a mesic ecosystem based on the ecosystem response model by Knapp et al. (2008). A comparison of the climatic conditions between northeast Colorado and Lethbridge did show that MAP was almost identical (Colorado: 321.0 mm, Lethbridge: 386.3 mm), but MAT is lower (5.9 vs. 8.6°C) and maximum rain event size is higher (50.1 vs. 18.2 mm) in Lethbridge, resulting in lower evaporative demand and possibly higher soil moisture content at the grassland in Lethbridge. A similar response was observed in a native tallgrass prairie in Kansas, USA where fewer but larger rain events also resulted in a 18% reduction in ANPP compared to the ambient rainfall treatment (Harper et al. 2005).

The lack of significant treatment effects on soil respiration rates in the first experiment can be explained by several factors. First, the treatments had no significant effect on soil moisture content or soil temperature, the primary drivers of soil respiration (Raich and Schlesinger 1992, Lloyd and Taylor 1994). This may be related to the fact that larger but less frequent rain events have been shown to recharge deep soils (Harper et al. 2005), resulting in no overall difference in soil water content between NN and NR. Based on these findings, one would assume that soil respiration should not differ between the two treatments, as its principal drivers did not portray significant changes. Previous

studies also suggest that soil respiration might be temporarily suppressed under altered precipitation treatments with high rainfall intensity, because the air spaces in the soil can be occupied by water, inhibiting the diffusion of CO_2 out of the soil (Bouma and Bryla 2000). Furthermore, extended time periods of water stress that lead to increased plant and microbial stress could decrease soil respiration (Bremer et al. 1998). Responses of soil respiration to larger less frequent rain events are highly complex and vary spatially depending on regional soil water conditions. Semi-arid grasslands have been shown to display rapid positive responses of soil respiration to precipitation pulses, caused by stimulated microbial activity and mineralization of organic matter, after extended dry periods (Liu et al. 2002, Maestre and Cortina 2003, Chen et al. 2008). According to Chen et al. (2008), soil respiration increased within 24 hours of the water application and respiration rates for the extreme precipitation treatments (50, 100 mm) remained significantly higher in comparison to previous respiration rates (Chen et al. 2008). Conversely, a negative response to larger, less frequent rain events was reported in a mesic tallgrass prairie in Kansas, USA where soil respiration declined by 20% in comparison to the control (Harper et al. 2005). This was due to extended dry periods between rain events reducing C assimilation (Fay et al. 2002, Knapp et al. 2002), which limited root growth and substrate supply to microbial communities in the rhizosphere (Johnson and Matchett 2001), resulting in reduced microbial and root-associated respiration (Harper et al. 2005). These contrasting responses of soil respiration to altered precipitation patterns in semiarid and mesic grasslands provide additional evidence that the study site of this experiment may be better described as an intermediate grassland as the soil respiration response observed was situated in between the typical responses of a semi-arid and mesic grassland. High soil respiration rates at the beginning of the

experiment can be explained by the developing plant community stimulating photosynthetic activity and root growth, which was fueled by high soil moisture availability. Furthermore, elevated soil temperatures in June increased soil respiration while soil moisture content was still high. Soil respiration rates then declined throughout July and August due to low water availability limiting photosynthetic activity, root growth and soil microbial activity.

2.4.2.2. *Experiment two: reduced precipitation*

Contrary to expectations, no significant difference occurred in aboveground biomass between the normal (RN: 158.3 ± 8.7 g m^{-2}) and reduced frequency treatment (RR: 139.2 ± 11.0 g m^{-2}) for the 50% reduced growing season precipitation experiment. Overall plant biomass for RR was about 12% lower than for RN. Similarly, a northern mixed grass prairie in eastern Montana showed no response of ANPP to rainfall manipulations towards larger, but fewer rain events (Wilcox et al. 2015). Previous research also suggests that northern mixed grasslands are relatively insensitive to extreme wet growing seasons and droughts, which could explain the lack of response observed in our experiment (Heitschmidt et al. 1999, Frank 2007, White et al. 2014). This lack of sensitivity has been attributed to early-season growth dynamics of C3-dominated grasslands (Ehleringer 1978, Pearcy et al. 1981, Vermeire et al. 2008) and greater reliance on soil moisture input from the winter and spring (Vermeire et al. 2008). Primary factors in influencing sensitivities to altered precipitation patterns in these grasslands might be the functional composition of the plant community, rooting depth and the timing of rain events (Wilcox et al. 2015). On the other hand, a factorial combination of 30% reduced

rainfall quantity and 50% greater inter rainfall dry periods in a tallgrass prairie in northeast Kansas, USA resulted in reduced ANPP and soil respiration in response to increased inter-rainfall intervals, but reduced rainfall quantity had no impact (Fay et al. 2000). As biomass production did not significantly decrease, or increase, in response to larger, less frequent rain events based on 50% reduced precipitation, we can assume that the altered rainfall frequency partially compensated for productivity losses associated with dry conditions, but it was not enough to surpass the ANPP of RN at the end of the growing season.

No difference in the seasonal course of soil moisture content, soil temperature, and gcc values were found in comparison to the first experiment. Overall soil moisture content in the reduced precipitation experiment (RN vs. RR) was slightly higher, and gcc values were slightly lower, than in the climate normal precipitation experiment (NN vs. NR). Previous research has shown a strong correlation between greenness indices and leaf area index (LAI) (Keenan et al. 2014). Given that, we can assume lower LAI for the reduced precipitation experiment based on measured gcc values. I suggest that lower LAI resulted in lower evapotranspiration and, therefore, higher residual soil moisture in the reduced precipitation experiment. The lack of significant treatment effects on soil moisture content, soil temperature and gcc were consistent with the results of the first experiment, indicating that changes in precipitation patterns towards larger less frequent rain events had no impact on these processes, even under extremely dry conditions.

There was a significant treatment effect on soil respiration, the respiration rates for RR were significantly higher than those of RN, showing clear peaks in respiration that coincided with the bi-weekly water additions (Fig. 13b, Table 6). These peaks represent the rapid increases in soil respiration that follow precipitation pulses due to wetting of dry

soils that can be observed in grasslands (Kieft et al. 1987, Liu et al. 2002, Chen et al. 2008). These rapid increases in soil respiration were likely caused by an increase in the availability of microbial substrate, leading to a stimulation of microbial activity (Huxman et al. 2004, Chen et al. 2008). A modelling analysis of the effects of extreme drought (> 40% decrease in annual precipitation) on respiration showed that the main drivers of sensitivity of heterotrophic respiration to drought were soil drying, reduced carbon inputs and reductions in soil C content, which are comparably slow processes (Shi et al. 2014). The modelling analysis showed that at mesic sites, reducing the number of rain events under drought conditions lessened the decrease in heterotrophic respiration in comparison to a reduction in rainfall event size (Shi et al. 2014), a pattern consistent with my results. Low aboveground biomass and higher soil respiration rates observed in the reduced frequency treatment (RR) could also be an indicator for increased allocation of carbon from shoots to roots, in order to improve water uptake from deeper soil layers. Such processes have previously been observed during droughts and warming in other grasslands (Poorter and Nagel 2000, Xu et al. 2012).

2.4.2.3. *Combined effects of normal and reduced precipitation treatments*

Aboveground biomass was significantly higher for the normal precipitation treatments (203.9 ± 11.0 g m^{-2}) in comparison to the reduced precipitation treatments (148.8 ± 7.3 g m^{-2}), resulting in a 27% reduction of ANPP under 50% reduced precipitation. Similar responses have been reported by Xu et al. (2013) showing that 50% reduced precipitation decreased ANPP by an average of 13.5 % in a mixed-grass prairie in Oklahoma, USA. Generally the consensus is that aboveground biomass production

decreases in conjunction with reduced precipitation (Fay et al. 2000, Penuelas et al. 2004). This is due to the fact that precipitation is the major driver of ANPP in grassland ecosystems (Mowll et al. 2015). For the second half of the experiment, a significant treatment effect on the greenness values with significantly higher gcc values for the normal precipitation treatment was observed. These outcomes are consistent with the biomass results, providing a temporal context for the divergence in the development of plant biomass between the normal and reduced precipitation treatments.

The significant interaction effect of treatment and time on soil temperature can be attributed to small early season effects. Potentially lower evapotranspiration in the reduced precipitation treatments due to lower plant biomass and LAI could explain why soil moisture content did not vary between the climate normal and reduced precipitation experiment.

Soil respiration rates for the normal precipitation treatment were significantly higher in comparison to the reduced precipitation treatment, suggesting that reduced precipitation inputs limited soil respiration. This was particularly obvious due to the spikes in soil respiration rates that were recorded in conjunction with the biweekly water additions (Fig. 13c). Similar responses have also been reported in a native tallgrass prairie in Kansas, USA where soil respiration decreased by 8% under a 30% reduction of growing season rainfall (Harper et al. 2005). They also reported reduced plant productivity in response to reduced rainfall quantity and altered rainfall timing, consistent with my biomass results (Fig. 11). Harper et al. (2005) showed that soil respiration was correlated with both soil water content and soil temperature, but these factors explained only 38-48% of the variability of soil respiration under altered rainfall timing. This observation suggests that other factors like substrate availability and plant or microbial

stress may limit soil respiration under these climatic conditions. A similar rationale might explain why reduced soil respiration rates in accordance with the reduced precipitation treatments were observed, even though no difference in soil water content was found between the reduced and normal precipitation treatments. As previously mentioned, the reduced biomass production and gcc values in the reduced precipitation treatment plots explained the lack of significant treatment effect on soil moisture content, but this could also explain the reduced soil respiration rates. Lower biomass production implies lower photosynthetic activity, which could reduce the amount of carbohydrates supplied to plant roots for growth, resulting in a limited availability of root exudates to microbes, which subsequently resulted in lower root and soil microbial respiration (Jentsch et al. 2011, Flanagan et al. 2013).

2.4.3. *Stable carbon isotope composition and total plant nitrogen: Implications for water-use efficiency and photosynthetic capacity*

Contrary to expectations, no significant differences between the stable carbon isotope composition of plant shoot biomass ($\delta^{13}C$) for the normal and reduced frequency treatments for the first and second experiment was observed. This lack of response suggests that changes in peak aboveground biomass were not the result of a limitation in stomatal conductance, but rather due to reduced photosynthetic capacity. However, the normal precipitation experiment did show significantly lower $\delta^{13}C$ values in comparison to the reduced precipitation experiment. This suggests that the differences in biomass production between the normal and reduced precipitation treatments were due to a combination of reduced stomatal conductance and photosynthetic capacity.

Flanagan (2009) previously showed a negative correlation between peak aboveground biomass and $\delta^{13}C$ values at the grassland in Lethbridge, indicating that lower biomass production was the result of reduced water availability. This is a typical response that can be observed particularly in semi-arid regions, as water availability is the primary limiting factor in these grasslands. The carbon isotope composition of plant tissue becomes depleted in ^{13}C (lower $\delta^{13}C$ values), when stomatal conductance and the uptake of carbon dioxide for photosynthesis increases. Conversely, the carbon isotope composition of plant tissue becomes enriched in ^{13}C (higher $\delta^{13}C$ values) when stomatal conductance is reduced relative to photosynthetic capacity (Flanagan 2009). Therefore, low water availability leads to reduced stomatal conductance, which decreases photosynthetic activity and results in lower biomass production and higher $\delta^{13}C$ values (Flanagan 2009). Similarly, Jentsch et al. (2011) found that severe drought imposed on a grassland community resulted in reduced leaf water potential, leaf gas exchange and leaf protein content, with a corresponding increase in $\delta^{13}C$ values. However, Jentsch et al. (2011) also reported no response of above- and belowground primary production to drought.

In comparison with other plant functional types, grasses have been found to have higher ci/ca values and lower $\delta^{13}C$ values as well as lower WUE, because they are short-lived species that take advantage of soil moisture when it is available and go into dormancy during times of water stress (Smedley et al. 1991, Ehleringer 1993, Ponton et al. 2006). Based on these findings we would expect that WUE is reduced during times of sufficient moisture availability and is increased when plants are under water stress. Farquhar and Sharkey (1982) also found that WUE is higher when stomatal conductance

is reduced because it limits water loss through transpiration more than CO_2 assimilation. Generally, the $\delta^{13}C$ values showed no variation between the different precipitation treatments, but did show lower $\delta^{13}C$ values in correspondence with higher biomass for the normal in comparison to the reduced precipitation treatments. This indicates that reduced biomass production was mainly a result of reduced photosynthetic capacity, resulting in higher WUE in the reduced biomass plots. Measurements of stable carbon isotope composition of plant tissue are therefore a valuable tool to investigate variation in physiological responses to changing climate conditions (Flanagan 2009).

The measurement of total plant nitrogen content provides additional information on the photosynthetic capacity of plants and further context to assess the impact of altered precipitation patterns on ecosystem function. The comparison of ambient peak aboveground biomass for 2017 to previous years shows that plant productivity was low, similar to previous dry years (Fig. 17a). This also resulted in lower total plant nitrogen content that was even below the average nitrogen concentration previously recorded in this grassland during dry years (Fig. 17b). Plant nitrogen content was not significantly different for the normal and reduced frequency treatment of experiment one and two, suggesting that reduced plant productivity of the NR treatment was not a result of lower plant nitrogen content, but was limited by water availability directly. As expected, nitrogen concentration of plant tissue was significantly higher for the normal precipitation experiment, indicating that increased nitrogen concentration of plant tissue contributed to higher photosynthetic capacity and increased plant production (Koerselman & Meuleman 1996, Burke et al. 1997). Similarly, Heisler-White (2009) suggested that enhanced wetting and drying cycles in semi-arid grasslands can lead to increased soil microbial activity and nitrogen release, causing greater foliar N content and leaf level carbon

fixation that results in higher plant biomass. Furthermore, Gu and Riley (2010) suggested that the effect of soil texture on soil moisture dynamics is a major factor influencing the response of nitrogen cycling to changes in precipitation patterns.

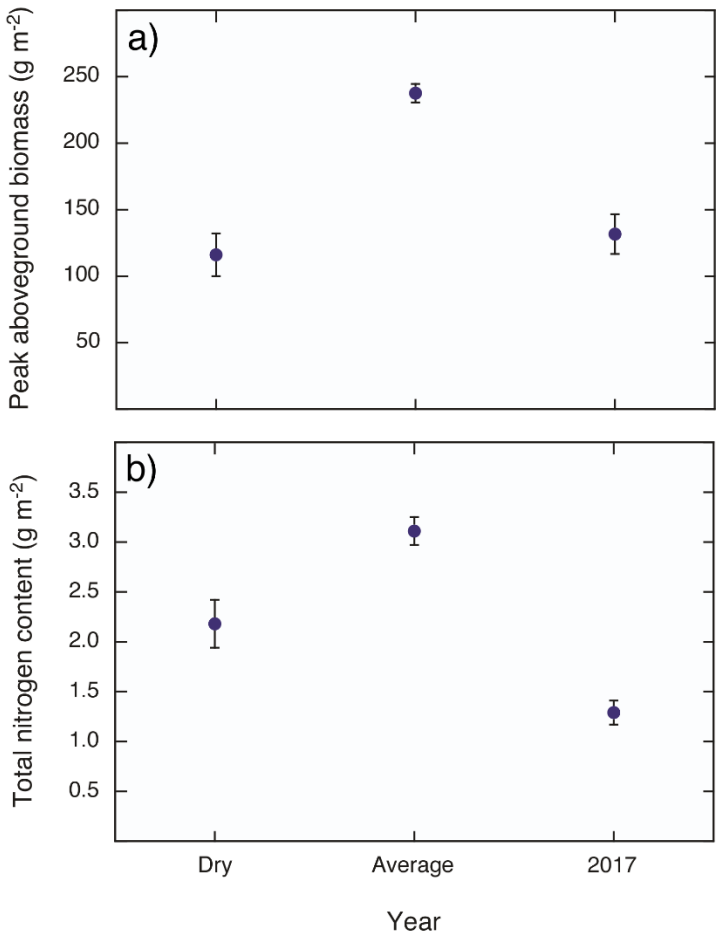

Fig. 17 Comparison of dry and average years with 2017 for **a)** peak aboveground biomass, and **b)** total plant nitrogen content at the time of peak biomass measured at the grassland site near Lethbridge, Alberta. Points represent the mean ± SE (n=4). Data for previous years provided by Flanagan & Adkinson (2011).

2.5. Conclusion

The response of aboveground plant biomass to larger less frequent rain events based on climate normal precipitation followed the ANPP response of a mesic ecosystem based on the ecosystem response model developed by Knapp et al. (2008). This unexpected ANPP response suggests that the study site is closer to a mesic grassland, rather than a semi-arid grassland based on the response classification by Knapp et al. (2008). Larger less frequent rain events only partially compensated for productivity losses associated with drier conditions based on 50% reduced precipitation. This response does not confirm my hypothesis, but it also does not completely reject it, as ANPP did not further decline under reduced rain frequency, but was stabilized. It was, however, consistent with responses that would be expected in a semi-arid ecosystem, suggesting that the reduced precipitation treatment created conditions closer to an actual semi-arid grassland.

Soil respiration rates were significantly impacted by larger, less frequent rain events based on 50% reduced precipitation and stayed constant under climate normal precipitation. In combination with the biomass results, soil respiration under altered precipitation timing and quantity could have a negative impact on carbon cycling processes, if NEE does not increase, resulting in a net ecosystem carbon loss.

Water-use efficiency proved to be relatively high when plants were confronted with water stress, which was induced by the altered precipitation treatments and caused reduced biomass production. The precipitation treatments had no effect on the stable carbon isotope composition of plant tissue, confirming that low biomass production was the result of water stress caused by low water availability.

CHAPTER 3. SUMMARY AND KEY FINDINGS

Aboveground net primary production (ANPP) in this northern Great Plains grassland responded negatively to larger, less frequent rain events based on climate normal precipitation, suggesting that this grassland might be better classified as a mesic, rather than semi-arid grassland according to Knapp's ecosystem response model. Under dry conditions (50% reduced precipitation) larger, less frequent rain events partially compensated for the productivity losses expected with reduced water availability, suggesting that aboveground productivity may not further decline under future drier conditions in this region. Changes in plant productivity impact the ecosystem carbon cycle by directly (root respiration) and indirectly (root exudation and root litter inputs) contributing to the belowground CO_2 production (Harper et al. 2005) leading to alterations of carbon budgets.

Soil respiration was insensitive to larger, but less frequent rain events under climate normal precipitation. However, it was significantly increased when rainfall quantity was reduced and combined with altered rainfall timing, towards larger less frequent events. Because ANPP and soil respiration are two major processes in the carbon cycle, even small variations in these parameters will have large implications for the global carbon budget due to the vast geographical extent of this biome (Heisler-White et al. 2009, Arredondo et al. 2016). In terrestrial ecosystems, soil is the largest source of CO_2 (Bahn et al. 2009), therefore the increased respiration rates observed in this experiment in combination with reduced biomass production are expected to result in a positive feedback effect (ecosystem net loss of CO_2) to the atmosphere, under warmer and drier conditions predicted for the future (Karl and Trenberth 2003). This effect was confirmed by Chou et al. (2008), who observed that heterotrophic respiratory losses exceeded

increases in NPP, due to the high sensitivity of R_h to changes in the seasonal timing of rainfall, resulting in an overall ecosystem carbon loss.

Water-use efficiency proved to be increased under intensified water stress and low biomass production was associated with high stable carbon isotope composition. This decrease in biomass production in response to larger less frequent rain events was attributed to restricted water availability.

Investigating the impact that climate change induced alterations of the global hydrological cycle have on ecosystem functions is of high importance. The relationship between precipitation (PPT) and aboveground net primary production (ANPP) is highly complex and has climatological, ecological and socioeconomic implications (Knapp et al. 2017). Climatologically this relationship is significant because it is directly linked to the global carbon cycle, as terrestrial ecosystems contribute two-thirds of the global net primary production (NPP) and the terrestrial plant biomass is holding the equivalent of 70% of the carbon stored in the atmosphere (Houghton 2007). Therefore, any variations in aboveground primary production will have a direct feedback effect on the global carbon cycle by altering atmospheric CO_2 levels. The ecological significance lies in understanding the mechanisms that control ecosystem processes, allowing us to improve climate models to accurately predict changes in ecosystem functions to future climatic changes. As humans, we also rely on ANPP for food, building materials, fuel and as forage for livestock (Haberl et al. 2007) to sustain our livelihood, which marks the socioeconomic importance of this research.

This experiment was intended to address multiple knowledge gaps that have been identified through previous research. First, the PPT-ANPP relationship has been found to be nonlinear under changing climatic conditions, highlighting the need for experiments

that investigate the impact of extreme precipitation anomalies (Knapp et al. 2017), because of their disproportionally large consequences for the global carbon cycle (Hoover and Rogers 2016). Second, due to the large spatial and temporal variation of warming and precipitation modifications caused by climate change, and the complexity of interactive effects of climate drivers, extrapolations from one region to another are not possible. Therefore local and regional experiments are needed that take site specific differences in soil characteristics, plant communities and land-use practices into account to accurately assess ecosystem responses to climate change drivers and their interactive effects (Fay et al. 2008, Polley et al. 2013, Reyer et al. 2013).

The biggest limitation of this experiment was the duration, as short-term experiments cannot inform about long-term consequences (Beier et al. 2012). For instance, some ecosystem responses only take effect after several years, including changes in plant abundance and community species composition.

The need for coordinated regional experiments that follow an experimental protocol with a specific set of metrics has been voiced previously (Smith et al. 2017), with the intention to increase the precision of climate models to accurately forecast ecosystem responses to climate change. Multi-year experiments are useful as they provide insights into long-term ecosystem responses to short-term extremes as well as continuous directional changes in environmental conditions. Multiple rain manipulation experiments based on climate normal precipitation have been conducted, highlighting the need for experiments focusing on extreme precipitation anomalies (Beier et al. 2012). Extreme events have been observed more frequently indicating a shift in precipitation patterns (IPCC 2007, New et al. 2001, Groisman et al. 2005). Furthermore, water stress thresholds for different ecosystems leading to declines and collapses in ecosystem functions are

largely unknown and have significant implications for the assessment of changes regarding the global carbon cycle (Reichstein et al. 2013, Estiarte et al. 2016).

Coordinated regional precipitation experiments are necessary that address the identification of water stress thresholds and how their exceedance affects ANPP, as well as the determination of maximum ANPP levels, the environmental conditions leading to plant mortalities, as well as the impact of extreme precipitation anomalies on ecosystem functions. Insights from these regional experiments should significantly improve our ability to accurately forecast ecosystem responses associated with changes in the global hydrological cycle and their impact on carbon budgets.

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Appendix A: Analysis of Historic Precipitation Data

1.1. Parameters and definitions

The daily precipitation data for Lethbridge from 1951 to 2015 was obtained from Environment Canada to analyze the historic precipitation record for the number of rain events, mean rain event size, maximum rain event size, mean dry interval between rain events and total precipitation during the time of 1 May - 31 August. This time frame marks the time frame for our experimental manipulation that is based on the analysis of the historic precipitation record which provides the baseline for creating the four experimental treatments.

To make the analysis comparable to previous studies we followed the definition of a rain event established by Heisler-White (2008). Precipitation larger than or equal to 2 mm was defined as a rain event, any event smaller than 2 mm was not considered biologically effective and therefore not included. Up to three consecutive days of rain make up one rain event, if there are more than three days of rain registered in a row they are split into at least two events and the date of the event is assigned to the day with the highest precipitation received (Heisler-White et al. 2008).

1.2. Historic precipitation analysis

The analysis of this 64-year period (1951 - 2015) showed an average of 14.03 rain events during the 1 May - 31 August peak growing season. The mean rain event size was 14.13 mm with a mean maximum rain event of 52.27 mm. The mean dry interval length was 7.45 days and the mean growing season precipitation (GSP) during 1 May - 31 August was 199.58 mm.

The lowest number of peak growing season rainfall events was recorded in 2001 with 6 events and the highest in 1995 with 23 events. Total rainfall during 1 May - 31 August showed a low of 50.4 mm in 2001 and a high of 425.6 mm in 1993. Table A1 shows a comparison of the analysis of meteorological data for Lethbridge and Colorado (Heisler-White, 2008).

Table A1. Comparison of growing season (26 May-11 Sep) meteorological data for Northeast Colorado (Heisler-White, 2008) and southern Alberta.

	COLORADO	LETHBRIDGE
LOCATION	Central Plains, North East Colorado	Great Plains, Southern Alberta
GRASSLAND TYPE	Shortgrass steppe, semi-arid	Short/mixed grass, semi-arid
MAP	321 mm	386.3 mm
MAT	8.6 °C	5.9 °C
GSP	177.5 mm	176.9 mm
AVG. NUMBER OF EVENTS	14 events	14 events
AVG. EVENT SIZE	12.9 mm	12.6 mm
AVG. DRY INTERVAL	8.4 days	7.9 days
AVG. MAX EVENT	18.2 mm	50.1 mm

1.3. Frequency analysis

The average rain event frequency lies between 14 to 15 events, which occurred in 8 and 10 years during the 64-year period and reflects the climate normal event frequency in our experimental manipulation. The treatment of 7 rain events occurs once during the 64-year period at the extreme drought end of the scale and represents our 50 % reduced frequency in our experimental manipulation (Fig. A1).

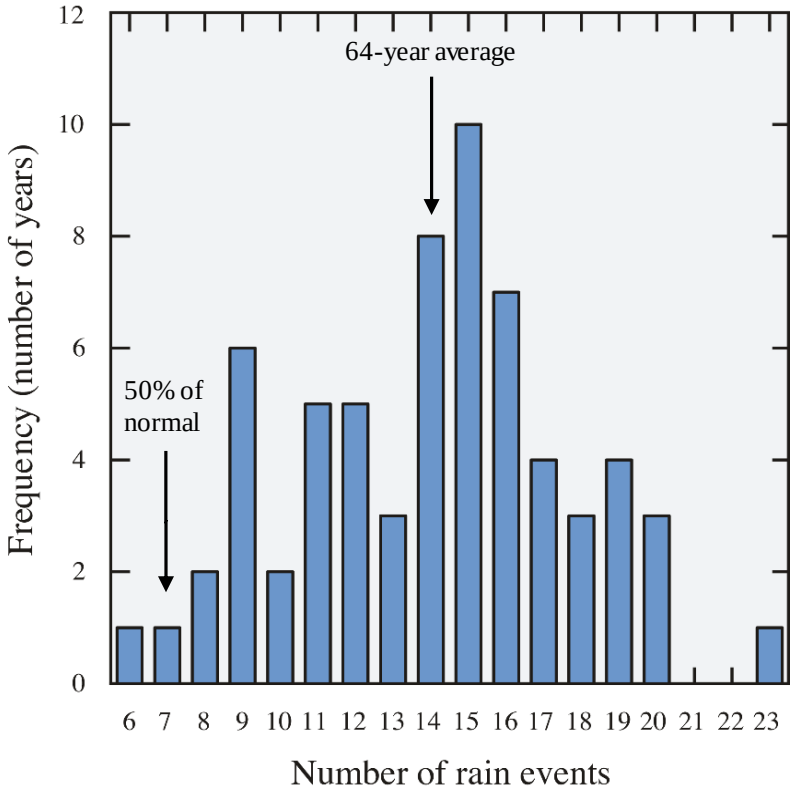

Fig. A1 Distribution of the total number of rainfall events from 1951-2015 during the time of May 1-Aug 31 and the frequency of years in which they occur. The experimental treatments of 14 and 7 events are indicated.

The selected rain event numbers of 7 and 15 events for the experiment make up a 14 and 7 day dry interval in between rain events which represent the climate normal number of dry days in between rain events (7 days) that occurred during 14 years of the 64-year period and the 50 % increase of dry days (14 days) which occurred in 3 years during the 64-year period towards the extreme high end of the distribution (Fig. A2).

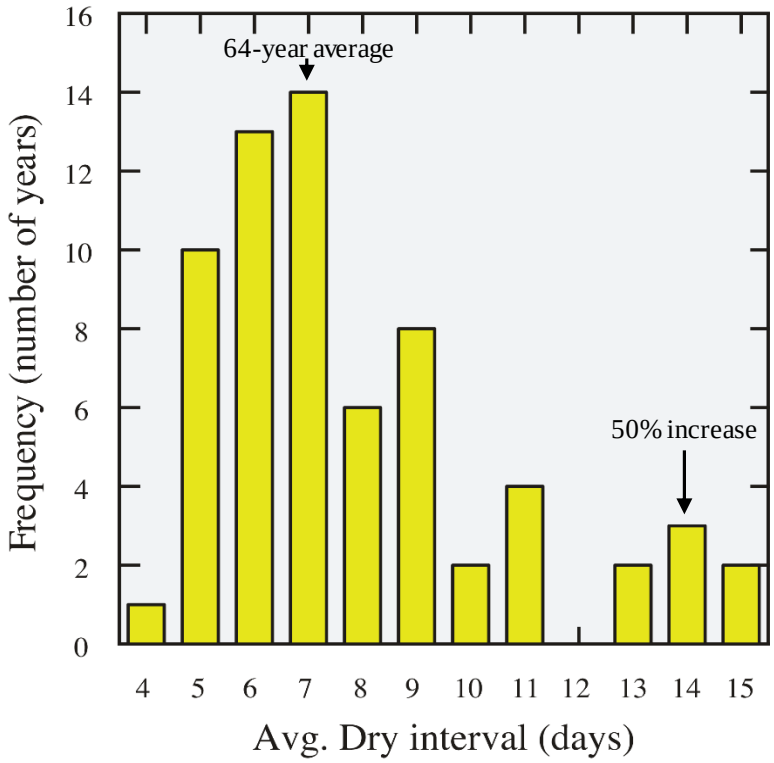

Fig. A2 Distribution of the average number of dry days in between rainfall events from 1951-2015 during the time of May 1-Aug 31 and the frequency of years in which they occur. The dry intervals of 7 and 14 days for the experiment are indicated.

The average rain event size lies around 14 mm and occurred in 6 years during the 64-year period and represents our climate normal precipitation and frequency treatment (NN) as well as the reduced precipitation and frequency treatment (RR) in the experiment. The 7 mm event size for our 50 % reduced precipitation treatment (RN) occurred once during the 64-year period at the extreme low end to the left of the distribution. Contrasting to that stands the 30 mm event size for the reduced frequency under climate normal precipitation treatment (NR) that is represented once as well during the 64-year period but at the extreme high end to the right of the distribution (Fig. A3).

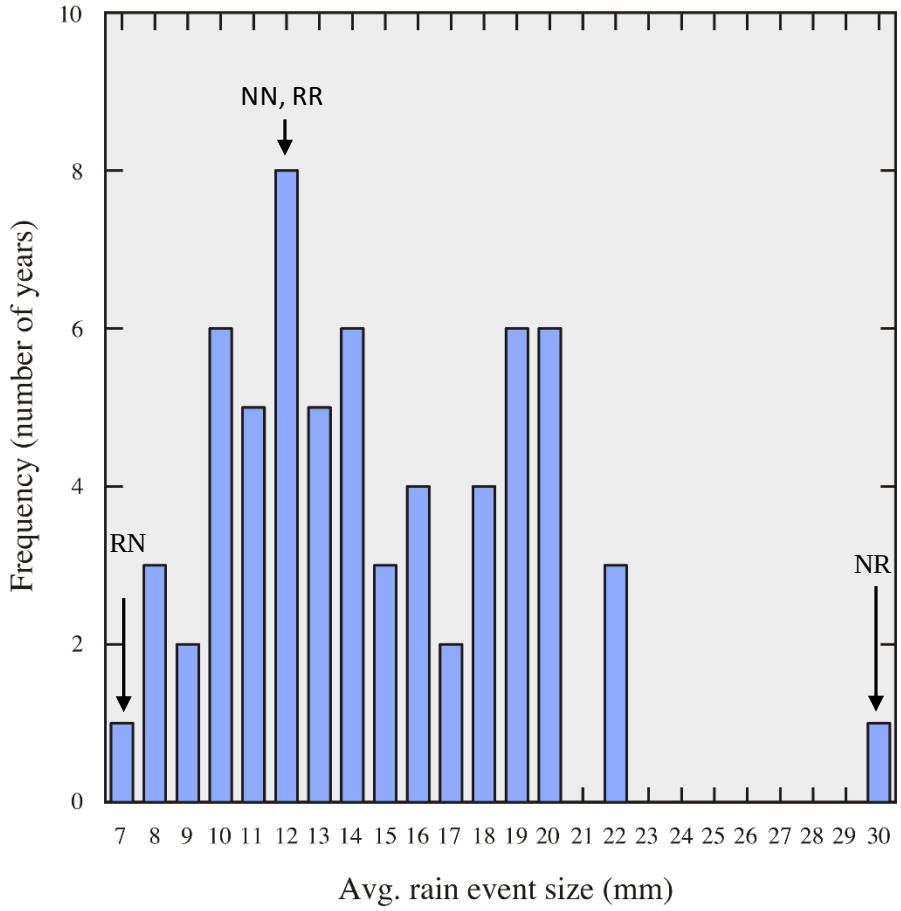

Fig. A3 Distribution of the average rain event size from 1951-2015 during the time of May 1-Aug 31 and the frequency of years in which they occur. The experimental treatments of 7, 14 and 30 mm are indicated.

The precipitation amounts selected for the experimental manipulation for the time frame of 1 May - 31 August of 210 mm for the climate normal treatment occurred in 8 years during the 64-year period and the 50 % reduced precipitation treatment of 105 mm occurs during two years of the 64-year period towards the extreme dry end of the distribution (Fig. A4).

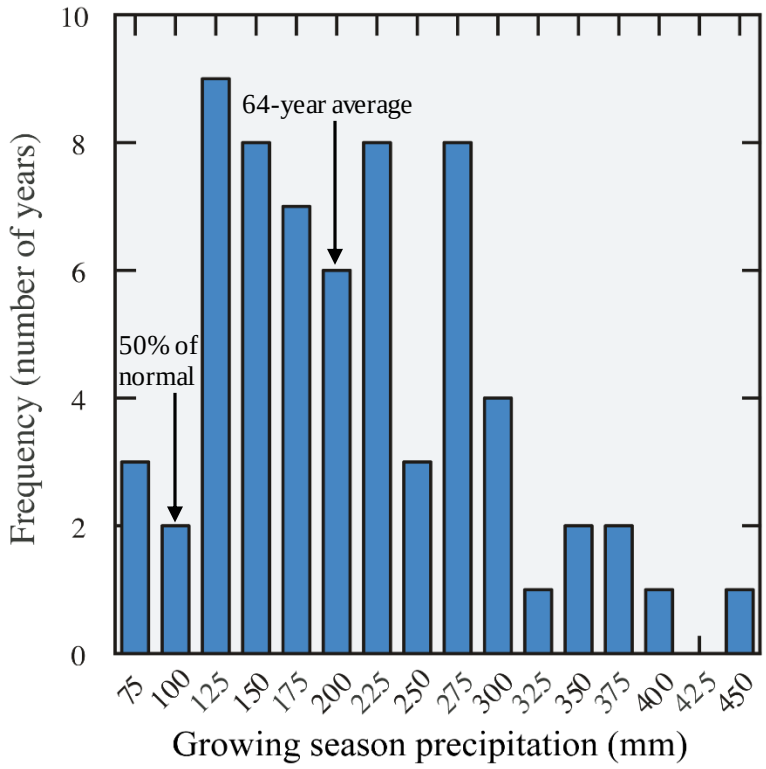

Fig. A4 Distribution of the growing season precipitation (GSP) received from 1951-2015 during the time of May 1-Aug 31 and the frequency of years in which they occur. The precipitation amounts of 105 mm (RN, RR) and 210 mm (NN, NR) for the experiment are indicated.

Appendix B: Shelter effects on Microclimate

1.1. Experimental set up

Shelter effects on the microclimate of our experimental plots were assessed by running three cycles of paired measurements underneath and outside the rain out shelter to evaluate to what degree the polycarbonate sheeting of the shelter roofs influences the amount of photosynthetically active radiation received and the air temperature underneath the shelters. Measurements were taken every 5 seconds and averaged every 30 min and stored on a Data logger (CR23X, Campbell Scientific Ltd.) which was powered by a battery connected to a solar panel (Fig. B1b). The experiment was conducted during the time of 30 June – 24 July 2016.

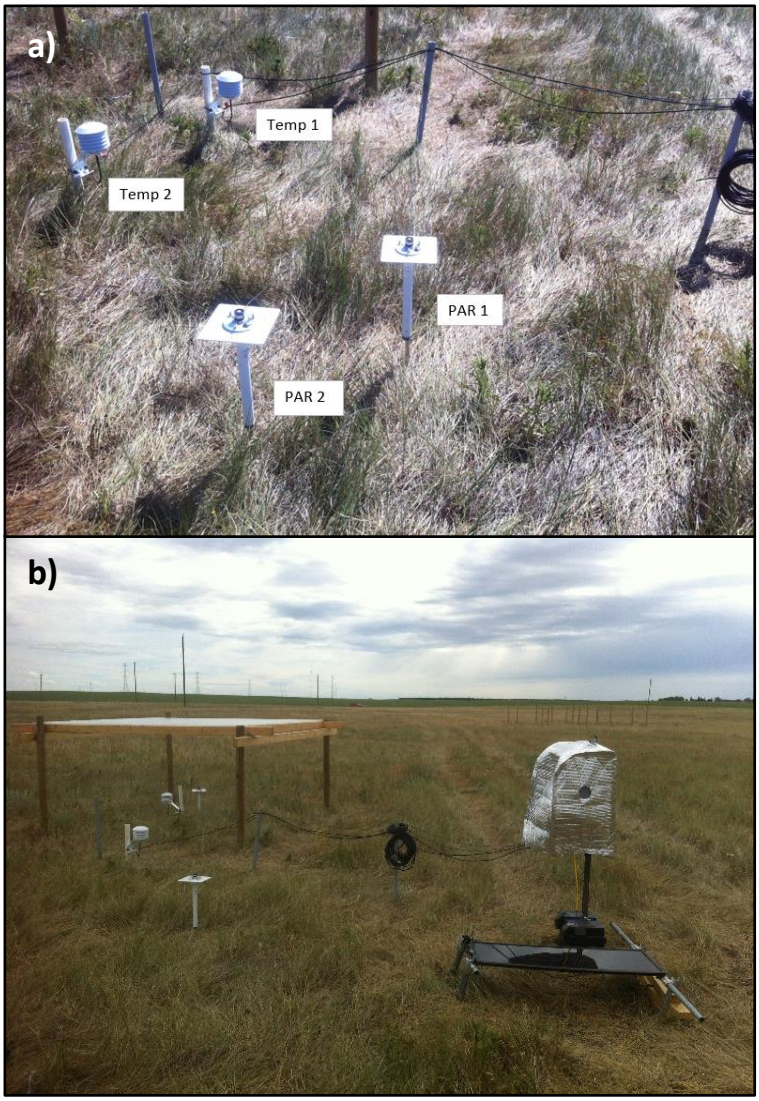

Fig. B1 Shelter effects experimental set up **a)** both PAR sensors and Air temperature sensors outside the rainout shelter **b)** paired measurements with one PAR/Air Temperature sensor under and one of each sensor outside the shelter.

1.2. Photosynthetically active radiation (PAR)

The photosynthetically active radiation (PAR) was measured underneath and outside the shelter using a Quantum PAR sensor (LI-190R, LI-COR) which was mounted plumb 40 cm above the ground on a metal stake on a platform. Percent transmittance was calculated as a ratio of the PAR values underneath and outside the shelter during the time frame of 11am - 5pm. The sensors were first tested outside the shelter to ensure accuracy of the measurements which resulted in 98 % conformity (Fig. B1a, 2). For the experiment the PAR values were integrated to receive the actual number of photons coming in during the 11am to 5pm time frame underneath and outside of the shelter. The analysis of PAR sensor 1 outside the shelter and PAR sensor 2 underneath the shelter (n = 7 days) showed an average of 29.07 mol m^{-2} $period^{-1}$ (11am – 5pm) for sensor 1 and an average of 24.96 mol m^{-2} $period^{-1}$ (11am – 5pm) for sensor 2 with a ratio of 85 % transmittance (Fig. B3a). After switching the sensors to sensor 1 underneath the shelter and sensor 2 outside the shelter (n=9 days) the average for sensor 1 was 24.61 mol m^{-2} $period^{-1}$ (11am – 5pm) and 29.92 mol m^{-2} $period^{-1}$ (11am – 5pm) for sensor 2 with a ratio of 82 % transmittance (Fig. B3b).
Both paired measurements combined resulted in an average of 83 % transmittance, which is similar to previous studies of shelter effects on incoming photosynthetically active radiation (Fay et al. 2000, Yahdjian and Sala 2002, Heisler-White et al. 2008).

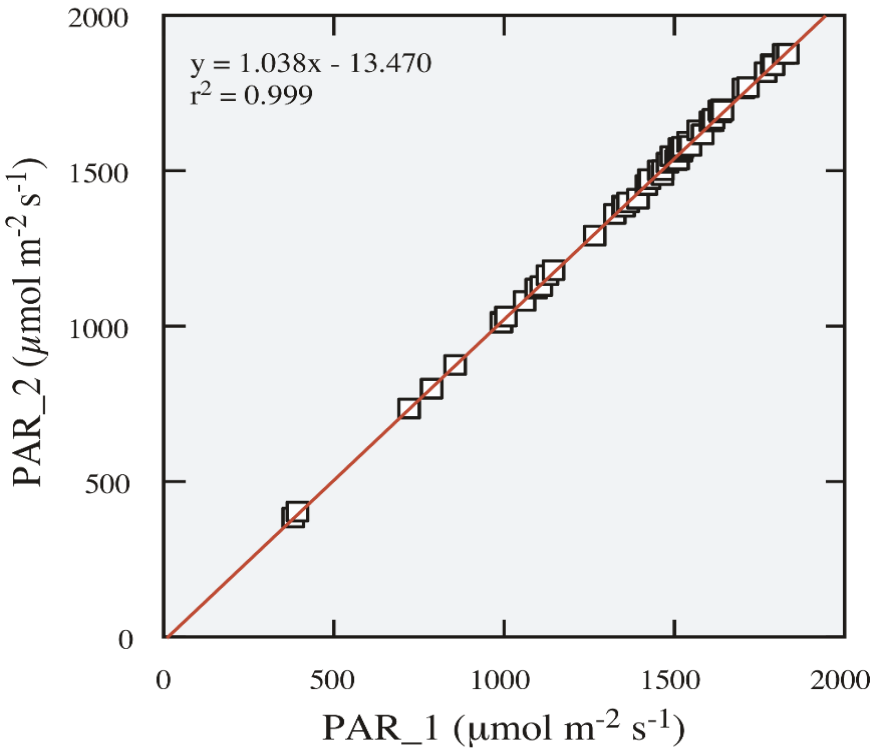

Fig. B2 Regression of PAR sensor 1 (PAR_IN_1) and PAR sensor 2 (PAR_IN_2) outside the shelter during the time of 11am to 5 pm from 30 June – 4 July 2016.

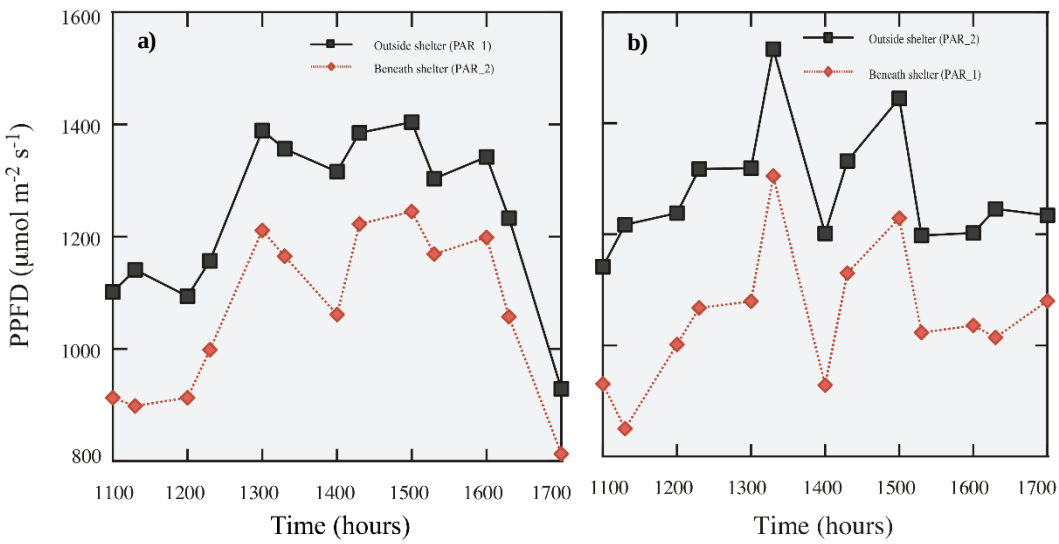

Fig. B3 Effect of rainout shelter on microclimate. Photosynthetic Photon Flux Density (PPFD) under and outside the shelter (ambient) with **a)** Sensor 1 outside (blue) and Sensor 2 under the shelter (red) **b)** Sensor 1 under (red) and Sensor 2 outside the shelter (blue).

1.3. Air temperature

In addition we measured the air temperature underneath and outside the shelter using a Temperature probe (107-T, Campbell Scientific Ltd.) mounted on a pipe 30 cm above the ground. The ratio of the temperatures measured underneath and outside the shelter were calculated. The analysis resulted in an average of 99 % across all three paired measurements for both sensor 1(Temp 1) outside the shelter and sensor 2 (Temp 2) underneath the shelter as well as sensor 1 underneath the shelter and sensor 2 outside the shelter. Overall air temperature showed little variations when comparing the difference between air temperatures underneath the shelter to ambient temperatures. The average daily difference between the air temperature underneath and outside the shelter varied between -0.72 to 0.26 °C depending on the time of day (Fig. B4). These indicate no effect of the rainout shelter on air temperature and were comparable to the results of previous studies (Yahdjian and Sala 2002).

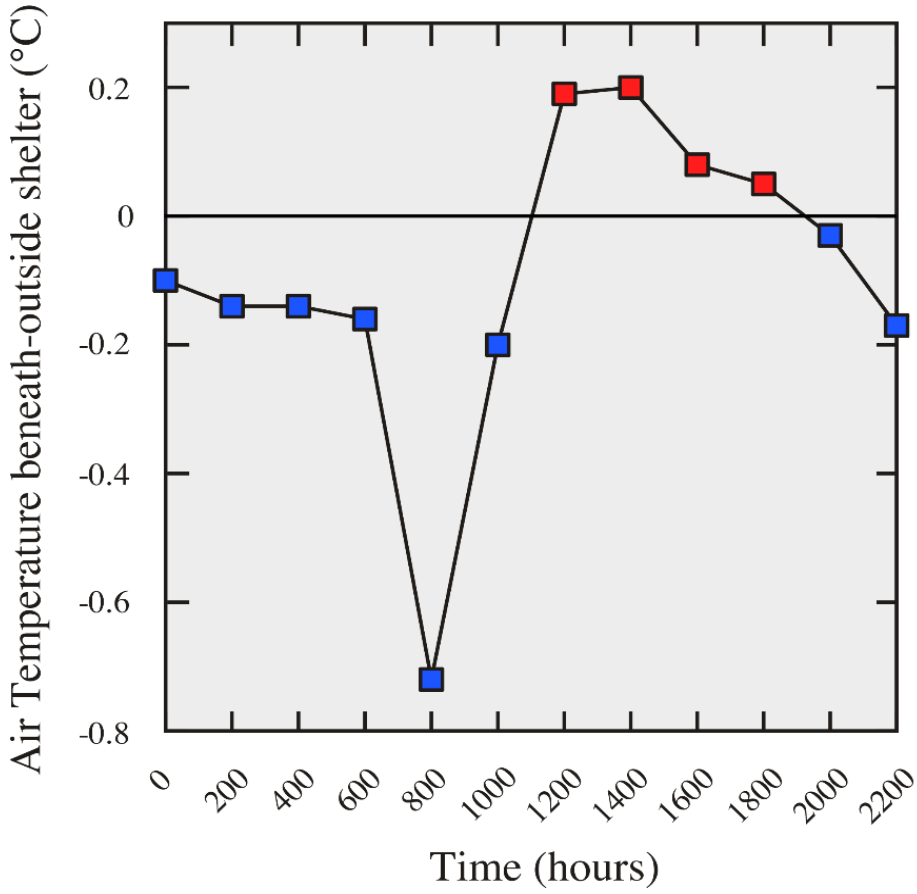

Fig. B4 Effect of rainout shelter on microclimate. Daily Air Temperature difference underneath and outside the rainout shelter during 5 -24 July 2016.

Treatment 1: Control	Average Precip/week 1951-2015													
(1add/week), Tuesdays	May 9th	May 16th	May 23rd	May 30th	Jun 6th	Jun 13th	Jun 20th	Jun 27th	Jul 4rd	Jul 11th	Jul 18th	Jul 25th	Aug 1st	Aug 8th	SUM
Per Event (mm)	11,5	10,3	11,3	12,7	18,6	20,0	22,0	16,4	14,2	9,9	9,2	7,6	7,4	8,6	180
Per Event (cm)	1,15	1,0	1,1	1,3	1,9	2,0	2,2	1,6	1,4	1,0	0,9	0,8	0,7	0,9	
Plot Area (2.14 m x 2.14 m)	45796	cm2													
Per Event (mL/plot)	52711,196	46940,9	51841,072	58069,328	85180,56	91729,39	100522,2	75242,83	65076,12	45292,244	42178,116	34850,756	33889,04	39567,744	
Per Event (L/plot)	**52,7**	**46,9**	**51,8**	**58,1**	**85,2**	**91,7**	**100,5**	**75,2**	**65,1**	**45,3**	**42,2**	**34,9**	**33,9**	**39,6**	
***5 replicates**	**263,6**	**234,7**	**259,2**	**290,3**	**425,9**	**458,6**	**502,6**	**376,2**	**325,4**	**226,5**	**210,9**	**174,3**	**169,4**	**197,8**	

Treatment 2: 100% rain	Average Precip/week 1951-2015							
(1 add every 2 weeks), Tuesdays	May 16th	May 30th	Jun 13th	Jun 27th	Jul 11th	Jul 25th	Aug 8th	SUM
Per Event (mm)	21,8	24,0	38,6	38,4	24,1	16,8	16,0	180
Per Event (cm)	2,18	2,4	3,9	3,8	2,4	1,7	1,6	
Plot Area (2.14 m x 2.14 m)	45796	cm2						
Per Event (mL/plot)	99652,096	109910,4	176909,95	175765,05	110368,4	77028,87	73456,78	
Per Event (L/plot)	**99,7**	**109,9**	**176,9**	**175,8**	**110,4**	**77,0**	**73,5**	
***5 replicates**	**498,3**	**549,6**	**884,5**	**878,8**	**551,8**	**385,1**	**367,3**	

Treatment 3: 50% rain	Average Precip/week 1951-2015													
(1 add/week, Tuesdays	May 9th	May 16th	May 23rd	May 30th	Jun 6th	Jun 13th	Jun 20th	Jun 27th	Jul 4rd	Jul 11th	Jul 18th	Jul 25th	Aug 1st	Aug 8th	SUM
Per Event (mm)	5,8	5,1	5,7	6,3	9,3	10,0	11,0	8,2	7,1	4,9	4,6	3,8	3,7	4,3	90
Per Event (cm)	0,58	0,5	0,6	0,6	0,9	1,0	1,1	0,8	0,7	0,5	0,5	0,4	0,4	0,4	
Plot Area (2.14 m x 2.14 m)	45796	cm2													
Per Event (mL/plot)	26355,598	23470,45	25920,536	29034,664	42590,28	45864,69	50261,11	37621,41	32538,06	22646,122	21089,058	17425,378	16944,52	19783,872	
Per Event (L/plot)	**26,4**	**23,5**	**25,9**	**29,0**	**42,6**	**45,9**	**50,3**	**37,6**	**32,5**	**22,6**	**21,1**	**17,4**	**16,9**	**19,8**	
***5 replicates**	**131,8**	**117,4**	**129,6**	**145,2**	**213,0**	**229,3**	**251,3**	**188,1**	**162,7**	**113,2**	**105,4**	**87,1**	**84,7**	**98,9**	

Treatment 4: 50% rain	Average Precip/week 1951-2015							
(1 add every 2 weeks, Tuesdays	May 16th	May 30th	Jun 13th	Jun 27th	Jul 11th	Jul 25th	Aug 8th	SUM
Per Event (mm)	10,9	12,0	19,3	19,2	12,1	8,4	8,0	90
Per Event (cm)	1,09	1,2	1,9	1,9	1,2	0,8	0,8	
Plot Area (2.14 m x 2.14 m)	45796	cm2						
Per Event (mL/plot)	49826,048	54955,2	88454,974	87882,524	55184,18	38514,44	36728,39	
Per Event (L/plot)	**49,8**	**55,0**	**88,5**	**87,9**	**55,2**	**38,5**	**36,7**	
***5 replicates**	**249,1**	**274,8**	**442,3**	**439,4**	**275,9**	**192,6**	**183,6**	

Watering day	May 9th	May 16th	May 23rd	May 30th	Jun 6th	Jun 13th	Jun 20th	Jun 27th	Jul 4rd	Jul 11th	Jul 18th	Jul 25th	Aug 1st	Aug 8th	Total
Total litres (n=10 or 20 plots)	395,3	1125,1	388,8	1259,8	638,9	2014,8	753,9	1882,6	488,1	1167,5	316,3	839,1	254,2	847,7	**12.372**

Week	Day (Tues)	DOY			Day	DOY	Precip amounts to be added (mm) NN	NR	RN	RR
	2-May-17	122	Shelter On							
1	9-May-17	129	1		9-May-17	129	11,5		5,8	
2	16-May-17	136	2		16-May-17	136	10,3	21,8	5,1	10,9
3	23-May-17	143	3		23-May-17	143	11,3		5,7	
4	30-May-17	150	4		30-May-17	150	12,7	24,0	6,3	12,0
5	6-Jun-17	157	5		6-Jun-17	157	18,6		9,3	
6	13-Jun-17	164	6		13-Jun-17	164	20,0	38,6	10,0	19,3
7	20-Jun-17	171	7		20-Jun-17	171	22,0		11,0	
8	27-Jun-17	178	8		27-Jun-17	178	16,4	38,4	8,2	19,2
9	4-Jul-17	185	9		4-Jul-17	185	14,2		7,1	
10	11-Jul-17	192	10		11-Jul-17	192	9,9	24,1	4,9	12,1
11	18-Jul-17	199	11		18-Jul-17	199	9,2		4,6	
12	25-Jul-17	206	12		25-Jul-17	206	7,6	16,8	3,8	8,4
13	1-Aug-17	213	13		1-Aug-17	213	7,4		3,7	
14	8-Aug-17	220	14		8-Aug-17	220	8,6	16,0	4,3	8,0
	15-Aug-17	227	Harvest							
					Avg event (mm)		12,8	25,7	6,4	12,8
Soil Resp on Thursdays					**Total precip (mm)**		180	180	90	90
							Spread rain over 2 days			

Appendix D: MATLAB scripts

Image analysis script for the greenness index (gcc):

```
clear; homedir=pwd; format short g;
global answer

dirach=uigetdir('','Choose picture (jpeg) directory');
cd (dirach);
jpgfiles=[dirach '\*.jpg'];
d=dir(jpgfiles);
if isempty(d)==1; disp('Selected directory does not contain any jpg files; aborting
program!'); return; end;
str = {d.name};
[s,v] = listdlg('PromptString','Select jpg input file',...
                'SelectionMode','multiple',...
                'ListString',str);
outdata(s,:)=0;
filename(s,:)=[cellstr('test')];
nfiles = length(s);
for i = 1:nfiles;
   filein=char(str(s(i)));
   x=imread(filein);
   dx=double(x);
   red=dx(:,:,1);
   green=dx(:,:,2);
   blue=dx(:,:,3);
   subred=dx(1000:2000,500:1500,1);
   subgreen=dx(1000:2000,500:1500,2);
   subblue=dx(1000:2000,500:1500,3);
   totalred=sum(sum(subred));
   totalgreen=sum(sum(subgreen));
   totalblue=sum(sum(subblue));
   gcc=totalgreen/(totalred+totalgreen+totalblue);
   outdata(i)=gcc;
   filename(i)=[cellstr(filein)];
end;
title1=[cellstr('gcc value') cellstr('file name')];
fileout=['gcc_Results'];
xlswrite(fileout,title1,1,'A1');
xlswrite(fileout,outdata,1,'A2');
xlswrite(fileout,filename,1,'B2');
```

Repeated-measures ANOVA script:

```
function [p, table] = anova_rm(X, displayopt)
%   [p, table] = anova_rm(X, displayopt)
%   Single factor, repeated measures ANOVA.
%
%   [p, table] = anova_rm(X, displayopt) performs a repeated measures ANOVA
%   for comparing the means of two or more columns (time) in one or more
%   samples(groups). Unbalanced samples (i.e. different number of subjects
%   per group) is supported though the number of columns (followups)should
%   be the same.
%
%   DISPLAYOPT can be 'on' (the default) to display the ANOVA table, or
%   'off' to skip the display. For a design with only one group and two or
%   more follow-ups, X should be a matrix with one row for each subject.
%   In a design with multiple groups, X should be a cell array of matrixes.
%
%   Example: Gait-Cycle-times of a group of 7 PD patients have been
%   measured 3 times, in one baseline and two follow-ups:
%
%   patients = [
%    1.1015    1.0675    1.1264
%    0.9850    1.0061    1.0230
%    1.2253    1.2021    1.1248
%    1.0231    1.0573    1.0529
%    1.0612    1.0055    1.0600
%    1.0389    1.0219    1.0793
%    1.0869    1.1619    1.0827 ];
%
%   more over, a group of 8 controls has been measured in the same protocol:
%
%   controls = [
%     0.9646    0.9821    0.9709
%     0.9768    0.9735    0.9576
%     1.0140    0.9689    0.9328
%     0.9391    0.9532    0.9237
%     1.0207    1.0306    0.9482
%     0.9684    0.9398    0.9501
%     1.0692    1.0601    1.0766
%     1.0187    1.0534    1.0802 ];
%
%   We are interested to see if the performance of the patients for the
%   followups were the same or not:
%
%   p = anova_rm(patients);
%
%   By considering the both groups, we can also check to see if the
%   follow-ups were significantly different and also check two see that the
%   two groups had a different performance:
%
%   p = anova_rm({patients controls});
```

```
%
%
%   ref: Statistical Methods for the Analysis of Repeated Measurements,
%     C. S. Daivs, Springer, 2002
%
%   Copyright 2008, Arash Salarian
%   mailto://arash.salarian@ieee.org
%

if nargin < 2
    displayopt = 'on';
end

if ~iscell(X)
    X = {X};
end

%number of groups
s = size(X,2);

%subjects per group
n_h = zeros(s, 1);
for h=1:s
    n_h(h) = size(X{h}, 1);
end
n = sum(n_h);

%number of follow-ups
t = size(X{1},2);

% overall mean
y = 0;
for h=1:s
    y = y + sum(sum(X{h}));
end
y = y / (n * t);

% allocate means
y_h = zeros(s,1);
y_j = zeros(t,1);
y_hj = zeros(s,t);
y_hi = cell(s,1);
for h=1:s
    y_hi{h} = zeros(n_h(h),1);
end

% group means
for h=1:s
    y_h(h) = sum(sum(X{h})) / (n_h(h) * t);
end
```

```
% follow-up means
for j=1:t
   y_j(j) = 0;
   for h=1:s
      y_j(j) = y_j(j) + sum(X{h}(:,j));
   end
   y_j(j) = y_j(j) / n;
end

% group h and time j mean
for h=1:s
   for j=1:t
      y_hj(h,j) = sum(X{h}(:,j)  / n_h(h));
   end
end

% subject i'th of group h mean
for h=1:s
   for i=1:n_h(h)
      y_hi{h}(i) = sum(X{h}(i,:))  / t;
   end
end

% calculate the sum of squares
ssG = 0;
ssSG = 0;
ssT = 0;
ssGT = 0;
ssR = 0;

for h=1:s
   for i=1:n_h(h)
      for j=1:t
         ssG  = ssG  + (y_h(h) - y)^2;
         ssSG = ssSG + (y_hi{h}(i) - y_h(h))^2;
         ssT  = ssT  + (y_j(j) - y)^2;
         ssGT = ssGT + (y_hj(h,j) - y_h(h) - y_j(j) + y)^2;
         ssR  = ssR  + (X{h}(i,j) - y_hj(h,j) - y_hi{h}(i) + y_h(h))^2;
      end
   end
end

% calculate means
if s > 1
   msG  = ssG  / (s-1);
   msGT = ssGT / ((s-1)*(t-1));
end
msSG = ssSG / (n-s);
msT  = ssT  / (t-1);
msR  = ssR  / ((n-s)*(t-1));
```

```
% calculate the F-statistics
if s > 1
   FG  = msG  / msSG;
   FGT = msGT / msR;
end
FT  = msT  / msR;
FSG = msSG / msR;


% single or multiple sample designs?
if s > 1
   % case for multiple samples
   pG  = 1 - fcdf(FG, s-1, n-s);
   pT  = 1 - fcdf(FT, t-1, (n-s)*(t-1));
   pGT = 1 - fcdf(FGT, (s-1)*(t-1), (n-s)*(t-1));
   pSG = 1 - fcdf(FSG, n-s, (n-s)*(t-1));

   p = [pT, pG, pSG, pGT];

   table = { 'Source' 'SS' 'df' 'MS' 'F' 'Prob>F'
      'Time'  ssT t-1 msT FT pT
      'Group' ssG s-1 msG FG pG
      'Interaction' ssGT (s-1)*(t-1) msGT FGT pGT
      'Subjects (matching)' ssSG n-s msSG FSG pSG
      'Error' ssR (n-s)*(t-1) msR  [] []
      'Total' [] [] [] [] []
      };
   table{end, 2} = sum([table{2:end-1,2}]);
   table{end, 3} = sum([table{2:end-1,3}]);

   if (isequal(displayopt, 'on'))
      digits = [-1 -1 0 -1 2 4];
      statdisptable(table, 'multi-sample repeated measures ANOVA', 'ANOVA Table', ''
digits);
   end
else
   % case for only one sample
   pT  = 1 - fcdf(FT, t-1, (n-s)*(t-1));
   pSG = 1 - fcdf(FSG, n-s, (n-s)*(t-1));

   p = [pT, pSG];

   table = { 'Source' 'SS' 'df' 'MS' 'F' 'Prob>F'
      'Time'  ssT t-1 msT FT pT
      'Subjects (matching)' ssSG n-s msSG FSG pSG
      'Error' ssR (n-s)*(t-1) msR  [] []
      'Total' [] [] [] [] []
      };
   table{end, 2} = sum([table{2:end-1,2}]);
   table{end, 3} = sum([table{2:end-1,3}]);
```

```
    if (isequal(displayopt, 'on'))
        digits = [-1 -1 0 -1 2 4];
        statdisptable(table, 'repeated measures ANOVA', 'ANOVA Table', '', digits);
    end
end
```

Appendix E: Data logger programs CR23X

Data logger program for measuring shelter effects on the microclimate:

```
;{CR23X}
;*************************************************************
; FLUXNET CANADA SITE
; CR23X program for assessing shelter effects at the Fluxnet site
; Lavinia Haase
; created 16/06/2016
;*************************************************************
;*****************************************
; Record sensor readings every 5 seconds
;*****************************************
*Table 1 Program
01: 5 Execution Interval (seconds)
;*****************************************
; Measure data logger internal temperature
;*****************************************
1: Panel Temperature (P17)
1: 1 Loc [ Ref_Temp ]
2: Batt Voltage (P10)
1: 2 Loc [ Battery ]
;*************************************
; Measure 107 Temperature Probe 1
; measure sensor using SE channel 1
;*************************************
3: Temp (107) (P11)
1: 1 Reps
2: 1 SE Channel
3: 1 Excite all reps w/E1
4: 3 Loc [ Temp_107a ]
5: 1.0 Multiplier
6: 0.0 Offset
;*************************************
; Measure 107 Temperature Probe 2
; measure sensor using SE channel 3
;*************************************
4: Temp (107) (P11)
1: 1 Reps
2: 3 SE Channel
3: 2 Excite all reps w/E2
4: 4 Loc [ Temp_107b ]
5: 1.0 Multiplier
6: 0.0 Offset

;*****************************************
; Measure Quantum PAR sensor 1
; measure sensor using analog channel DIFF3
;*****************************************
5: Volt (Diff) (P2)
1: 1 Reps
```

```
2: 22 50 mV, 60 Hz Reject, Slow Range
3: 3 DIFF Channel
4: 5 Loc [ PAR_IN_1 ]
5: 292.1 Multiplier
6: 0.0 Offset
;*******************************************
; Measure Quantum PAR sensor 2
; measure sensor using analog channel DIFF4
;*******************************************
6: Volt (Diff) (P2)
1: 1 Reps
2: 22 50 mV, 60 Hz Reject, Slow Range
3: 4 DIFF Channel
4: 6 Loc [ PAR_IN_2 ]
5: 292.1 Multiplier
6: 0.0 Offset
;*******************************************
; Data Output
; output data to final storage every 30 min.
; average values for all variables
;*******************************************
7: If time is (P92)
1: 0 Minutes (Seconds --) into a
2: 30 Interval (same units as above)
3: 10 Set Output Flag High (Flag 0)
8: Real Time (P77)
1: 1110 Year,Day,Hour/Minute (midnight = 0000)
9: Average (P71)
1: 6 Reps
2: 1 Loc [ Ref_Temp ]

End Program
```

Datalogger program for collecting enviornmental data during the main experiment:

```
;{CR23X}
; ***************************************************************************************
;
; {CR23X}
; ROOD GRASSLAND
; CS23X program - environmental data, rain manipulation experiment
; Lavinia Haase
; created Feb 3rd 2017; corrected and finalized Feb 21st 2017
; ***************************************************************************************
;

; ***************************************************************************************
;
; Record sensor readings every 5 seconds
; ***************************************************************************************
;

*Table 1 Program
  01: 5        Execution Interval (seconds)


; ***************************************************************************************
;
; Measure data logger internal temperature and battery voltage
; ***************************************************************************************
;

1:  Panel Temperature (P17)
 1: 1        Loc [ Ref_Temp  ]

2:  Batt Voltage (P10)
 1: 2        Loc [ Battery_V ]


; ***************************************************************************************
;
; Measure 107 Air Temperature probe
; measure sensor using SE channel 1
; ***************************************************************************************
;

3:  Temp (107) (P11)
 1: 1        Reps
 2: 1        SE Channel
 3: 1        Excite all reps w/E1
 4: 3        Loc [ Air_Temp  ]
 5: 1.0      Multiplier
 6: 0.0      Offset


; ***************************************************************************************
;
; Measure Thermocouple Soil Temperature (7.5 cm depth) probes 1-4
; measure sensor using DIFF channel 2-4
; ***************************************************************************************
;
```

```
4:  Thermocouple Temp (DIFF) (P14)
 1: 4        Reps
 2: 21       10 mV, 60 Hz Reject, Slow Range
 3: 2        DIFF Channel
 4: 1        Type T (Copper-Constantan)
 5: 1        Ref Temp (Deg. C) Loc [ Ref_Temp  ]
 6: 4        Loc [ S_Temp_1  ]
 7: 1.0      Multiplier
 8: 0.0      Offset


; ********************************************************************************************
; Measure CS-616 Water Content Reflectometer 1-4 at 15 cm depth
; 5: measure sensors at all 4 locations using SE channel 11-14 and control port 5
; 6: create soil reference temperature (20C)
; 7-10: calculate temperature factor (t) correction for each location
; 11-19: Soil moisture temperature correction for probe 1-4
; 20: convert output period to volumetric water content (VWC)
; ********************************************************************************************

5:  CS616 Water Content Reflectometer (P138)
 1: 4        Reps
 2: 11       SE Channel
 3: 15       All reps use C5
 4: 20       Loc [ SM_1      ]
 5: 1.0      Multiplier
 6: 0.0      Offset

6:  Z=F x 10^n (P30)
 1: 20       F
 2: 00       n, Exponent of 10
 3: 95       Z Loc [ SoilT_Ref ]

7:  Z=X-Y (P35)
 1: 95       X Loc [ SoilT_Ref ]
 2: 4        Y Loc [ S_Temp_1  ]
 3: 100      Z Loc [ TFactor_1 ]

8:  Z=X-Y (P35)
 1: 95       X Loc [ SoilT_Ref ]
 2: 5        Y Loc [ S_Temp_2  ]
 3: 101      Z Loc [ TFactor_2 ]

9:  Z=X-Y (P35)
 1: 95       X Loc [ SoilT_Ref ]
 2: 6        Y Loc [ S_Temp_3  ]
 3: 102      Z Loc [ TFactor_3 ]

10:  Z=X-Y (P35)
 1: 95       X Loc [ SoilT_Ref ]
 2: 7        Y Loc [ S_Temp_4  ]
 3: 103      Z Loc [ TFactor_4 ]
```

```
11:  Polynomial (P55)
 1: 4        Reps
 2: 20       X Loc [ SM_1      ]
 3: 104      F(X) Loc [ pSM_1     ]
 4: 0.526    C0
 5: -0.052   C1
 6: 0.00136  C2
 7: 0.0      C3
 8: 0.0      C4
 9: 0.0      C5

12:  Z=X*Y (P36)
 1: 100      X Loc [ TFactor_1 ]
 2: 104      Y Loc [ pSM_1     ]
 3: 108      Z Loc [ tSM_1     ]


13:  Z=X+Y (P33)
 1: 20       X Loc [ SM_1      ]
 2: 108      Y Loc [ tSM_1     ]
 3: 108      Z Loc [ tSM_1     ]

14:  Z=X*Y (P36)
 1: 101      X Loc [ TFactor_2 ]
 2: 105      Y Loc [ pSM_2     ]
 3: 109      Z Loc [ tSM_2     ]

15:  Z=X+Y (P33)
 1: 21       X Loc [ SM_2      ]
 2: 109      Y Loc [ tSM_2     ]
 3: 109      Z Loc [ tSM_2     ]

16:  Z=X*Y (P36)
 1: 102      X Loc [ TFactor_3 ]
 2: 106      Y Loc [ pSM_3     ]
 3: 110      Z Loc [ tSM_3     ]

17:  Z=X+Y (P33)
 1: 22       X Loc [ SM_3      ]
 2: 110      Y Loc [ tSM_3     ]
 3: 110      Z Loc [ tSM_3     ]

18:  Z=X*Y (P36)
 1: 103      X Loc [ TFactor_4 ]
 2: 107      Y Loc [ pSM_4     ]
 3: 111      Z Loc [ tSM_4     ]

19:  Z=X+Y (P33)
 1: 23       X Loc [ SM_4      ]
 2: 111      Y Loc [ tSM_4     ]
 3: 111      Z Loc [ tSM_4     ]
```

```
20:  Polynomial (P55)
 1: 4        Reps
 2: 108      X Loc [ tSM_1     ]
 3: 8        F(X) Loc [ SM_VWC_1  ]
 4: -0.4724  C0
 5: 0.0233   C1
 6: 0        C2
 7: 0.0      C3
 8: 0.0      C4
 9: 0.0      C5

; ************************************************************************************************************************
; Data Output
; average every 30 min.
; ************************************************************************************************************************

21:  If time is (P92)
 1: 0        Minutes (Seconds --) into a
 2: 30       Interval (same units as above)
 3: 10       Set Output Flag High (Flag 0)

22:  Real Time (P77)
 1: 1110     Year,Day,Hour/Minute (midnight = 0000)

23:  Average (P71)
 1: 11       Reps
 2: 1        Loc [ Ref_Temp  ]

24:  Average (P71)
 1: 4        Reps
 2: 20       Loc [ SM_1      ]


*Table 2 Program
  02: 0.0000    Execution Interval (seconds)

*Table 3 Subroutines

End Program
```