

**Climate Change and the Bunchgrass Prairie: The Effect of Shifting
Precipitation and Temperature Regimes on Prairie Nutrient Cycling
and Plant Communities**

Zoe Volenec

Introduction

Climate Change and its Consequences for the 21st Century

Since 1880 the global surface temperature has risen by 0.85°C, leading to unprecedented environmental changes beginning in the 1950s (IPCC 2014; Karl et al 2009). The International Panel on Climate Change, IPCC, (2014) cites increases in anthropogenic greenhouse gases as being “extremely likely” to have played the dominant role in global temperature increase. The effects of climate change are far reaching, resulting in altered hydrological systems, increases in extreme weather events, and shifting species’ ranges (IPCC 2014). The global climate will continue to respond to anthropogenic emissions, with IPCC models (2014) predicting anywhere from a 0.3°C to 4.8°C increase in temperature over the course of the 21st century. The variability captured by the range in these projections is due to the use of models based on differing future emissions levels and contrasting emphasis on positive feedback cycles. For example, a 0.3°C increase represents an estimate made with a model assumption that emissions will remain at their 2000 level, while models that predict a low level increase in emissions forecast a minimum 1.1°C increase (IPCC 2007). These temperature increases, similar to the climate change that has already taken place, will not be evenly distributed across the earth’s surface. Temperature change will be concentrated at the Polar regions (Fig 1a), while precipitation change will be highly variable, with upper latitude areas experiencing increased rainfall levels and tropical regions seeing a decrease in yearly rainfall (Fig 1b).

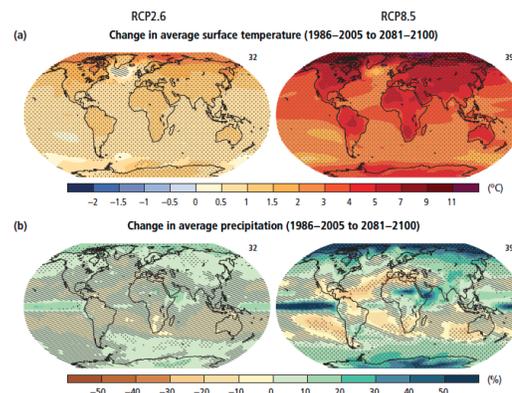


Figure 1. Global map displaying the geographic variation in climate change, drawn from the IPCC’s *Climate Change 2014: Synthesis Report*.

The predicted changes in climate will alter the physical landscape and thus have the potential to affect the living communities of species that rely upon it. Shifting temperature and precipitation regimes are predicted to result in physiological, distributional, and phenological adaptations in today’s species (Hughes 2000). Plants form the foundation for all terrestrial food webs, thus shifts in their phenology and productivity have the potential to alter ecosystem functions and drive bottom-up community effects (Cleland et al 2007). Of particular interest are grasslands, which comprise 31-43% of ice-free land surfaces, and contribute significantly to maintaining earth’s carbon balance (Zhou et al 2012). Temperature and precipitation changes have the highest potential to alter grassland ecosystems as temperature affects carbon as well as nitrogen cycling and precipitation can change soil quality, leading to shifts in biodiversity and species productivity (Beier et al 2004). Thus, climate change has the potential to modify multiple ecological cycles.

First, increased temperature and increased concentrations of carbon dioxide, a key greenhouse gas, can change the fluxes of the carbon cycle through the effect these factors exert on terrestrial carbon sinks. Some models predict that anthropogenic climate change will result in lowered carbon sequestration ability through reduced rates of net primary productivity (NPP), a measure of photosynthetic biomass production (Friedlingstein et al 2001; Luo 2009). This prediction is based on three factors:

- 1) While increased atmospheric carbon dioxide concentrations will enhance photosynthetic rates, as carbon dioxide can be a limiting factor in photosynthesis and leads to increased water efficiency use via decreased stomatal conductance, this effect will plateau at high atmospheric carbon dioxide levels (Nowak et al 2004; Cramer et al 2001). Thus, the increased photosynthetic potential will be short lived.
- 2) Higher temperatures will lead to an increased rate of heterotrophic respiration and plant metabolic maintenance requirements, reducing the gross primary productivity available for biomass production (Cramer et al 2001).
- 3) Higher temperatures allow for the chemical reactions of photosynthesis to operate at higher rates only if no other growth factors are limiting (Wu et al 2011). However, this response depends on the optimum temperature for photosynthesis, which differs among latitudes, and is conditional on water availability, which could be aggravated by elevated temperatures.

Combined with an increased rate of soil carbon decomposition as rising temperatures accelerate microbe activity, this could lead to a positive feedback cycle between climate change and carbon cycling (Davidson and Janssens 2006). Even if increased temperatures elevate plants' NPP, Kirschbaum (1995) found that soil decomposition exhibits higher temperature sensitivity, still implying that the terrestrial carbon sink would shrink.

Second, climate change will impact the nitrogen cycle. Not only is nitrogen considered the main limiting factor for plant growth in terrestrial and marine ecosystems, but also changes in the nitrogen cycle stand to affect the carbon cycle, since nitrogen and carbon cycles in terrestrial ecosystems are generally coupled (Wan et al 2005). The rate of anthropogenic nitrogen release into the global nitrogen cycle has been amplified by the widespread use of synthetic fertilizers and increased industrial processes, including the use of fossil fuels (Suddick et al 2013). These actions have already significantly altered the nitrogen cycle, leading to increases in nitrous oxide, a potent greenhouse gas (Vitousek et al 1997). Rising air temperatures will only function to exacerbate the issue of nitrogen pollution as ammonia emissions from agriculture will increase and nitrogen efficiency use from livestock production decreases (Suddick et al 2013). While enhanced nitrogen levels could plausibly lead to increased photosynthetic capabilities of plants and thus an increased terrestrial carbon sink capacity, nitrogen saturation in terrestrial ecosystems poses a serious problem due to leaching of nutrients such as calcium and potassium as well as soil acidification (Wan et al 2005; Vitousek et al 1997).

The availability of this increased nitrogen for plant use in photosynthesis will depend upon the response of soil microbes to climate change. Nitrogen mineralization is the process by which organic nitrogen, the predominant form of nitrogen present in soils, is converted into inorganic nitrogen by soil microorganisms. Increased temperature has been shown to increase nitrogen mineralization activity by soil microbes (Zak et al 1999; Rustad et al 2001). In addition, nitrogen mineralization rate is optimized in soils at almost saturated moisture capacity due to positive effects of moisture on microbial growth and their ability to reach nitrogen substrates in the soil (Stanford and Epstein 1974). However, too much precipitation may lead to leaching of nitrogen

(Lehmann and Schroth 2003), and the rainfall quantity responsible for leaching will likely vary among ecosystems that differ in naturally available soil moisture. Plants require this inorganic nitrogen such as nitrate and ammonium for growth processes and will be sensitive to any changes in its concentration.

Finally, biodiversity is predicted to decrease due to climate change as temperature and precipitation regimes shift, leaving species outside their optimal geographic growth range (Sala et al 2000; Vitousek 1994). Increased nitrogen deposition as well as warmer temperatures may lead to nutrient enrichment of ecosystems, as discussed above, an environment that favors species with a fast growth competitive advantage and could lead to additional biodiversity losses (Wan et al 2005). Grassland biodiversity is particularly at risk compared to other ecosystems' biodiversity levels due to its elevated sensitivity to changes in multiple drivers of biodiversity, including land use, nitrogen deposition, and atmospheric carbon dioxide concentrations (Sala et al 2000). Reductions in biodiversity create a positive feedback cycle as biodiversity functions to reduce the susceptibility of ecosystems to the large-scale environmental changes that can drive additional biodiversity loss (Chapin et al 2000). Furthermore, biodiversity plays a key role for many ecosystem services. It has long been hypothesized that the relationship between biodiversity and productivity is best described by a unimodal curve, as low levels of biodiversity do not have enough ecosystem service providers and at high levels of biodiversity competitive exclusion limits productivity. The optimum level of biodiversity may differ between ecosystems, but ideally diversity should be shared across ecosystem service roles. In a review of over 1,700 papers on the subject, Cardinale et al (2012) found that biodiversity was crucial for efficient biomass production and nutrient recycling and that increasing biodiversity losses can lead to an acceleration in the loss of these ecosystem functions. Tilman et al (2012) found that decreases in biodiversity resulted in productivity losses comparable to those induced by changes in nitrogen, water, and carbon dioxide levels, and biodiversity changes remained a dominant driver of productivity through time.

Uncertainties in Predicting the Interactions of Climate Variables

Although some of the trends of shifts in ecosystem function due to climate change, like those described above, are largely agreed upon, the interactive effects of temperature and precipitation can be hard to predict in this context (Wu 2011). Wu et al (2011) found that in a synthesis of 85 studies, only a small number of experiments investigated the interaction of temperature and precipitation in producing ecosystem responses to climate change. The results of their meta-analysis, confirmed by these multifactor studies, found that the combined effect of temperature and precipitation changes produced smaller responses than those predicted from additive, single-factor studies. Wu et al (2011) recommended that more multifactor experiments be conducted, with study sites representing a wider range of ecosystems, due to the differing responses of vegetation across biomes.

The individual effects of temperature and precipitation on NPP and senescence, a measure of the rate of die-off of vegetation, have been explored more thoroughly through manipulative experimentation. Studies have demonstrated the general trends of increased net primary productivity with increased precipitation at ambient temperatures and decreased NPP with increased temperature at ambient precipitation levels due to aggravated water stress (Wu et al 2011; Fay et al 2003; Zhou et al 2007). Senescence tends to vary more than productivity, though, and while increased temperature serves to drive increased senescence, it remains largely undetermined how senescence responds to altered precipitation regimes (Cleland et al 2007).

Although Harpole et al (2007) demonstrated that a late season rainfall event was correlated with continued carbon uptake by vegetation in a study of spring productivity, it is widely hypothesized that temperature is the main determinant of die-off rate in many ecosystems, like grasslands (Belovsky and Slade 2013). Conversely, the interactive effects of precipitation and temperature are unclear and will likely depend on the ratio of precipitation to temperature. The interaction of increased temperature with increased precipitation has not been definitively shown to promote or reduce NPP. Increased precipitation will not exert as strong an effect on plant growth if evapotranspiration rate, a function of temperature, is also increased and much of the precipitation is lost to the atmosphere (Manske 2001). The precipitation-temperature ratio serves to compare the amount of rainfall to the evapotranspiration demand, and an increase in this ratio should result in a relative increase in NPP in water-stressed ecosystems, as a greater amount of water is available for plant growth.

Even among multifactor climate change studies conducted solely in grassland ecosystems, the results of changes in plant productivity and phenology brought on by temperature and precipitation regime shifts have not been particularly consistent. This may largely stem from the variability in available soil moisture across different grasslands, necessitating studies for how different grassland types will respond to climate change. Common grassland types include tall grass, mixed grass, and shortgrass prairies, listed in order of decreasing available soil moisture. Bunchgrass prairie, more common historically in Northern America, has the lowest amount of available soil moisture and must spread its roots horizontally to collect adequate rainfall for photosynthesis. As the type of grassland present is largely dependent upon yearly rainfall, it follows that changes in available soil moisture hold the potential to explain differing grassland responses to climate change. For example, work in a mixed, short grass prairie conducted by Flanagan et al (2013) found that experimental warming in addition to a 50% increase in precipitation did not significantly affect aboveground NPP. Conversely, Shaw et al (2002) found that experimental warming and a 50% increase in precipitation for a California annual grassland produced an intermediate response with NPP increased to a level comparable with single-factor experiment responses. Although this annual grassland received more yearly precipitation, the short and mixed grassland studied by Flanagan et al (2013) received 30% of its yearly precipitation during the May-June growing season. Thus, the elevated NPP of the annual grassland in response to an increased precipitation treatment could demonstrate the increasing importance of available soil moisture for plant growth as the soil moisture deficit increases in an ecosystem.

The effect of changing temperature and precipitation on soil nitrogen levels may also be dependent on the ecosystem context, particularly the available soil moisture. Brzostek et al (2012) conducted a meta-analysis study on sixteen global change experiments and found that the response of nitrogen availability, measured in terms of the potential proteolytic enzyme activity of microbes, depended upon the soil moisture deficit, the difference between precipitation and evapotranspiration demand. Changes in temperature and precipitation that increased the deficit resulted in lower microbial enzyme potential, while those that decreased the soil moisture deficit increased microbial enzyme potential (Brzostek et al 2012). The direction of temperature and precipitation changes necessary to decrease the soil moisture deficit differed between ecosystems, especially among different latitudes, due to the variety of climates represented in the study. For example, mid-latitude grasslands had enhanced soil moisture debts after exposure to experimental warming, a result also found in warmer, temperate forests but in contrast to colder, mesic forests where warming did not exacerbate the soil moisture deficit. Thus, as with

NPP, the ratio between precipitation and temperature may be the operative variable in determining the microbes' ability to carry out the process of nitrogen mineralization.

Based off these findings, the knowledge gap in climate change studies can be identified as primarily stemming from a dearth of multifactor manipulative studies that could be used to pinpoint the interaction of precipitation and temperature regime changes across different ecosystems, especially ecosystems with varying soil moisture deficits. Thus, by designing a study that investigates the precipitation and temperature interaction in determining ecosystem response, ideally through use of the precipitation-temperature ratio, and carrying out the experimental manipulations in a novel habitat type, the results could be used to strengthen existing climate change models. By incorporating a novel habitat type, unrepresented by current climate change literature, the results will serve to demonstrate whether the ecosystems' responses to climate change variables will be uniform or whether a dynamic response must be incorporated into future modeling work.

Closing the Knowledge Gap

This study will examine the interaction of increased temperature and changes in precipitation levels and seasonality in the context of net primary production (NPP), species composition, and nitrogen cycling of a bunchgrass prairie system. The bunchgrass prairie of the National Bison Range of Montana was selected as the study site for this experiment due to the extreme climate it represents, occupying a climate between grassland and cold desert. Literature on the effects of climate change on the bunchgrass prairie is highly underrepresented among the studies conducted on the future of grassland habitats. Studies conducted to date tend to focus on shortgrass and tallgrass prairies, especially the Kansas Konza tallgrass, both of which are grassland types that receive higher annual precipitation than the bunchgrass prairie and may not be as susceptible to larger soil moisture deficits. Historical climate data indicates that over the past 100 years at the National Bison Range (NBR), it has been shown that temperature has increased by 0.6°C, while annual precipitation has decreased by 26% (Belovsky and Slade 2013), shown in Figures 2 and 3. In addition, over the last 35 years, as annual precipitation continues to decrease, precipitation has increased in the plants' May-June growing season (Belovsky and Slade 2013).

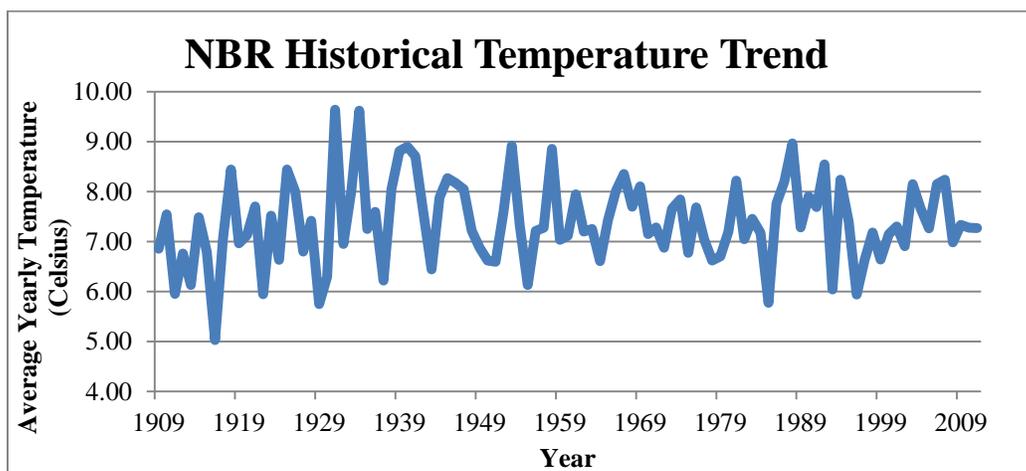


Figure 2. Trend of average yearly temperature increase at the National Bison Range, based off data collected by Belovsky and Slade (2013).

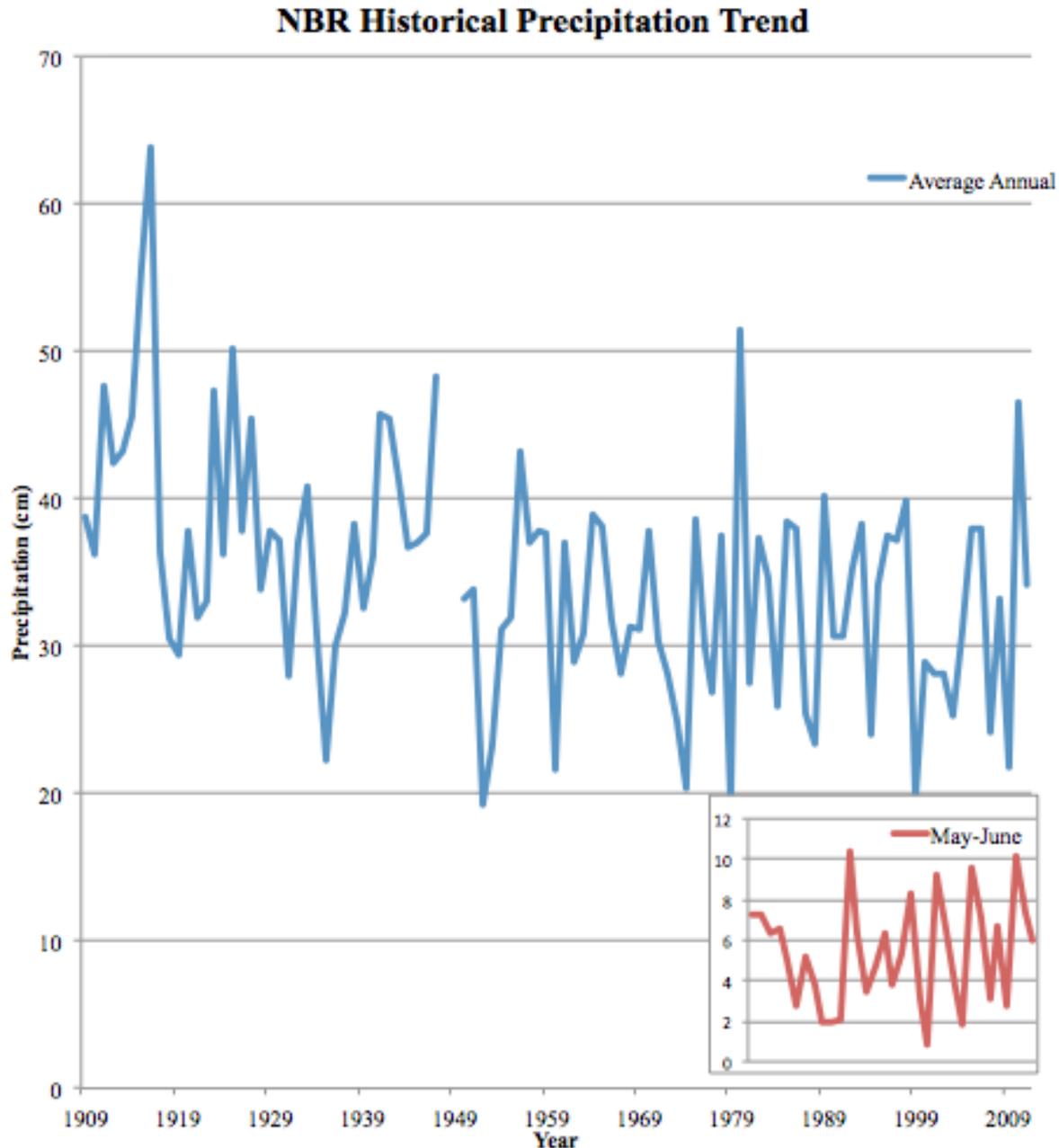


Figure 3A and 3B. Trends of decreased annual precipitation and increased growing season precipitation for the National Bison Range, drawn from Belovsky and Slade (2013).

Historically high spring rains and snow runoff have led to elevated spring productivity followed by a summer drought period that triggers a slow desiccation-driven die off. September rains provide the moisture needed for another period of plant growth, giving the bunchgrass prairie its unique bimodal growth period productivity. With the new trends in precipitation and temperature, spring productivity has been augmented and the summer die off is amplified, while the loss of fall precipitation has decreased the fall bought of productivity. According to regional climate change projections for the Northern United States, these trends will likely continue as warming exceeds 2°C over the next century, accompanied by increases of up to 20% in mean

annual precipitation, which will be delivered in heavier rainfall events (IPCC 2007). The National Bison Range was also selected due to its importance as a target to preserve biodiversity. The bunchgrass prairie land represented at the National Bison Range is only 1% of its original area, making it one of the United States' most endangered ecosystems (Johnson and O'Neil 2001). As invasive species such as *Linaria dalmatica* and *Vententata dubia* compete with native bunchgrass species, it will become increasingly important to predict plant responses to future climate change.

To assess the importance of the interactions created by multifactorial changes and their effect on environmental seasonality, this experiment will be conducted with a block design, shown below (Fig. 4). Summer nighttime temperature will be increased by 1.0°C using a passive warming system, simulating the predicted increase in daily minimum temperatures (National Academy of Sciences 2010; IPCC 2007). Nighttime temperatures are predicted to increase faster than daytime temperatures, as cold extremes are reduced and the diurnal temperature range contracts (IPCC 2007). Precipitation will be increased by 20% in June, the critical time for plant production, and over the course of the whole growth season, which is critical for senescence. A control treatment (no manipulation) will also be included, for a total of six treatments, which will be replicated five times. Experimentation will be conducted over two years to capture not only the initial effects, but also any lagged effects that may occur.

T+	T+ P+ June	T+ P+ summer
Buffer Zone		
Control	P+ June	P+ summer

Figure 4. Experimental design of the study, demonstrating the treatment combinations used to test the assumptions of multifactorial climate interaction models. (T+ = increased temperature, P+ June = increased precipitation by 20% in June, P+ summer = increased precipitation by 20% across the summer growing season)

Study Hypotheses

This study was designed to address three experimental aims regarding the effect climate change factors will exert on primary productivity and senescence of bunchgrass prairie vegetation, the nutrient cycling of nitrogen, and the subsequent shifts in plant community abundances as well as biodiversity. Hypotheses predict the variable response to treatment relative to the control.

1. The effects of increased temperature and increased precipitation on NPP and senescence have already been discussed at length and are summarized in Figure 5. Regarding the interactive effects of precipitation and temperature (Treatments 5 and 6), it was hypothesized that NPP could either increase or decrease, but the result would hinge on the precipitation-temperature ratio. Increases in this ratio will yield more available soil moisture, which should help to alleviate the soil moisture deficit.

	Ambient Temperature	Increased Temperature
Ambient Precipitation	1. Control	4. ↓ NPP, ↑ senescence
Increased Precipitation (June)	2. ↑NPP, no change senescence	5. ↑ or ↓ NPP, ↑ senescence
Increased Precipitation (summer)	3. ↑NPP, ↓ senescence	6. ↑ or ↓ NPP, ↑ senescence

Figure 5. Hypotheses relating NPP and senescence of treatment plots to the ambient temperature and precipitation control plot.

2. The rate of nitrogen mineralization should increase with corresponding increases in both growing season precipitation and temperature. The amount of inorganic nitrogen available to vegetation in treatment plots exposed to increases in both climate variables will depend on the interaction between temperature and precipitation effects, namely the operative precipitation-temperature ratio. This ratio will capture the soil moisture available for use by the microbes, as experimental plots exposed to increased precipitation in addition to increased temperature will lose some of the increased precipitation treatment through evaporation, the rate of which is positively correlated with temperature.

3. Examining the plant community as a whole, the diversity of grasses and forbs should decrease with exposure to elevated temperatures as drought resistant plants outcompete other species. This decrease in biodiversity will affect productivity differently depending where the bunchgrass prairie lies on the biodiversity-productivity curve. If the bunchgrass habitat lies to the right of the curve, this decrease in biodiversity could actually increase productivity. However, if the bunchgrass habitat lies on the peak of the curve or to the left, decreases in biodiversity would further lower productivity.

Grasses in general should become more abundant compared to forbs. Belovsky and Slade (2013) found that grass NPP responded negatively to forb NPP, and vice versa, indicating that the two groups are in competition for resources. In their study, grass NPP also responded negatively to June temperature, but positively to June precipitation. Forb NPP responded negatively to June temperature as well, but had no significant response to June precipitation. Thus increases in the precipitation-temperature ratio would favor grass production and provide a competitive advantage over forbs. However, species richness within both groups will likely decline as species with a competitive advantage, such as a higher optimum temperature for photosynthesis, come to dominate the community.

Within the grasses, C₃ species should remain dominant, although shifts within this grouping to a primarily Triticeae tribe (wheat grass) community may occur. As the bunchgrass prairie at the National Bison Range is dominated by C₃ grass species, any shift in grass community composition should take place within the C₃ group towards those species with a higher temperature range for optimum photosynthesis. Belovsky and Slade (2013) have shown that increasing temperatures over a 35 year time period led to increased Triticeae tribe composition and a decline in the relative abundance of Poaeae tribe (blue grass) species. Increased annual temperature should favor C₄ grasses (Epstein et al 1997) as high night time temperatures can impede C₃ seed development (Brown and Gersmehl 1985), but this bunchgrass prairie currently only has one C₄ species present at low concentration throughout the area.

By testing not only combinations of temperature and precipitation treatments, but also each factor individually, this study will determine how the bunchgrass prairie response to climate change relates to the results of climate change experiments conducted on tall grass and short grass prairies discussed earlier. The results of this experiment will be able to contribute to

climate change modeling by allowing incorporation of dynamic vegetation responses to regional shifts in temperature and precipitation.

Methods

Experimental Set Up

The same experimental set up will be used to address all the experimental aims. Experimentation will take place at the National Bison Range in Montana, where the study site will be located in a subsection of the range named the Triangle, which is at an elevation of 832m and UTM coordinates 713570E 5248100N (Belovsky and Slade 2013). The site at triangle has a fence to exclude large mammals such as deer or bison that could trample the experimental set up. Each of the five replicates will be conducted in a 3x3m enclosure that is divided into six 1x1m plots for each treatment or control, and with a one-meter buffer zone between the two rows of temperature treatment combinations, as shown below. The five replicate enclosures will be delineated by aluminum edging buried 0.25m into the ground, an effective depth for excluding borrowing rodents as well as roots of plants from outside the plot (Vantassel et al 2011). Inside the enclosure, aluminum will be buried 0.1m into the ground between the 1x1m plots to prevent plant roots from crossing treatment plots and to eliminate water movement and leaching across treatments, negating the need for a buffer zone between all treatment plots. Bedell and Buckhouse (1994) found that 0.03m of precipitation penetrated 0.12m into bunchgrass prairie soil, even when the area was cleared of vegetation. The 0.03m measurement is well above the precipitation treatments likely to be added, a conclusion reached based on calculations from the past ten years of National Bison Range weather data.

The temperature treatment will be implemented with a passive warming method achieved by placing louvered plastic slats on top of the enclosure (Germino and Smith 1999; Aronson and McNulty 2009). These plastic slats will be made of Optix acrylic and placed at a 45° angle every 10cm, angled in opposite directions so that they meet at a 90° angle in the center of the 3x1m temperature treatment row of the plot (Sherwood 2013). These slats will be held in place by a wooden beam on either side of the 3x1m row and supported by a PVC structure that will hold them 0.75m off the ground, high enough to prevent interference with grass growth. The PVC structure will remain in place during the winter, while the louvered slats will be removed in September to avoid snow loading and replaced in May of the following year for the second summer of data collection. Insect netting will be placed over the frame to exclude the presence of grasshoppers and other insects that could selectively eat the vegetation, skewing productivity results. In order to avoid the complications associated with grasshopper hatchlings within the experimental plots, molasses traps will be constructed in each of the five replicates (Klein and Wenner 2001).

In order to monitor the precipitation for the enclosures and provide the basis for the precipitation treatment, a National Geographic Deluxe weather station will be placed in the center of the 2-hectare study site. Rainfall will be increased by 20%: this will be done in the growing season plots by adding water only in June and for the summer plots by adding water in June and each month of the summer. The precipitation treatment will be added once a week, in order to avoid increasing the frequency of rainfall events, by evenly watering each of the increased precipitation plots.

Extreme weather events do have the potential to affect the experiment, and research has been done in order to format contingency plans for such circumstances. The passive warming method system that will be implemented is well suited to extreme temperature events, as it has been previously used with success in montane ecosystems of Idaho and Wyoming, where temperatures can vary from 3°C to 27°C over the course of a single day (Sherwood 2013; “Weather”). In addition, the structure supporting the warming system was secured with rebar posts dug into the soil to mitigate the effect of any strong winds brought by June storms. The case of flooding associated with these June storms was also considered. As too much water can stunt plant growth and lead to nitrogen leaching from the soil, flooding may eliminate the possibility of the intended increased June precipitation treatment as the treatment may have to be reduced

to 10% or have a delayed application (Rosenzweig et al 2002; Lehmann and Schroth 2003). As increased precipitation will result in larger precipitation treatments, large rainfall events may even call for precipitation treatments to be applied over the course of a week rather than in one episode. Conversely, there is the possibility for drought, especially in the later summer months. In case of weeks without rainfall, biweekly additions can be made, or if a prolonged drought occurs, the 20% calculation will be based on a ten-year historical average for weekly rainfall at the National Bison Range.

Measurements will be taken to ensure that the treatments are having the desired effect. A HOBOtemp data logger will be used on each temperature treatment side of the 3x3m plot to measure temperatures in order to gauge the direct effects of temperature treatments (Risch and Frank 2007). Measurements will be recorded every fifteen minutes then converted into hourly and daily averages. Soil moisture data for each plot will be obtained using gypsum blocks placed 0.05m below the ground's surface (Dobriyal et al 2012; Zhou et al 2006; De Valpine and Harte 2001). The moisture readings will be taken weekly, with additional measurements before and after water treatments have been added. If the National Bison Range experiences an extremely dry summer, though, a second method of moisture measurement such as a Hydrosense probe may need to be used in the drier summer months as gypsum blocks are not effective below a certain level of soil desiccation. In addition, gypsum block readings are affected by temperature, with increased temperature resulting in higher moisture readings, up to 3% per °C (Muñoz-Carpena 2004).

The statistical analysis of the temperature and soil moisture data, as well as primary productivity and nitrogen data, will be focused on two-way ANOVA. The two factors will be temperature (ambient, increased) and water treatment (ambient, increased June, increased seasonally). Before conducting the ANOVAs, the dependent quantitative variables will be tested to ensure they meet the assumptions of the ANOVA test. Although a 2³ factorial ANOVA test with temperature (warmed or ambient), precipitation (elevated or ambient), and seasonality of precipitation treatment (June or seasonal) would be ideal, all the combinations of variables are not possible as there will be no treatment comprised of ambient precipitation in June then increased precipitation in the rest of the summer months. Depending on the variable of interest, additional statistical tests may also be conducted. For daily temperature, repeated measures ANOVA (RM) will be used in order to remove the effect of time, as temperatures in Montana vary greatly between June and September. The two-way ANOVA and RM ANOVA will be run not only on the daily temperature averages, but also on daily minimum and maximum temperatures. Along with any transformations needed to meet ANOVA assumptions, soil moisture data will be converted into a measure of soil moisture relative to the control within each replicate since soil moisture varies spatially. This will remove the effect of variation between replicates, which could mask the true effects of the temperature and precipitation treatments.

Primary Productivity Measurement and Analysis

The experimental strategy specific to addressing Aim 1 of this study involves biweekly radiometer readings that will be used to calculate primary productivity. These radiometer readings will then be converted into biomass measurements by creating a regression of radiometer readings and clipped biomass from 0.1m² calibration plots located near the study plots (Belovsky and Slade 2013). The plots used to create this regression are chosen to include variation in vegetative cover, from low to high, and are chosen to represent the relative abundances of grasses and forbs within the experimental plots. Within each treatment plot, blue painted nails will be placed to form a 0.5m² area from which 3 overlapping radiometer readings will be taken with the device held approximately 1.5m over the ground (Belovsky and Slade 2013; Adamsen et al 1999). Regular readings during the season will be taken through the insect netting, so a conversion factor, determined by Belovsky and Slade (2013), will be used when calculating NPP. Additionally, one radiometer reading will be taken at the beginning and at the end of the experimental period without the insect netting in place, in case the netting has an unpredicted effect on readings.

After data for the summer has been collected, radiance readings will be converted to biomass measurements, which can then be used to calculate NPP and senescence. Net primary productivity for the

study period will be the sum of all the increases in living biomass from May to September. All chronological increases between any two biweekly measurements will be added together to arrive at NPP for the season. The rate of senescence will also be examined with the use of net primary productivity data obtained from radiometer readings (Belovsky and Slade 2013; Knapp et al 2002). Percent senescence will be calculated as $1 - \frac{\text{lowest observed green biomass}}{\text{peak observed green biomass}}$ (Belovsky and Slade 2013), and will likely have to be transformed using arcsine or a similar method in order to normalize the measurements. The two-way ANOVA setup detailed above will be used with NPP and senescence as the dependent variables. In addition, a similar two-way ANOVA will be run with the dependent variables transformed into relative values based on the control in each replicate. Lastly, a general linear regression model will be used to investigate the relationship between the precipitation-temperature ratio and NPP. This ratio will be constructed using the precipitation and temperature data already available to investigate the combined effect of the two factors.

Measuring Nutrient Cycling of Nitrogen

Three different measurements of nitrogen will be collected – organic soil nitrogen, inorganic soil nitrogen, and nitrogen present in the vegetation. Inorganic soil nitrogen will be measured using resin bags placed in each 1x1m experimental plot (Wyland 1993; Garten et al 2008). Resin bags placed in the spring will be removed in the fall, while those placed in the fall will be removed in the spring of the following year. Organic nitrogen will be measured using soil cores taken from each plot. The nitrogen content of the vegetation will be sampled using 5.0g of dried clippings collected from each 1x1m plot (An et al 2005). Soil cores and vegetation clipping will be collected in June and September of each year of the study. Lab analyses will include running KCl extractions on the resin bags and soil cores followed by Lachat spectrophotometer analyses (Robertson et al 1999). Grass clippings will be ground then run on the CEST Costech elemental analyzer (Ma et al 2012). Enough of the KCl extract and ground class clippings will be generated to run a second spectrophotometer or elemental analysis in case any equipment errors occur.

The same two-way ANOVA established in the previous experimental strategy section will be used on the three dependent variables of soil inorganic nitrogen, soil organic nitrogen, and plant nitrogen levels. In addition, a general linear regression model investigating the relationship between the precipitation-temperature ratio and the levels of nitrogen may be employed to determine the interactive effect of these two climate variables.

Assessing Biodiversity of Experimental Plots

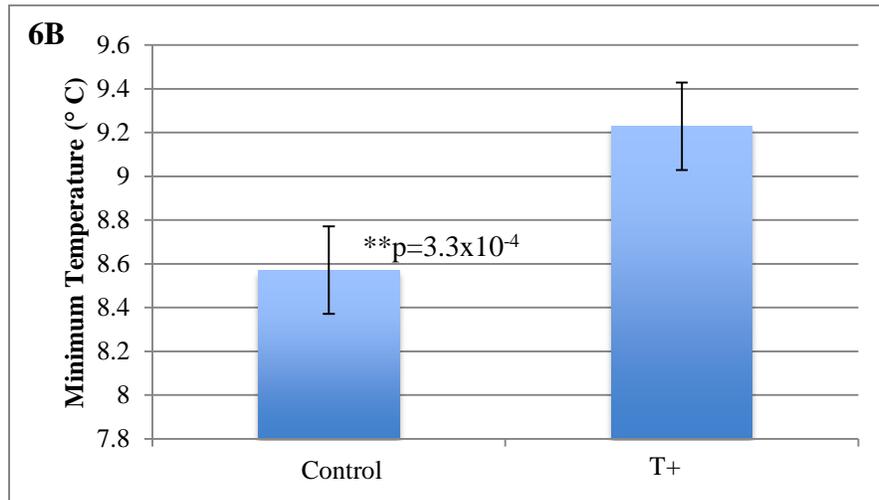
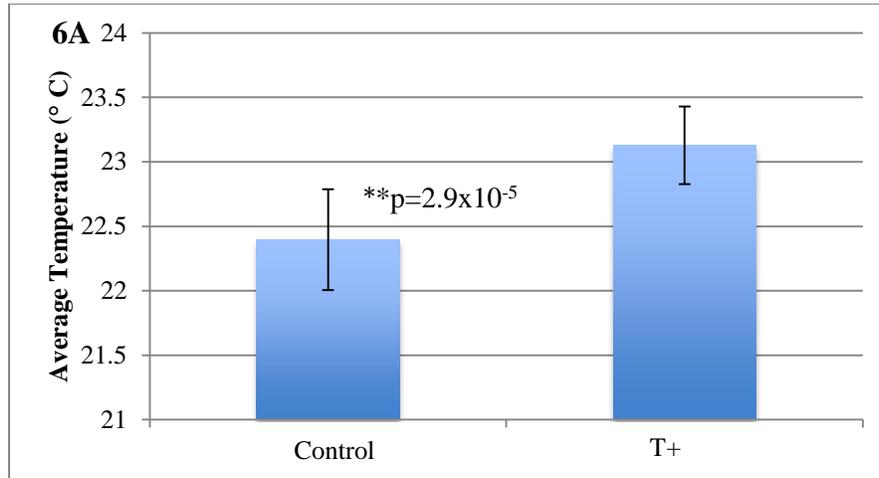
Species composition data will be measured in June and September using a point frame sampler, with 100 data points taken per 1x1m plot. The point frame sampler covers one quadrant of the plot at a time, so 25 readings will be taken from each quadrant, with care taken to avoid the immediate edge in order to eliminate any edge effects. Readings will be recorded as a plant species (grass or forb), bare ground, litter, moss, or lichen. Species composition shifts should be most apparent across the two-year study period. Thus comparisons of the relative composition of grass and forb groups can be made from the beginning to the end of the study period for each treatment group. Within each year, analysis of the changes in species compositions can also be compared across treatment groups. In addition, the Shannon-Weiner biodiversity index can also be analyzed between the treatment groups and study years.

Preliminary Results

Note that at this time results are only available for the first year of study and that, due to a backlog on the elemental analyzer at the University of Notre Dame, data on nitrogen cycling for 2014 is also unavailable. In addition, while biodiversity has been calculated, analysis will take place across study years, so results are not reported here.

Temperature Treatment

The temperature elevation treatment significantly increased average daily temperature, average minimum temperature, and average maximum temperature (see Figure 6a, b, c). Across the replicates, average daily temperature was increased by $0.73^{\circ}\text{C} \pm 0.47^{\circ}\text{C}$, average minimum daily temperature was increased by $0.66^{\circ}\text{C} \pm 0.26^{\circ}\text{C}$, and average maximum daily temperature was increased by $1.39^{\circ}\text{C} \pm 1.04^{\circ}\text{C}$.



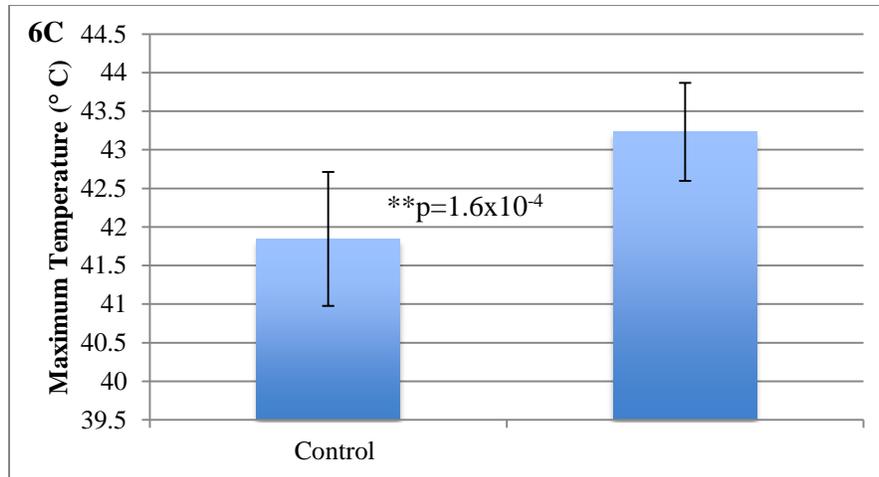


Figure 6. Graphs A, B, and C depict the average increase in daily average, daily minimum, and daily maximum temperature, respectively, for the 2014 data collection season.

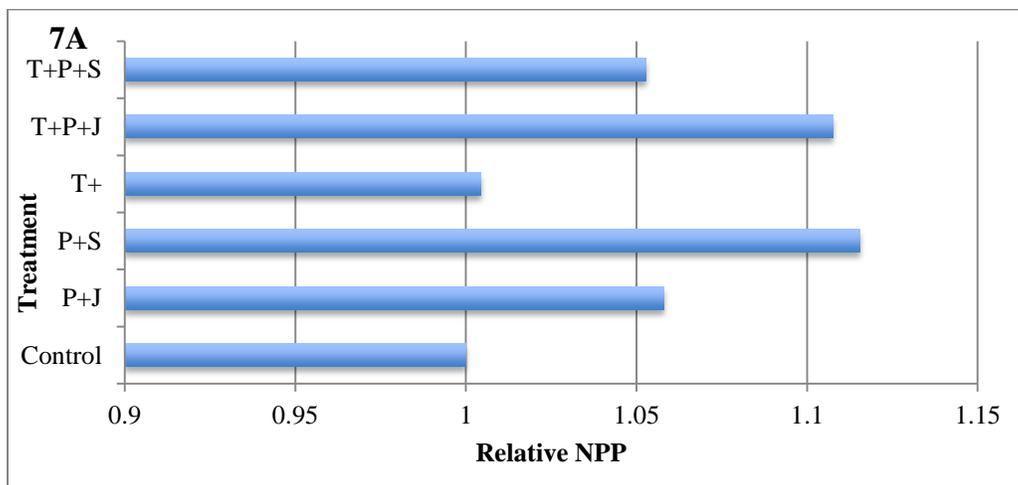
From data collected thus far in the 2015 field season, nighttime temperature has increased by $0.97 \pm 0.35^\circ\text{C}$ across the replicates.

Moisture Treatment

HOBOTemp data logger measurements were discontinued into the second year of experimentation due to the extreme level of soil desiccation present at the test site. Use of the HOBOTemp loggers yielded negative readings, which are outside of the interval that the manufacturer, Delmhorst, guarantees accurate measurements. The Hydrosense probe was also not sensitive enough to detect informative readings of soil moisture. This issue was exacerbated by the extreme drought and high temperatures the area experienced in 2015.

Primary Productivity and Senescence

A two-way ANOVA with the temperature and water treatments as the two factors found no significant effect of either individual variable or their interaction on NPP and senescence. However, the effect of the water treatment on NPP of the experimental plots, relative to the control, was trending towards significance ($p=0.13$) and the effect of the temperature treatment on relative senescence was also trending towards significance ($p=0.33$), shown in Figures 7A and 7B.



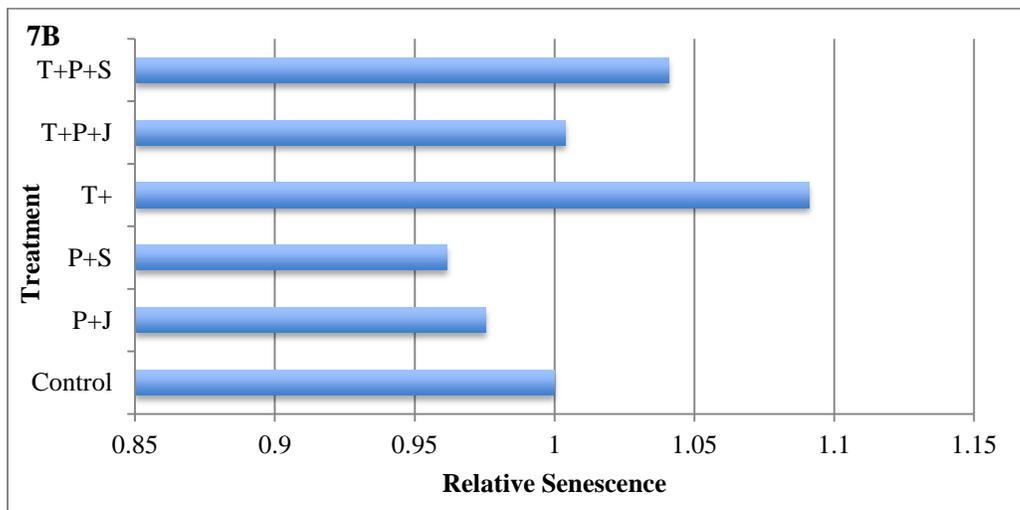


Figure 7. Graphs A and B show the NPP and senescence, relative to the control, in the treatment plots to provide a visualization of the treatment effects.

Preliminary results, found using general linear model regression, indicated that a significantly positive relationship existed between the net primary productivity of vegetation and the precipitation-temperature ratio of the plot ($p=0.01$), shown in Figure 8.

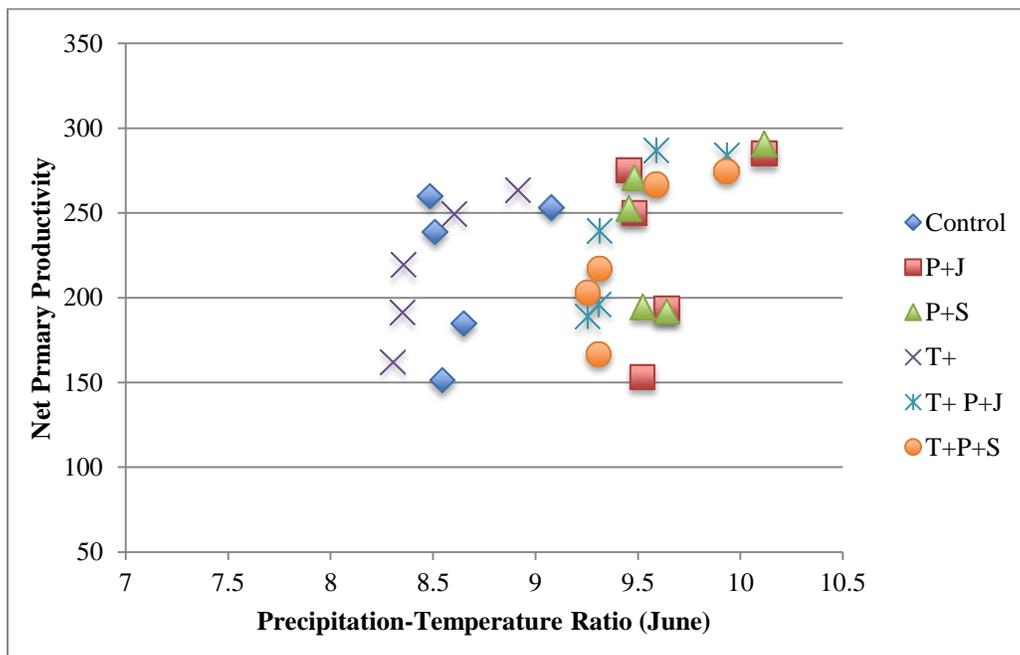


Figure 8. The significantly positive relationship ($p=0.02$) between the net primary productivity of plots and the precipitation-temperature ratio.

Discussion

Preliminary results indicate that the temperature treatment was effective in significantly raising not only the nighttime (minimum) temperature, but also the daily maximum and average temperature. Variation in the degree of temperature elevation for the treatment was present across the replicates, which

may be a result of the natural variation in surface temperatures, despite experimenter effort to choose a homogenous plot area. Other experiments using a passive warming system have measured subsurface temperature to confirm temperature increase, which tends to be more stable, but this would not have been feasible in the current circumstance, due to the difficulty encountered installing subsurface probes in the extremely dry soil.

The level of desiccation at the experimental site also made the use of gypsum blocks and other soil moisture measurement techniques unfeasible. Gypsum block readings were negative even after significant rainfall and Hydrosense probe readings consistently measured 0% across all treatments. While this impeded the ability to investigate available soil moisture, the ratio of precipitation-temperature was still used to investigate the available moisture for plant growth.

Although only in its first year of implementation, the study found trends in net primary productivity that may be amplified by another year of study. Results suggest that precipitation is a major driver of net primary productivity, which also indicates the key role of water stress as a determinant of the future role of precipitation levels. Temperature also appeared to accelerate the rate of senescence, as predicted. In addition, the role of the precipitation-temperature ratio was confirmed with a GLM regression that indicated a significantly positive relationship between net primary productivity and the June precipitation-temperature ratio.

While the role of the precipitation-temperature ratio as a representation of available soil moisture appears to be well-founded, analysis of nitrogen data and the second year of net primary productivity data will be crucial to establishing its role in ecosystem function.

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