

DISSERTATION

GRASSLAND RESPONSES TO SEASONAL SHIFTS
IN WATER AVAILABILITY

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ABSTRACT

GRASSLAND RESPONSES TO SEASONAL SHIFTS IN WATER AVAILABILITY

Climate change is altering seasonal dynamics across a wide range of ecosystems with consequences that include shifts in phenology, timing of nutrient availability, and changes in plant community composition. Current research has primarily focused on temperature as the key driver for these shifts because of the strong directional trend with climate warming, however, alterations in the availability of water across seasons is an unappreciated aspect of climate change that can significantly influence ecosystem functioning. While changes in the seasonal availability of water are expected to be globally pervasive, grasslands may be particularly vulnerable because these ecosystems are often water-limited and have species with distinct seasons of growth. Therefore, my dissertation examined how seasonal patterns of water availability may shift with climate change in the grasslands of the US Great Plains and the ecological consequences of these shifts. I first explored several mechanisms by which climate change is altering the seasonal water balance, using the Great Plains as a case study. Building on that, I then designed two field experiments in semi-arid grasslands that altered seasonal patterns of water availability to understand how these shifts affected ecosystem function and structure (primarily C_3 vs C_4 grasses). Overall, the results from both field experiments suggest that shifts in the seasonality of water availability with climate change will alter carbon cycling dynamics, shift seasonal patterns of canopy albedo, and differentially impact C_3 vs. C_4 species in the semi-arid grasslands of the US Great Plains. Thus, my research confirms the importance of this aspect

of climate change and provides evidence that seasonal shifts in water availability can alter ecosystem processes and drive compositional changes. Since grasslands provide many economically and ecologically valuable services, understanding how climate change will impact these systems is critical for land managers and policymakers to make informed decisions.

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CHAPTER 1: INTRODUCTION

Anthropogenic emissions are rapidly increasing the atmospheric concentrations of several greenhouse gases such as carbon dioxide (CO₂) and methane (CH₄), and these are warming temperatures globally (Canadell et al., 2021; Eyring et al., 2021). Since a warmer atmosphere has a greater water-holding capacity (Trenberth, 2011), climate change is intensifying the hydrologic cycle with important ecosystem consequences. In particular, there has been an increase in the number of extreme dry and wet periods (Groisman & Knight, 2008; Fischer et al., 2013; Sillmann et al., 2015; Eyring et al., 2021), exemplified by the ongoing American southwest megadrought (Williams et al., 2022). Extreme wet periods are often accompanied by extreme rainfall events (Fischer et al., 2013; Kunkel et al., 2013; Sillmann et al., 2015; Donat et al., 2016), or deluges, which can have especially serious economic and ecological impacts. With these novel climate scenarios, there is a tendency to focus on changes in the means (i.e., is this region becoming wetter or dryer with climate change?; Chou et al., 2013; Huang et al., 2016; Overpeck & Udall, 2020). Understanding these trends is meaningful because there are well-established relationships between total amount of precipitation and many ecosystem functions. Globally, mean annual precipitation (MAP) does a good job delineating the distribution of the different biomes and their productivity (Knapp & Smith, 2001; Chapin et al., 2012). Regionally, other important trends emerge; for example, in the North American Great Plains, there is a strong positive relationship between MAP and aboveground net primary production (ANPP, Sala et al., 1988; Huxman et al., 2004). But climate change is multi-dimensional, and many factors in addition to total amounts of precipitation affect ecosystem responses.

For many ecosystems, the seasonal timing of precipitation strongly controls the dynamics of ecosystem functioning, primarily because it determines when water is available for use. Importantly, the seasonal timing of water availability influences the dominant vegetation type (Stephenson, 1990; Paruelo & Lauenroth, 1996; Seliger et al., 2020; Palmquist et al., 2021) and controls many ecosystem processes, such as carbon and nutrient cycling (Schimel & Parton, 1986; Epstein et al., 1999; Pérez-Ruiz et al., 2022). However, changes in the timing of water availability at the seasonal timescale are often overlooked as a key driver of ecosystem shifts due to climate change, in part because seasonal predictions are complex (White & Hastings, 2020) and there are many potential mechanisms that can shift seasonality (Stephenson, 1990). This may be especially true for grasslands - where previous work in this biome has demonstrated its strong sensitivity to the timing of water availability (Petrie et al., 2016, Parton et al., 2012).

Grasslands are one of the most widespread and diverse ecosystems on the planet, covering over a third of the world's terrestrial surface (Bardgett et al., 2021). They play a crucial role in global biodiversity and provide many ecosystem services, such as carbon storage (Pendall et al., 2018), in addition to supporting a wide range of human activities, including agriculture, livestock grazing, and recreation (Bengtsson et al., 2019). But many grasslands are also water-limited (Lauenroth & Burke, 2008) and particularly vulnerable to changes in precipitation patterns due to climate change (Petrie et al., 2016). Understanding how these semi-arid grasslands respond to climate change will be critical for managing and preserving these invaluable ecosystems in a sustainable manner.

The semi-arid grasslands of the North American Great Plains are characterized by soils that are frequently dry with intermittent wet periods (Sala et al., 1992), and as a result, their functions are tightly coupled with the timing of water availability (Noy Meir 1973; Schwinning

& Sala, 2004; Heisler-White et al., 2008). Previous work has demonstrated that many ecosystem responses depend on both the patterns and timing of precipitation. For example, several recent studies in the shortgrass steppe of Colorado have highlighted the potential for deluges to enhance both ANPP and belowground net primary production, modulate the dynamics of canopy greenness and soil respiration, and increase flowering of the dominant grass species, with the seasonal timing important for controlling the magnitude of response (Heisler-White et al., 2008; Post & Knapp, 2019; 2020; 2021; Hoover et al., 2022). While it is clear that the temporal patterns of water availability modulate ecosystem function in semi-arid grasslands within the growing season, how shifts outside of the growing season influence ecosystem function is unknown, and other important changes may still emerge from altered seasonal patterns of water availability.

The semi-arid grasslands of the US Great Plains are also home to both C₃ and C₄ grasses, often referred to as cool and warm season grasses, respectively. Predicting the future ranges of these two functional groups with climate change is critical for land managers and decision makers, as these grasses differ in forage quality (Barbehenn et al., 2004; Chamaille-Jammes and Bond, 2010), phenology (Goodin & Henbrey 1997; Piao et al., 2019), and climate feedbacks (i.e., water and energy fluxes; Richardson et al., 2013). With climate change, most studies predict expansion of C₄ grasses because of their advantages at higher temperatures (Teeri & Stowe 1976; Ehleringer & Björkman 1977; Epstein et al., 1997; Still et al. 2003; Yamori et al. 2014). However, shifts in the seasonal patterns of water availability may play an underappreciated role in determining the future distribution of these grasses; previous work suggests that where these grasses coexist, the seasonal partitioning of soil moisture may be a dominant driver of their relative abundances and productivities (Winslow et al., 2003; Knapp et

al., 2020; Xie et al., 2022). Thus, the potential for changes in seasonal patterns of water availability to alter the structure of these grasslands would have important implications for carbon cycling and feedbacks into the atmosphere (Richardson et al., 2013; Piao et al., 2019; Xie et al., 2022), yet experimental evidence for this is still limited.

In the following chapters, I address the above climate change issues through three studies. First, I explore how the seasonal availability of water might shift in the North American Great Plains and discuss potential consequences for ecosystem functioning and structure. I provide a review on this topic and include an analysis of long-term weather records from the central US Great Plains as a case study for identifying mechanisms altering seasonal water patterns. This chapter provides the rationale for both of my field experiments, which aim to test ecosystem responses to altered soil moisture patterns in the semi-arid grasslands of the US Great Plains. For my first field study (Chapter 3), I experimentally shifted seasonal precipitation patterns without changing total precipitation amount. This enabled me to isolate the impacts of shifts in precipitation seasonality on C_3 vs. C_4 grass dynamics and ecosystem functions. My second field experiment (Chapter 4) was designed to determine if dormant season precipitation in the form of deluges would have carryover effects the following growing season. Due to a naturally occurring extreme drought in the following spring, this experiment provided a lens into seasonal legacy effects (Bastos et al., 2020) with compounded extremes (extreme wet followed by extreme dry periods). Combined, these three studies have allowed me to achieve the goal of this dissertation - to better understand how shifts in the seasonal availability of water may alter ecosystem dynamics. My hope is that the insights from this research will help us to better manage these important grassland ecosystems in the face of a rapidly changing climate.

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CHAPTER 2: SHIFTING SEASONAL PATTERNS OF WATER AVAILABILITY: ECOSYSTEM RESPONSES TO AN UNAPPRECIATED DIMENSION OF CLIMATE CHANGE¹

2.1 Summary

Seasonal patterns of water availability can differ dramatically among ecosystems, with well-known consequences for ecosystem structure and functioning. Less appreciated is that climate change can shift the seasonality of water availability (e.g., to wetter springs, drier summers), resulting in both subtle and profound ecological impacts. Here we (1) review evidence that the seasonal availability of water is being altered in ecosystems worldwide, (2) explore several mechanisms potentially driving these changes, and (3) highlight the breadth of ecological consequences resulting from shifts in the seasonality of water availability. We conclude that seasonal patterns of water availability are changing globally, but in regionally specific ways requiring more rigorous and nuanced assessments of ecosystem vulnerability as well as the ecological consequences.

2.2 Introduction

From a climatic perspective, seasonality is defined as regular and periodic changes in environmental conditions on an annual timescale (White & Hastings, 2020). Seasonality is evident to some degree in almost all ecosystems (Tonkin et al., 2017) and is relevant to most ecological studies (White & Hastings, 2020). Viewed from the perspective of annual oscillations in solar radiation and temperature, seasonality tends to increase in importance with distance from the equator, with mid-latitude ecosystems characterized by four relatively distinct seasons. At mid-latitudes, meteorological (temperature) and astronomical (solar radiation) seasons tend to

¹ Hajek, O. L., & Knapp, A. K. (2022). Shifting seasonal patterns of water availability: ecosystem responses to an unappreciated dimension of climate change. *New Phytologist*, 233(1), 119-125.

overlap (Trenberth, 1983) with periods of low (winter) and high (summer) temperature/solar radiation bridged by transitional seasons (spring and autumn). These four seasons generally correspond to a 365-day-long sine wave (Trenberth, 1983; Fig. 2.1a) with the amplitude of seasonality varying much more geographically than interannually. Despite evidence that increasing concentrations of greenhouse gases have altered both the mean and amplitude of seasonal cycles of air temperature (Xia et al., 2014; Santer et al., 2018), general relationships between seasons and temperature (i.e., summers are warmer than winters) are not expected to change with climate change, as a consequence of the relative invariance of the annual cycle of solar radiation inputs.

In contrast to temperature, seasonal patterns of precipitation are much more variable among ecosystems in space and time (Fig. 2.1b). Annual precipitation patterns can vary from strongly seasonal (e.g., winter- or summer-dominated) to bimodal to aseasonal (Fig. 2.1b). Indeed, geographic variation in ecosystem structure has long been attributed, in part, to differences in the seasonal timing of precipitation (Stephenson, 1990; Paruelo & Lauenroth, 1996; Seliger et al., 2021). The importance of precipitation seasonality is most evident when ecosystems with similar annual temperature and precipitation, but varying precipitation seasonality, are compared (Fig. 2.1c). In such cases, it is clear that precipitation seasonality strongly influences the overall dynamics of an ecosystem's water balance, determining which seasons have water surpluses or deficits and, in large part, driving the temporal dynamics of biotic activity (Stephenson, 1990).

With unprecedented rates of global warming, ecologists have justifiably focused on the biotic and biogeochemical consequences of temperature changes within particular seasons (e.g., spring warming and earlier leaf-out). However, research designed to better understand the

consequences of shifts in the seasonality of water availability is less common, despite the clear importance of this dimension of seasonality.

Our objectives are to (1) review evidence that the seasonal timing of water availability is shifting, or projected to shift, with climate change, (2) identify globally important mechanisms underpinning seasonal shifts in water availability, and (3) highlight the potential ecosystem consequences of these changes. Because studies on the ecological consequences of annual and seasonal warming have been reviewed previously (see Walther et al., 2002; Parmesan & Hanley, 2015), we incorporate warming in this review primarily through its indirect effects on the seasonality of water availability. Furthermore, to provide deeper insight into the causes and consequences of seasonal shifts in water availability, we highlight a region expected to be particularly vulnerable to alterations in the timing of water availability – the Central Grasslands of the US Great Plains.

2.3 How and why is the seasonal availability of water changing?

Ecosystem structure and functioning are better understood when the seasonal dynamics rather than the annual amount of available water are considered (Stephenson, 1990). Water availability is determined largely by the dynamic balance between (1) supply (dominated by precipitation inputs in most terrestrial ecosystems), (2) demand (atmospheric vapor pressure gradients driving evapotranspiration, ET; Grossiord et al., 2020), (3) soil water storage and groundwater availability, which can provide important buffers when supply and demand are out of sync (Stephenson, 1990), and (4) other hydrological losses (runoff, groundwater recharge). We will focus on supply and demand as determinants of seasonal patterns of water availability here because these are important in all terrestrial ecosystems, and both are strongly driven by meteorological variables (precipitation and temperature) affected by climate change.

Furthermore, although the dynamics of soil water content may more directly determine the availability of water to plants in most (but not all) ecosystems, meteorological variables have longer time series available for assessing change, greater spatial coverage, and require fewer assumptions (Young et al., 2021).

Much of the evidence for shifting seasonality – and, consequently, our review – focuses on precipitation inputs, based on the assumption that seasonal changes in precipitation will, in most cases, alter seasonal water availability. The most direct way that seasonal patterns of water availability can be altered is by a shift in the seasonality of precipitation inputs (i.e. more precipitation in one season, less in another). But warming temperatures, as well as altered solar irradiance (via cloud cover) and windspeed can affect ET and alter seasonal patterns of water availability – independent of changes in precipitation. Because interactions between energy and water strongly influence the overall water balance of ecosystems, changes to either supply or demand can result in water deficits or surpluses in any season (Stephenson, 1990, Fig. 2.1c). Thus, we evaluate both changing precipitation patterns and warming temperatures as drivers of shifting seasonality in water availability.

There is compelling evidence that seasonal patterns of water availability in ecosystems are changing (Padron et al., 2020), but whether particular seasons are becoming wetter or dryer is geographically variable. Some analyses of long-term precipitation records suggest that wet and dry seasons are becoming more pronounced globally (Chou et al., 2013; Hegerl et al., 2015), but others report that dry seasons are becoming wetter, reducing seasonality (Murray-Tortarolo et al., 2017). This lack of consensus is mirrored by regional-scale analyses. For example, precipitation has increased in the early summer months in parts of the US (Belovsky & Slade, 2020), but decreased in the UK (Fowler & Kilsby, 2003). Other notable shifts in seasonality include reports

of increased winter precipitation in China (Li et al., 2020), but decreased winter precipitation in Australia (BoM, 2020), and changes in the seasonal timing of precipitation inputs relative to temperature (Flanagan et al., 2017), consistent with projections for North American monsoon rainfall to shift from warmer to cooler months (Cook & Seager, 2013). In addition, increases in the frequency of extreme storms during summer (Moustakis et al., 2021) can either increase or decrease water availability to plants (depending on the ecosystem; Knapp et al., 2008; Zeppel et al., 2014), effectively rendering summers wetter or drier relative to other seasons. Although the above review is not comprehensive, it illustrates two key points: (1) seasonal patterns of water availability for ecosystem functioning have and are expected to continue to change, and (2) although most evidence is from changing precipitation inputs, shifts in this dimension of seasonality are complex and can have multiple potential drivers including warming temperatures (Padrón et al., 2020).

2.4 The US Great Plains – a case study for evaluating seasonality in water availability

In order to illustrate the diversity of potential mechanisms altering seasonal patterns of water availability, we focus on the Central US Great Plains. Like many grassland regions, much of the US Great Plains is water-limited (Epstein et al., 2002) and particularly sensitive to changes in water availability (Petrie et al., 2016). Furthermore, this region is dominated by plant functional types with distinct growing seasons – ‘cool season’ C₃ and ‘warm season’ C₄ grasses. Thus, shifts in the seasonal timing of water availability between spring and summer can be critical for modulating this region’s productivity and phenology (Epstein et al., 1999; Winslow et al., 2003).

The US Great Plains also is expected to be impacted by climate change in ways that encapsulate the many mechanisms altering seasonal patterns of water availability globally. For

example, this region is expected to become more arid overall because of increased temperatures, despite slight increases in total precipitation (Hufkens et al., 2016; Seager et al., 2018). But this synoptic view belies shifts in seasonal precipitation inputs that can vary substantially across the region (Seager et al., 2018). The central US region, in lockstep with much of the world, also is expected to experience an increase in climate extremes, including exceedingly wet and severe drought periods (Hegerl et al., 2015) which can alter seasonal precipitation patterns (Knapp et al., 2020). Furthermore, because extreme rainfall events can constitute a large fraction of annual precipitation, particularly in arid systems, the timing of when they occur can be an important driver of seasonality. Finally, atmospheric warming, whether uniform throughout the year or seasonally disparate, has the potential to differentially affect ET among seasons and, thus, alter seasonal patterns of water availability.

2.5 Assessing precipitation patterns, extremes and warming as drivers of seasonality

Given strong phenological and ecological differences in the dominant species spanning Northern ($C_3 > C_4$) to Southern ($C_4 > C_3$) Great Plains grasslands, we evaluated long-term (c. 100 year) precipitation records along a latitudinal gradient to determine if precipitation seasonality has changed in spring (MAM) vs summer (JJA) months (Fig. 2.2a,b). Despite slight increases in spring and summer precipitation totals (consistent with Hufkens et al., 2016, and Seager et al., 2018), we found no evidence that the relative contribution of spring and summer precipitation has changed over time for this region (i.e. the slopes of relationships did not differ, Fig. 2.2c,d). Consequently, we explored other drivers influencing the seasonal timing of water availability.

We next evaluated how the seasonal distribution of precipitation in spring and summer changes during extreme wet and dry years. We focused on the wettest ($> 90^{\text{th}}$ percentile) and

driest (< 10th percentile) years, based on probability distribution functions for each site, and compared these with average years (40th to 60th percentile). During extreme drought years in the Southern Great Plains, there were strong seasonal shifts from summer- to more spring-dominated precipitation patterns, a pattern not seen in the North and Central Great Plains (Fig. 2.3). By contrast, there were no significant changes in seasonality during extreme wet years. Thus, as precipitation variability increases and extreme hydrologic periods become more common, Southern Great Plains ecosystems will experience asymmetric shifts in the seasonal timing of water availability.

Finally, we assessed how warming, and thus increased atmospheric demand for water, can alter seasonal patterns of water availability. Because of geographic uncertainty in the degree of asymmetry across seasons in warming (Xia et al., 2014), we assumed equal warming for both spring and summer – the seasons of interest for C₃ vs C₄ plants. For this analysis, we determined potential ET (PET) for each site based on past climate data (<https://www.worldclim.org/>) and then increased monthly temperatures by 2°C to quantify the effect of warming on PET.

As expected, PET increased disproportionately in the summer vs the spring (as much as three-fold) despite uniform warming in both seasons (Fig. 2.4). The larger increase in summer PET is driven by the exponential relationship between air temperature and atmospheric vapor pressure deficits (Fig. 2.4, inset). Thus, the nature of this thermodynamic relationship will effectively alter seasonal patterns of water availability without any change in precipitation (Padrón et al., 2020). Importantly, this shift will be greatest where it is already warmest – the Southern Great Plains, compounding this region’s vulnerability to climate change.

2.6 Ecosystem consequences of shifting seasonality

There is compelling evidence that even relatively subtle shifts in seasonal patterns of water availability can have profound ecological consequences. Indeed, almost all major ecosystem processes including primary productivity, respiration, microbial activity, decomposition, and net ecosystem exchange will be impacted by changes in the seasonal timing of water availability (Epstein et al., 2002; Morgan et al., 2016; Ru et al., 2018). In arid and semi-arid systems, the seasonal timing of water availability is especially critical for ecosystem structure (i.e., community composition; Epstein et al., 1999; Gremer et al., 2018; Knapp et al., 2020) and functioning (Peng et al., 2013; Densmore-McCulloch et al., 2016; Hovenden et al., 2019). In parts of the Great Plains for example, spring precipitation is the primary determinant of annual primary production (Derner & Hart, 2007; Morgan et al., 2016). Thus, alterations in spring water availability may impact ecosystem functioning more than changes during other seasons. Key seasons for water availability are ecosystem-specific (e.g., winter rainfall in Mediterranean climates; Fig. 2.1c), but the net impact of seasonal shifts in water availability on productivity can be complex everywhere and a challenge to predict. For example, reductions in water availability in one season (summer) can be offset by increased plant growth during another (e.g., spring; Hufkens et al., 2016).

Shifts in phenological patterns, particularly earlier leaf-out and delayed senescence, typically are viewed as evidence of climate warming, but shifts in the timing of water availability can influence plant phenology directly (Shen et al., 2015; Fu et al., 2021) as well as through changes in plant community composition (Winslow et al., 2003). There is evidence, for example, that the abundance of shrubs vs grasses (Germino & Reinhardt, 2014; Gremer et al., 2018) and cool vs warm season species (Knapp et al., 2020) can be altered by shifting the seasonal timing

of water availability with consequences for forage production, nutrient cycling, and water catchment dynamics (Epstein et al., 1999; Palmquist et al., 2021). As noted previously, extreme droughts can reduce summer dominance of rainfall in Great Plains grasslands, and this can lead to shifts from C_4 to C_3 dominance (Knapp et al., 2020). Given that C_3 grasses leaf-out weeks earlier than C_4 species, such phenological changes will significantly impact ecosystem–biosphere interactions, altering seasonal patterns of canopy albedo, conductance, and ecosystem fluxes (Richardson et al., 2013; Piao et al., 2019). Furthermore, as plants leaf-out earlier, increased early season ET earlier can create ‘seasonal legacy effects’ (Bastos et al., 2020), potentially amplifying summer drought as enhanced spring growth preempts soil moisture from being available in later seasons. Greater soil drying during summer can, in turn, intensify heat waves (Lian et al., 2020).

More broadly, most ecosystems are populated by species that vary in their seasonal growth patterns and phenological activity (e.g. spring ephemerals vs fall flowering species). Shifts in the seasonality of water availability have the potential to alter the relative success of these species and their ecological interactions through a variety of mechanisms. These include altering the seasonal timing of nutrient availability (Ernakovich et al., 2014; Densmore-McCulloch et al., 2016), mismatches between plants and pollinators (Ovaskainen et al., 2013), misalignment in the timing of thermal and hydrological requirements for seedling establishment (Walck et al., 2011), altered competitive interactions between plants (Hallet et al., 2019; Palmquist et al., 2021) and increased opportunities for species invasions (Fridley, 2012). Although plasticity in plant traits may help to offset some of these consequences (Wang & Callaway, 2021), we posit that even relatively subtle shifts in the seasonal water balance of most ecosystems will have pervasive ecological consequences, potentially altering ecosystem boundaries as plant communities and functions change (Knapp et al., 2020).

2.7 Final comments and research needs

We have argued that seasonally shifting patterns of water availability are an underappreciated consequence of climate change – with diverse drivers and a broad array of ecological impacts. Water-limited ecosystems are global in extent, and arid and semi-arid lands are likely to be particularly sensitive to changes in the seasonality of water availability. However, even in more mesic temperate forests, the seasonal timing of water deficits vs surpluses will strongly impact the physiological dynamics of trees, and different seasonal water balances favor dominance by very different growth forms (deciduous vs coniferous; Stephenson, 1990). There also is limited evidence that mesic tropical ecosystems, with distinct wet and dry seasons, will respond to climate change-induced alterations in the seasonality of water availability (Yavitt et al., 2004). Finally, of the drivers reviewed, warming-induced increases in PET that are greater in warmer than cooler seasons may be the most widespread cause of seasonal shifts in the water balance of ecosystems, although as climatic extremes become more common, their importance as drivers of seasonality may increase. Unfortunately, despite substantial observational support that changes in the seasonal availability of water will have far-reaching ecological consequences, experimental quantification of these impacts is limited, and more mechanistic evaluations are needed. We urge climate change researchers to impose explicit manipulations of the seasonal timing of water availability not only to advance our understanding of the consequences of this neglected dimension of climate change, but also to help identify those ecosystems that are most vulnerable.

2.8 Figures

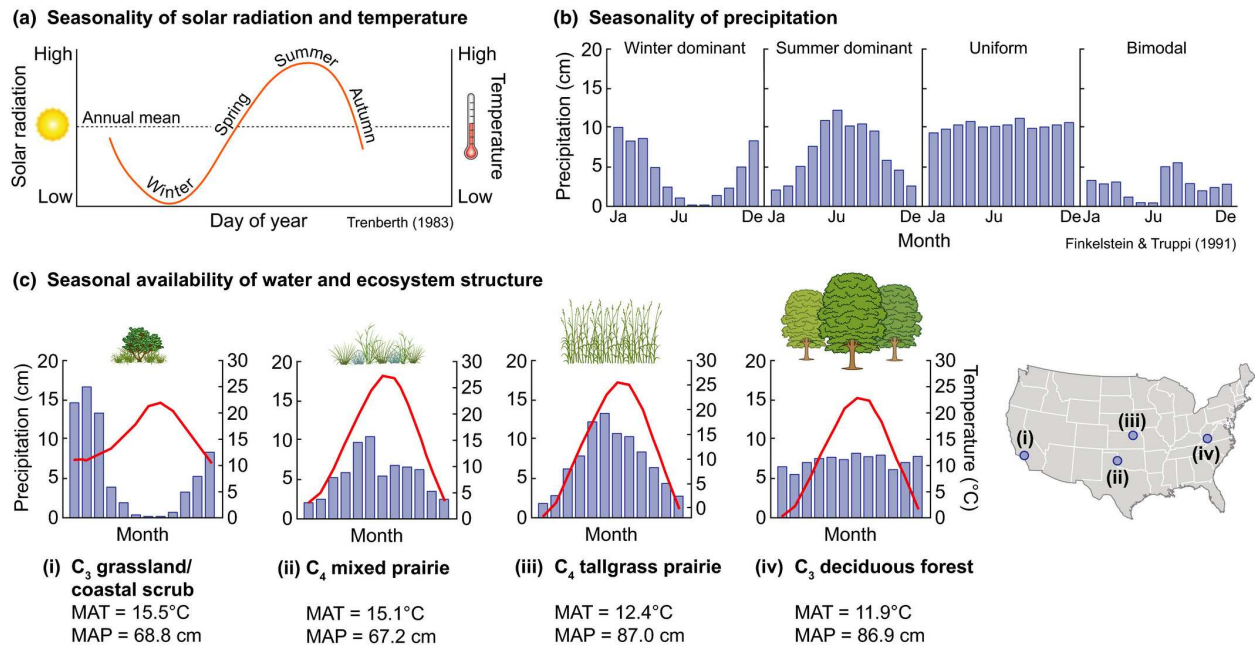


Figure 2.1. (a) Sine wave depiction of the annual time course of solar radiation and air temperature for the four seasons (~90-d periods at mid-latitudes). Whether defined astronomically (solar radiation) or meteorologically (temperature), the general temporal dynamics are similar (with temperature lagging behind solar radiation; see Trenberth, 1983). The horizontal dashed line represents mean annual solar radiation and air temperature. Note that although the amplitude of the sine wave varies, by definition, winter months are below the mean, summer above the mean, and spring and autumn represent transition seasons for most ecosystems. (b) Range of variation in seasonal patterns of precipitation across the United States (after Finkelstein & Truppi, 1991). Shown are a winter-dominated pattern (California), a summer rainfall pattern (Iowa), a uniform pattern (New York) and a bimodal pattern (Arizona). (c) Seasonal patterns of temperature (red line) and precipitation (blue bars) combined for ecosystems that span the US (points on the map correspond to panels from left to right). At monthly timescales, temperature is strongly related to potential evapotranspiration (Tegos et al., 2017), and thus these panels also depict how ecosystem demand for water (red line) corresponds to supply (blue bars). The balance of this supply and demand largely determines the seasonal availability of water for ecosystem functioning (Stephenson, 1990). Note that sites with similar mean annual temperature (MAT) and precipitation (MAP) support dramatically different plant communities and ecosystems, suggesting an important role for the seasonal availability of water. From L to R, sites are Ojai, CA; Black Kettle National Grassland, OK; Konza Prairie, KS; Washington and Jefferson National Forest, VA (climatic data from PRISM, <https://prism.oregonstate.edu/>).

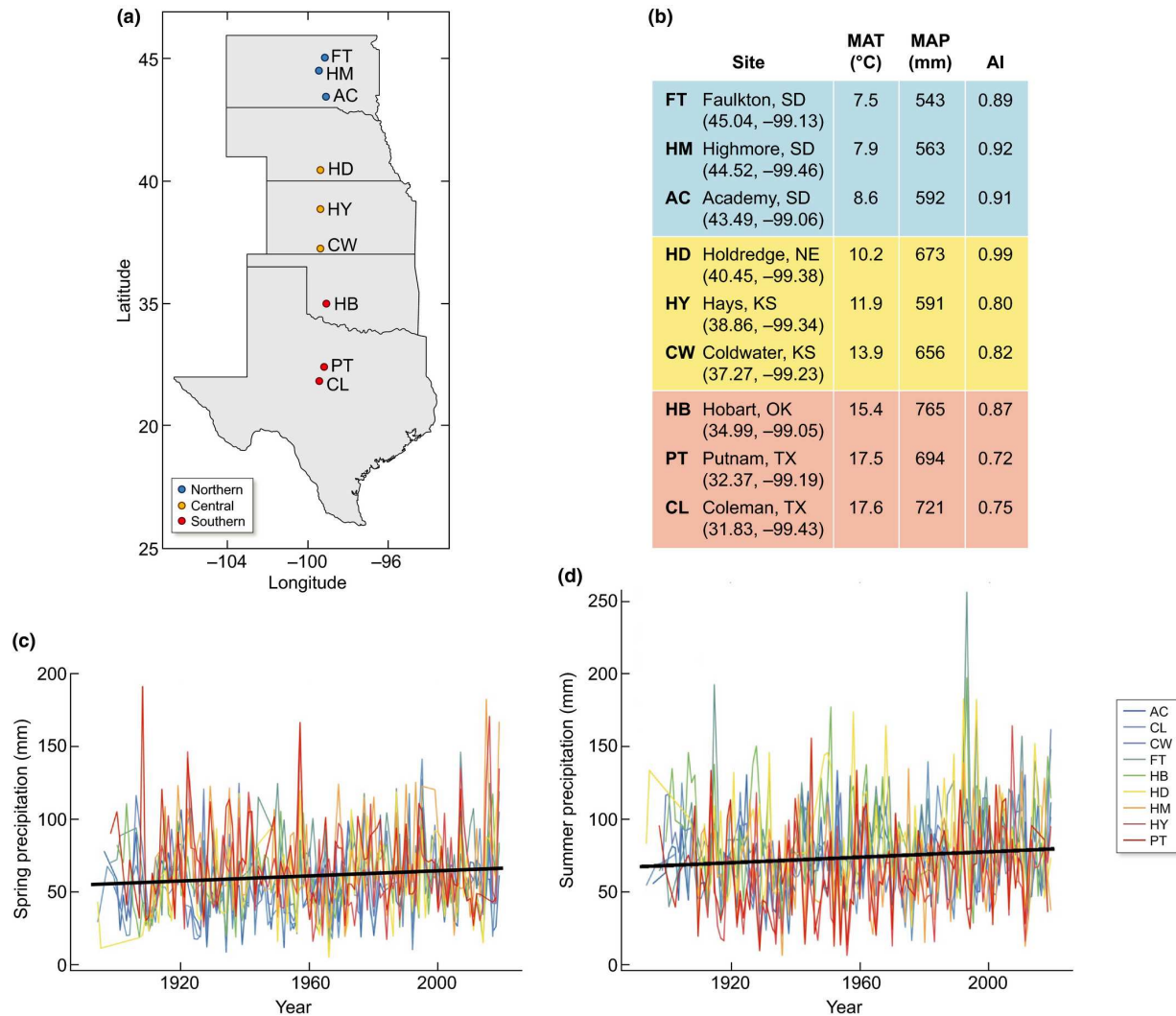


Figure 2.2. (a) Location of nine sites (with > 100-yr monthly weather data, retrieved from <https://www.ncei.noaa.gov/>) selected to represent a N–S transect across the Central US Great Plains. (b) Mean annual temperature (MAT), mean annual precipitation (MAP) and the aridity index ($AI = P/PET$; PET, potential evapotranspiration) for each site. Climate data derived from PRISM 30-yr means (1981–2010; <http://prism.oregonstate.edu>). (c, d) Long-term temporal trends in spring (MAM) and summer (JJA) precipitation for each site. A slight increase ($P < 0.05$) in both spring and summer precipitation is evident across all sites overall (solid black line). However, at the site level, spring precipitation increased significantly at only one site, and summer precipitation increased significantly at two sites. The overall rates of increase did not differ between spring and summer, indicating the relative contribution of spring and summer precipitation to the growing season total precipitation has remained unchanged.

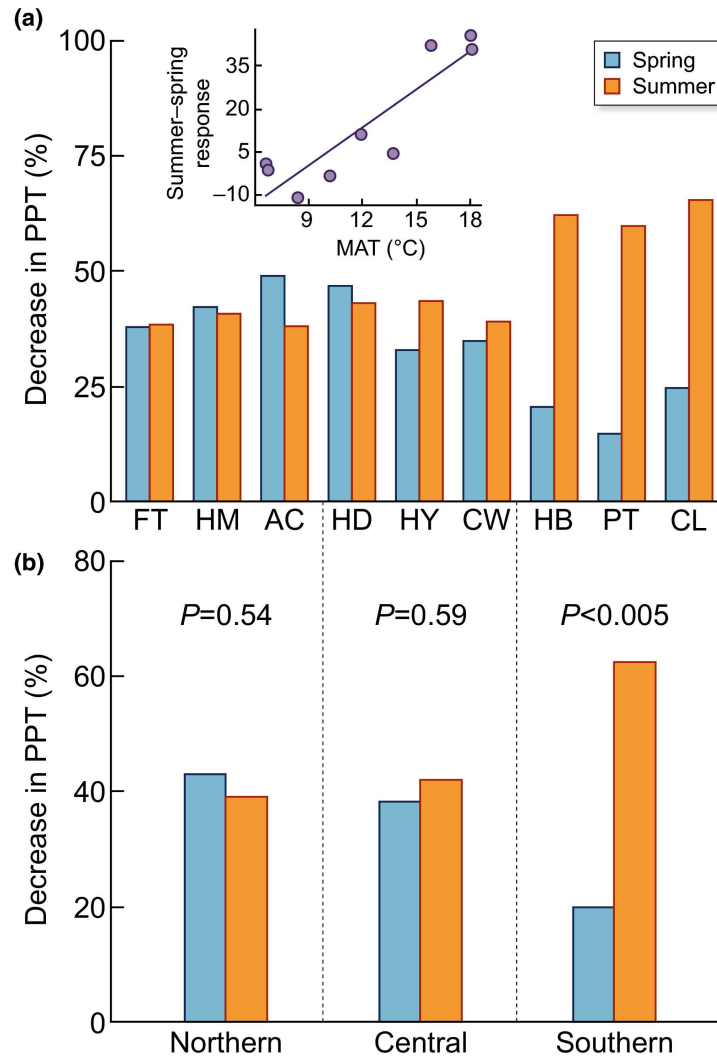


Figure 2.3. How seasonal precipitation (PPT) patterns are altered during extreme drought years across a N–S transect in the US Great Plains (see Fig. 2.1 for site abbreviations). Extreme drought years (approximately eight per site) were defined as those with annual PPT below the 10th percentile of site-based probability distribution functions (NOAA climate data). (a) Comparison of the proportional reduction in PPT from normal during spring (blue) vs summer (orange) seasons. Note that as sites become warmer from N to S, summer PPT is reduced relatively more than spring PPT during extreme drought years (inset). (b) This differential response in summer vs spring PPT suggests that if multiyear extreme droughts become more frequent, as predicted, the seasonal distribution of PPT will be altered substantially in the Southern Great Plains (three-fold greater reductions in summer vs spring).

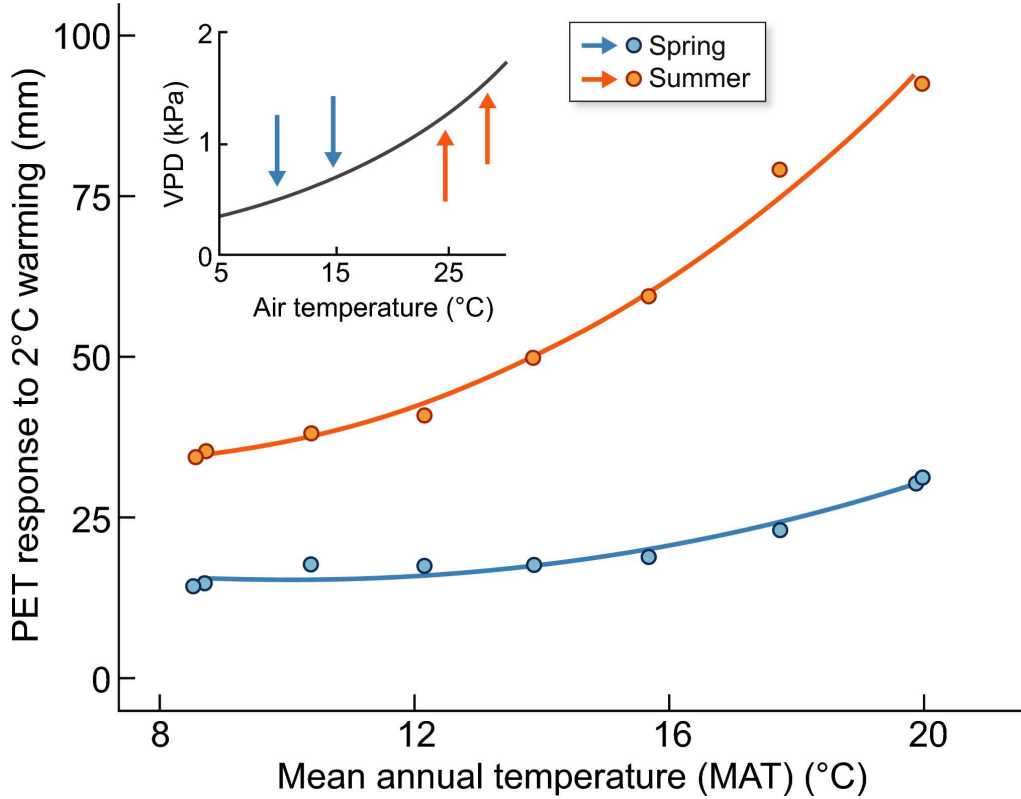


Figure 2.4. Responses in spring (blue) and summer (orange) potential evapotranspiration (PET) to a 2°C increase in temperature across a N–S mean annual temperature (MAT) gradient in the central United States (see Fig. 1). The increase in PET (based on the Thornthwaite (1948) method) was calculated as: $PET_{MAT+2^{\circ}C} - PET_{MAT}$ for each site. Note that even with uniform warming in all months, increases in summer PET are greater than in the spring, and that as MAT increases, the difference between summer vs spring PET increases. Both patterns can be explained by the exponential relationship (inset) between air temperature and the vapor pressure deficit (VPD, Grossiord et al., 2020). Arrows indicate the range of mean spring and summer air temperatures encompassed by the N–S transect (Fig. 1).

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CHAPTER 3: A TEST OF THE SEASONAL AVAILABILITY OF WATER HYPOTHESIS IN A C₃/C₄ MIXED GRASSLAND

3.1 Summary

Understanding how cool-season C₃ and warm-season C₄ grasses will respond to climate change is critical for predicting future grassland functioning. With warming, C₄ grasses are expected to increase relative to C₃ grasses. But, alterations in the seasonal availability of water may also influence C₃/C₄ dynamics because of their distinct seasons of growth. To better understand how shifts in the seasonal availability of water can affect ecosystem function in a northern mixed grass prairie in southeastern Wyoming, we reduced early season rainfall (April – June) using rainout shelters and added the same amount of excluded precipitation during the latter half of the growing season (July-September), effectively shifting spring rainfall to summer rainfall. As expected, this shift in precipitation seasonality influenced patterns of soil water availability in the experimental plots, leading to increased soil respiration in the summer months and sustained canopy greenness throughout the growing season. Despite these responses, there were no significant differences in C₃ aboveground net primary production (ANPP) between the seasonally shifted treatment (SEAS) and the plots that received ambient (AMB) precipitation. This was likely due to the high levels of spring soil moisture present before rainout shelters were deployed that sustained C₃ grass growth. However, in plots with high C₄ grass cover, C₄ ANPP increased significantly in response to increased summer rainfall. Overall, we provide the first experimental evidence that shifts in the seasonality of precipitation, with no change in temperature, will differentially impact C₃ vs. C₄ species, altering the dynamics of carbon cycling and canopy albedo in this extensive semi-arid grassland.

3.2 Introduction

Determinants of the distribution and abundance of C₃ vs. C₄ species have been studied for decades, with the relative success of plants that utilize these different photosynthetic pathways usually related to advantages that C₄ species have at warmer temperatures (Teeri & Stowe, 1976; Ehleringer & Björkman, 1977; Teeri, 1979; Pearcy & Ehleringer, 1984; Epstein et al., 1997; Tieszen et al., 1997; Still et al., 2003; Yamori et al., 2014). But nearly twenty years ago, Winslow et al. (2003) proposed the Seasonal Availability of Water (SAW) hypothesis to explain the global distribution of C₃ and C₄ grasses. This hypothesis emphasized differences in water availability between cool vs. warm seasons and posited that where C₃ and C₄ grasses co-exist, the seasonal partitioning of water is the primary determinant of their abundances and productivity. Simply put, where temperatures and precipitation vary seasonally, climates with greater precipitation inputs during the cool season will favor C₃ species whereas greater precipitation inputs during warmer months will favor C₄ species. At a global scale, the SAW model performed as well as temperature-driven physiologically based models (Collatz et al., 1998), supporting regional-scale conclusions that precipitation seasonality explained C₃/C₄ relative abundances as well or in some cases better than temperature (Paruelo & Lauenroth, 1996; Paruelo et al., 1998; Mitchell & Csillag, 2001). And further, the SAW algorithm was able to approximate the fraction of C₃ and C₄ biomass in mixed grasslands, providing an enhanced understanding of carbon cycling dynamics in these grasslands.

With climate change, the relative abundances and productivity of C₃ and C₄ species are likely to shift in grasslands worldwide (Epstein et al., 2002; Hajek & Knapp, 2022; Havrilla et al., 2022). In particular, a warming climate has been predicted to enhance the success of C₄ species because of their photosynthetic advantages and increased water use efficiency at high

temperatures (Collatz et al., 1998; von Fischer et al., 2008; Morgan et al., 2011; Palmquist et al., 2021; Havrilla et al., 2022). However, temperature and water availability are strongly coupled (Grossiord et al., 2020; Konapala et al., 2020; Padrón et al., 2020), and shifts in the seasonal availability of water due to both rising temperatures and changes to in the hydrologic cycle may lead to unexpected outcomes -- especially since some aspects of climate change, such as drought and exponentially increasing evaporative demand as temperatures increase, may disproportionately affect water availability in the warmest months (Hajek & Knapp, 2022). For example, Knapp et al. (2020) argued that during the 1930s' decadal scale "Dust Bowl" drought, C₃ grasses became more abundant than C₄ grasses in the central US because low precipitation coupled with abnormally high warm season temperatures and evaporative demand reduced water availability more in the summer than the spring. This relative shift in the seasonal timing of water availability reduced the abundance of C₄ grasses in favor of C₃ species, even with warmer temperatures overall. Such changes to seasonal patterns of water availability and resultant shifts in the relative abundances of C₃ and C₄ plants have the potential to alter the phenological dynamics of carbon uptake in ecosystems (Goodin & Henebrey, 1997; Polley et al., 2013; Richardson et al., 2013; Piao et al., 2019; Xie et al., 2022), affect forage production (Barbehenn et al., 2004; Chamaille-Jammes & Bond, 2010), a key ecosystem service provided by grasslands (Bengtsson et al., 2019), and may even impact total levels of primary production (Winslow et al., 2003; Xie et al., 2022). Overall, predicting responses to shifts in the seasonal availability of water is complex, but such changes are likely to be globally pervasive (Chou et al., 2013; Hegerl et al., 2015; Murray-Tortarolo et al., 2017; Padrón et al., 2020).

Despite the potential consequences of shifts in C₃ vs C₄ abundance for grassland structure and function, and support for the SAW hypothesis at the global scale, field experiments that

explicitly assess the sensitivity of ecosystems to shifts in the seasonal dynamics of water availability are lacking, particularly in ecosystems where both C₃ and C₄ grasses coexist. Such experiments are needed to empirically test the SAW hypothesis at local (ecosystem) scales and provide insight into how C₃/C₄ dominated ecosystems might respond to future shifts in the seasonality of water availability, an understudied dimension of climate change (Hajek & Knapp, 2022).

Here we report results from a field experiment designed to evaluate how alterations in seasonal patterns of water availability affected the relative success of C₃ and C₄ grasses, overall productivity, and soil CO₂ efflux (soil respiration) in a semi-arid grassland in Wyoming. We altered precipitation inputs by excluding early season precipitation and then adding an equivalent amount of precipitation during the warmer months to shift the typical seasonal pattern of soil moisture from “spring wet, summer dry” to “spring dry, summer wet” in this native grassland. We expected that this seasonal shift in water availability would significantly alter C₃ vs. C₄ grass production and overall C cycling dynamics, even during a single growing season (Skinner et al., 2002). In particular, we hypothesized that the C₄ grass productivity would increase in the seasonally shifted plots (SEAS; “spring dry, summer wet”) relative to the plots receiving ambient precipitation while C₃ productivity would be reduced relative to ambient plots. Further, the seasonally shifted plots would see overall greater rates of soil respiration because additional moisture during the warmer months would stimulate microbial activity. Such results would support the key role of the seasonality of soil moisture in grasslands, independent of changes in temperature.

3.3 Methods

3.3.1 Site Description

Research was conducted in an undisturbed, mixed grass prairie at the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) High Plains Research Station (41.20, -104.88). In this native grassland, mean annual precipitation is 415 mm, and mean annual temperature is 7.9 °C (Griffin-Nolan et al., 2018). Approximately 70% of the annual precipitation occurs during the growing season (Apr. - Aug.; Fig. A1.1) with Apr.-June typically the wettest period. Long-term average aboveground net primary production (ANPP) is approximately $\sim 140 \text{ g m}^{-2}$ (Griffin-Nolan et al., 2018), and although the site has a history of moderate levels of livestock grazing, the area where this experiment was conducted had been protected from large ruminant grazing since 2013.

We selected this grassland for several reasons. First, although it is dominated by C_3 perennial grasses such as *Pascopyrum smithii* and *Hesperstipa comata*, C_4 grasses (primarily *Bouteloua gracilis*) are widespread, and there are areas where they co-dominate. Second, the ratio of C_3/C_4 grasses has been shown to respond to precipitation manipulations (Skinner et al., 2002), including extreme drought (Knapp et al., 2020). And finally, long-term productivity records suggest that C_3/C_4 ratios at this site are sensitive to interannual variability in precipitation seasonality (Fig. 3.1). For example, in years when the relative proportion of spring precipitation is higher than usual, the ratio of C_3/C_4 ANPP tends to increase, with the opposite response evident after relatively dry springs. Of course, other climatic attributes (i.e., temperature, previous year's precipitation) vary during these years in addition to precipitation seasonality, which motivated us to conduct an experiment that isolated the influence of the seasonality of water availability.

3.3.2 Experimental Design

Before the 2021 growing season, we established twenty 1 m² plots (n=10 per treatment). Plots were separated by at least 3 m, and aluminum flashing was installed (10 cm belowground and 5 cm aboveground) 20 cm outside of the plot perimeter to reduce surface and shallow soil water movement into and out of each plot. Rainout shelter roofs (2.44 m × 3.05 m made of clear corrugated polycarbonate, Suntuf, Palram Americas) that were larger than the 1 m² plots were then placed over ten of the plots. Roofs were initially installed 80 cm above the ground at a slight angle to allow water to drain away from the plot; later in the season, the shelters were raised to 100 cm. Although previous work has demonstrated that these shelters have minimal influence on the microclimate (Loik et al., 2019; Post & Knapp, 2020; Hoover et al., 2022), we monitored soil temperatures at 10 cm weekly and evaluated light transmission under the roofs using a 1-m linear quantum light sensor (Decagon AccuPAR, model LP-80).

We altered the seasonal dynamics of soil water availability (seasonally shifted treatment, SEAS) by using the clear roofs to exclude all precipitation from April 10 – June 30 (some minor blow-in of precipitation during storms was inevitable). We removed the shelters July 1 and added the equivalent amount of water excluded in the spring to these plots in addition to the ambient precipitation received. The additional precipitation was applied manually throughout July – September replicating the distribution of rainfall events from the spring (*i.e.*, additions were similar in event frequency and magnitude, Figure A1.2). This ensured that total growing season precipitation was similar for the SEAS and the ambient (AMB) treatment, which received natural precipitation for the entire growing season. Precipitation was recorded at a nearby NOAA weather station (Cheyenne Weather Forecast Office, 41.1516, -104.80622). Treatments (n=10) were randomly assigned, and because there was some minor topographical variation in the

landscape (10 plots were slightly uphill from the others), we assigned treatments within two blocks to control for any effects topography. Block effects were non-significant in our analyses, but after plants became active, we noted that the cover of C₃ and C₄ functional groups varied widely among plots. Thus, we estimated total plant cover by species in each plot to account for this variation in our analyses.

3.3.3 Measured Responses

We measured soil moisture (volumetric water content, %VWC) weekly in all plots throughout the experiment (April 10 – September 23) with a 20 cm handheld soil moisture time-domain reflectometry probe (Campbell-Scientific Hydrosense II). This instrument integrates soil moisture in the top 20 cm of soil where most of the root biomass in this grassland is located (Sun et al., 1997; Carillo et al., 2014). To assess treatment effects on plant water status, we estimated mid-day (12:00 - 14:00hr MST) leaf water potential with a Scholander pressure chamber (PMS instruments) for a dominant C₃ grasses, *Pascopyrum smithii*, and the dominant C₄ grass, *Bouteloua gracilis*. Fully expanded, mature canopy leaves (1-2 leaves per plot, n=6 per treatment) were collected each week. Because the C₃ and C₄ grasses become active at different times of the growing season, we measured *P. smithii* water status from May 27 – Sept. 16 and *B. gracilis* from June 15 - Sept. 16.

To evaluate how differences in the seasonal availability of water influenced C₃ and C₄ dynamics and ecosystem function, we measured canopy greenness and soil CO₂ efflux weekly, photosynthetic rates for the primary C₃ and C₄ species in June and July, and ANPP at the end of the growing season (September).

Canopy greenness, measured to assess canopy-scale phenological responses and to serve as a proxy for potential ecosystem carbon uptake, was estimated with repeat digital photography

(following the methods of Post & Knapp, 2020, Hoover et al., 2022). Briefly, an iPhone camera was positioned directly above a marked 50 cm x 50 cm frame in a corner of each plot, and each photograph was then cropped to contain only the interior area of the frame. These cropped photos were processed using the R package EBImage (Pau et al., 2010) to calculate the average green chromatic coordinate (GCC) index (Filippa et al., 2016). The GCC index accounts for variation in pixel brightness (Filippa et al., 2016), thus avoiding background light levels and potential infrastructure impacts.

Soil respiration was measured weekly (May 5 – September 23) to quantify how the treatments influenced this important carbon flux (Hashimoto et al., 2015). Permanent PVC collars (10 cm in diameter, n=6 per treatment) were installed in locations between grasses at the end of April (2.4 cm belowground and 2 cm aboveground), and all vegetation within the collars was removed (clipped at the base). Before each measurement, any new vegetation growth was also gently removed. Soil respiration was measured using a 6400-09 soil flux chamber attached to an LI-6400XT (LiCor., Inc, Lincoln NE, USA). Measurements were taken mid-day (between 8:30hr – 12:30hr MST) at ambient CO₂ concentration, humidity, and temperature.

Leaf gas-exchange was measured (June 23-24) prior to roofs coming off and after the roofs were removed (July 24-25). On each date, a portable photosynthesis system (LI-6400, LiCor., Inc, Lincoln NE, USA) was used to measure the CO₂ uptake (net photosynthesis, or A) on 12 fully expanded mature upper canopy leaves for both C₃ (*P. smithii*) and C₄ (*B. gracilis*) individuals in each treatment.

The LI-6400 was fitted with a 3×2 cm cuvette head and a red-blue LED light source. For all measurements, flow rate was held constant at 600 μmol s⁻¹. The temperature exchanger was set to an average midday summer temperature of 30 °C. Leaf temperature (T_{leaf}) was measured

with a thermocouple and averaged 31 ± 1.7 °C (standard deviation) across all measurement dates. Relative humidity conditions in the chamber were controlled near ambient levels but did vary slightly depending upon water vapor fluxes from the leaf. Photosynthetic photon flux density in the chamber was set at $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$, approximating full-sun conditions to measure light-saturated net photosynthesis (A_{sat}) and stomatal conductance to water vapor (g_s , Fig. A1.3) at a chamber reference $[\text{CO}_2]$ of $420 \mu\text{mol mol}^{-1}$. All measurements occurred between 10:00hr and 15:00hr MST

Finally, ANPP was estimated near the end of the growing season (Sept. 1-2) in all plots as plants began to senesce. For each plot, all aboveground vegetation within two 0.1 m^2 subplots was harvested to ground height, sorted by functional group (C_3 grass, C_4 grass, forb, woody, or annual grass), and then dried at 60 °C for 48 hours before being weighed to the nearest 0.01g. Previous year's growth was easily distinguished from current year growth and was not included.

3.3.4 Statistical Analyses

All analyses were performed using R (version 4.1.3). For 20 cm soil moisture, GCC, water potential, and soil respiration data, we used a repeated measures mixed model analysis of variance (lme4 package, Bates et al., 2015) with treatment and sampling date as fixed effects, plot as a random effect, and an interaction term between date and treatment. Block was originally included, but due to a lack of statistical significance, it was excluded from the final models. We also used *t*-tests to compare 20 cm soil moisture, GCC, water potential, and soil respiration measurements between treatments for both periods of the experiment – before and after roof removal. For ANPP measures, we used an ANCOVA with percent C_4 cover as a categorical variable to account for the variation in C_3/C_4 cover. High C_4 cover was defined as plots with C_4 cover greater than 25% ($n=3$ for AMB and $n=4$ for SEAS), with the remaining plots considered

low C₄ cover (< 5%, n=7 for AMB and n=6 for SEAS). This categorical predictor was used in conjunction with treatment to test the difference in total ANPP as well as functional group differences, namely C₃ and C₄ ANPP. To evaluate significant differences among these groups, we calculated *post-hoc* Tukey's pairwise differences.

3.4 Results

3.4.1 Environmental Context

Based on long-term historical records (PRISM Climate Group, 2022), spring and early season precipitation in 2021 was higher than normal (193 mm in 2021 versus 163 mm average for Apr. – June; Fig. A1.1). These wet conditions were then followed by a dry warm season period (46 mm in 2021 versus 139 mm average for July – Sept.; Fig. A1.1). Not only was the early season particularly wet, but March was also exceptionally wet compared with long-term records, totaling nearly 89 mm in 2021 compared to a normal of 22 mm. For the duration of the experiment (Apr. 10 – Sept. 23; Fig. A1.1), total precipitation was ~240 mm for both treatments. When roofs were in place, Apr. 10 - June 30, total precipitation was 193 mm. Subsequently, we manually applied 193 mm of water over 14 events (average event size of added water was 14 ± 10 mm) to the SEAS plots from July 1 – Sept. 23 in addition to the ambient precipitation received during this time (46 mm). Thus, the SEAS plots received all 240 mm of precipitation during the latter half of the growing season, while the AMB plots received 193 mm of precipitation during the first half and only 46 mm during the second (Fig. A1.2).

Due to the exceptionally wet March, soil moisture levels were already high when the shelters were initially deployed. Despite the antecedent soil moisture conditions, the shelters were effective at preventing most precipitation from Apr. 10 – June 30, however, there was evidence of some blow-in, particularly with late spring snowstorms (see soil moisture data, Fig.

3.2). Soil temperature was slightly elevated under the shelters during the spring (Fig. 3.4 inset), and light transmission was $81.1 \pm 1.3\%$ of ambient light, which corresponds with values observed previously (Post & Knapp, 2019).

3.4.2 Treatment Effects

Both treatments had similar soil moisture levels at the beginning of the experiment in the upper 20cm (AMB: $29.0 \pm 2.0\%$, SEAS: $26.5 \pm 1.6\%$ (standard error), Fig. 3.2). However, soil moisture levels quickly diverged during the spring such that mean soil moisture in the AMB treatment was $29.4 \pm 0.7\%$ vs. $20.8 \pm 0.6\%$ for the SEAS treatment during the period that shelters were in place (Apr. 10 – June 30). After roofs were removed (June 30) and water additions began, soil moisture increased in the SEAS treatment as expected (Fig. 3.2); average soil moisture levels for the warm season period of the experiment were $15.4 \pm 0.6\%$ for SEAS vs. $11.9 \pm 0.4\%$ for AMB. Overall, our experiment was successful in altering seasonal soil moisture patterns significantly (Fig. 3.2, Table 3.1).

Mid-day leaf water potentials provide additional insight into how soil moisture levels translate into potential plant stress (Rodriguez-Dominguez et al., 2022; Fig. 3.3). As expected, mid-day leaf water potential values were generally less negative during the spring cool season than the warmer summer period. Averaged over the spring and prior to shelter removal, leaf water potentials for the C₄ grass, *B. gracilis*, did differ not between treatments; however for *P. smithii*, water potentials were significantly more positive in the AMB than SEAS plots in the spring ($P = 0.0006$). After the shelters were removed, and as expected, mid-day leaf water potentials for both the C₃ and C₄ grasses in the SEAS treatment were less negative than the AMB treatment (Fig. 3.3), indicating more favorable water status in both grasses when the summer period was wetter ($P < 0.0001$ for both treatments).

Plant photosynthetic responses to the seasonal shift in soil water content differed based on functional group (Fig. 3.4 inset, Fig. A1.3). The C₃ grass *P. smithii* responded as expected; light-saturated net photosynthesis (A_{sat}), stomatal conductance to water vapor (g_s), and the maximum rate of electron transport for RuBP regeneration (J_{max}) were all significantly higher in the AMB plots in June. After removal of the roofs and the addition of water to the SEAS plots, these leaf-level physiological measurements increased in the SEAS vs. AMB plots for *P. smithii*. The C₄ grass *B. gracilis* did not respond as expected. Instead, this species had higher A_{sat} , g_s , and J_{max} in both June and July in the SEAS plots. This unexpected June response may be attributable to the slight warming effect of the shelters and overall high spring soil moisture levels even in the SEAS plots.

The dynamics of canopy greenness were consistent with these physiological responses (Fig. 3.4). Canopy greenness for both treatments was similar at the beginning of the growing season, and we were able to capture the initial green-up for the growing season, which was primarily driven by the C₃ grasses. The SEAS treatment plots had a marginally enhanced green-up compared with the AMB treatment, and greenness remained higher in the SEAS plots relative to the AMB plots for most of the cool spring period, attributable to the slight elevation in temperatures beneath shelters during this time (Fig. 3.4 inset). After roof removal, temperature differences diminished between the treatments, but greenness in the SEAS plots subsequently increased markedly in response to the warm season soil moisture increases. At the end of season, the two treatments converged, but the SEAS plots still showed slightly higher greenness levels than the AMB treatment. Despite these apparent differences, a repeated measures mixed model ANOVA for the two treatments indicated significance only for sampling date and not treatment (Table 3.1). However, if average greenness prior to and following shelter removal are assessed

separately, there were significant differences between treatments, with the SEAS plots particularly greener than the AMB plots during the warm season (after shelter removal: $P < 0.0001$).

Despite differences in soil water patterns, canopy greenness, and plant physiological responses, there were no significant differences in total ANPP between the AMB and SEAS treatments (AMB = $158.2 \pm 12.9 \text{ g m}^{-2}$, SEAS = $184.7 \pm 14.6 \text{ g m}^{-2}$; $P = 0.19$, Fig. 3.5). Total ANPP values were primarily driven by C_3 ANPP which accounted for ~75% of total ANPP. However, there was a wide range in the relative abundances of C_3 and C_4 grasses across all plots; C_4 grasses in particular ranged from <5% to ~70% cover among plots. Because these divergent C_3/C_4 ratios were not obvious when plots were established, and because it is unreasonable to expect C_4 grass responses in plots with few C_4 grasses, we *post-hoc* categorized the plots into either high (> 25%, 7 plots) or low (< 5%, 13 plots) C_4 cover to better understand C_3 vs C_4 ANPP responses to the treatments. Even when accounting for variability in C_4 cover, there were no significant differences in C_3 ANPP between the AMB and SEAS treatments, although C_3 grass production was slightly higher in the SEAS plots. However, in plots with high C_4 cover, C_4 ANPP was significantly greater in SEAS plots than AMB plots (AMB = $32.75 \pm 7.4 \text{ g m}^{-2}$, SEAS = $58.81 \pm 12.9 \text{ g m}^{-2}$; $P = 0.013$).

Finally, soil CO_2 efflux patterns mirrored seasonal trends in soil moisture. Soil respiration was reduced in the SEAS treatment in the early season compared to the AMB treatment but became elevated later in the growing season (Fig. 3.6). When averaged across the two treatment periods, soil CO_2 efflux in the AMB treatment was significantly greater than the SEAS treatment in the first half of the growing season ($P < 0.0001$), but during the second half, the SEAS treatment had higher rates of soil CO_2 efflux ($P < 0.0001$), leading to overall greater losses of

soil CO₂ for the SEAS plots during the growing season ($P = 0.0017$; Fig. 3.6). Soil respiration peaked for the AMB treatment near the end of May, and then slowly declined throughout the rest of the growing season, a trend that corresponds to the observed soil moisture patterns. For the SEAS treatment, soil respiration peaked near the end of July when temperatures were greatest and soil moisture values were high. Further, the maximum rates of soil respiration were greater in the SEAS treatment than the AMB treatment (AMB= $4.7 \pm 0.85 \mu\text{mol m}^{-2}\text{s}^{-1}$, SEAS= $5.9 \pm 1.5 \mu\text{mol m}^{-2}\text{s}^{-1}$), indicating enhanced CO₂ loss during the warmer months. A repeated measures mixed model ANOVA for the two treatments indicated that treatment, date, and their interaction were all significant (Table 3.1).

3.5 Discussion

With climate change, shifts in the seasonal availability of water are likely to arise from a variety of mechanism (Stephenson, 1990; Hajek & Knapp, 2022). Because ecosystems with co-occurring C₃ and C₄ species are potentially sensitive to changes in the seasonal availability of water (Paruelo & Lauenroth, 1996; Paruelo et al., 1998; Skinner et al., 2002, Knapp et al., 2020, Havrilla et al., 2022), our objective was to experimentally test the Seasonal Availability of Water (SAW) hypothesis as proposed by Winslow et al. (2003). Thus, we directly manipulated the seasonality of water availability in a semi-arid, C₃/C₄ grassland for an entire growing season. Although our results are from a single site, implications from this study may extend further given that ecosystems with co-occurring C₃ and C₄ species are globally widespread (Still et al., 2003; Xie et al., 2022). Importantly, although much is known about the physiological, phenological and ecological traits of C₃ vs C₄ species (Ehleringer et al., 1997; Epstein et al., 1997; Tieszen et al., 1997; Collatz et al., 1998; Winslow et al., 2003), this study provides, to our knowledge, the first experimental test of the SAW hypothesis.

Overall, our results support the Seasonal Availability of Water hypothesis; a shift in the seasonal availability of water did affect the relative dynamics of C₃ and C₄ grasses and ecosystem functioning overall. Our treatments imposed contrasting patterns of soil moisture; the AMB plots had higher levels of water availability in the spring and much reduced soil water in the summer, whereas the addition of ~ 200 mm of precipitation to SEAS plots in the summer led to a substantial increase in soil moisture later in the growing season (Fig. 3.2). However, because of the above-average precipitation inputs at this site in the late winter/spring (March precipitation was >4-fold the historical average, March through May precipitation was ~ 175% of average), average soil water in the SEAS treatment in the spring was still higher than in the summer. In other words, while the rain-out shelters did reduce spring soil water in the SEAS vs. AMB plots, these levels were still relatively high compared to summer values. Thus, our treatments were AMB = “spring very wet, summer dry”, and SEAS = “spring moderately wet, summer wet”.

With these seasonal patterns, we found that for plots with substantial C₄ grass cover, C₄ production was significantly greater in the SEAS plots, indicating that enhanced water availability during the warmer period increased C₄ growth. In contrast, neither total ANPP nor C₃ ANPP differed significantly between the treatments, even when accounting for C₄ cover. This lack of response is likely a result of the extremely wet spring conditions for all plots. Similarly, mid-day leaf water potentials suggest that neither the C₃ nor the C₄ grasses in either treatment were water limited during the spring (Fig. 3.3), even though leaf water potential in the C₃ grass was reduced in the SEAS plots. Although not significant, the SEAS plots showed both an earlier and enhanced green-up compared with the AMB plots, likely driven by slightly warmer soil temperatures underneath the shelters. Since green-up in these grasslands is modulated by both temperature and soil moisture (Zhu & Meng, 2015; Post et al., 2022; Ren et al., 2022), once key

temperature thresholds are passed and there is sufficient moisture, plants can respond quickly (Moore et al., 2015). Thus, since the rainfall shelter infrastructure led to slightly warmer soil temperatures with sufficient soil moisture, the SEAS plots were able green-up slightly sooner in the spring.

In contrast to the wet spring, summer conditions were warm and dry, allowing for an amplification of treatment effects on soil moisture and greenness. Although maximum canopy greenness values were similar between treatments overall, the SEAS plots had an extended peak in greenness by several weeks relative to the AMB plots because of the seasonally shifted precipitation regime. Somewhat surprisingly, these differing phenology patterns did not translate into greater total ANPP for the SEAS treatment. Total ANPP was largely driven by C₃ ANPP at the study site ($76 \pm 6\%$ of total ANPP was C₃ production across all plots), and as noted above, C₃ ANPP did not show significant responses to the treatments despite reductions in their physiological status in SEAS vs. AMB treatments in the spring (Fig. 3.5). This is consistent with previous studies that indicate that C₃ production in grasslands has low sensitivity to warm-season precipitation levels (Heitschmidt et al., 1999; Skinner et al., 2002; Frank, 2007; von Fischer et al., 2008; White et al., 2014; Wilcox et al., 2015; Havrilla et al., 2022). However, responses in C₄ grasses were largely supportive of the SAW hypothesis. An increase in warm season soil moisture resulted in greater C₄ grass productivity (Fig. 3.5). Indeed, C₄ grasses in the SEAS treatment were less water-stressed and had higher rates of photosynthesis during their peak growing season compared to the AMB treatment (Fig. 3.4, Fig. A1.3).

Shifts in the seasonal timing of water availability due to climate change will likely alter the carbon balance of grasslands irrespective of C₃ vs C₄ responses because soil respiration, the primary flux of carbon out of grasslands, is sensitive to the timing and amount of precipitation

(Chen et al., 2008; Huxman et al., 2004; Munson et al., 2010; Song et al., 2012; Petrie et al., 2018; Post & Knapp, 2019; Hoover et al., 2022). Although we only saw moderate differences in ANPP with this experiment, we did find that soil CO₂ efflux responded very strongly to shifts in the seasonal availability of water (Fig. 3.6). The rates of soil respiration were highest in the SEAS treatment during the summer likely because warmer conditions in the summer coupled with increased soil moisture stimulated microbial activity and thus, increased soil respiration. We suspect that microbial activity may have contributed more to this increase in soil respiration for the SEAS treatment during the summer than root respiration because belowground productivity is typically less responsive to precipitation manipulations in this system (Wilcox et al., 2015) and warmer temperatures are a key driver for microbial activity (Li et al., 2018). Regardless, since a large portion of the carbon stored can be released with soil respiration in these grasslands (Pendall et al., 2013; Yan et al., 2014; Hashimoto et al., 2015; Song et al., 2019), changes to the timing of water availability are likely to alter the carbon storage of this ecosystem.

Overall, understanding grassland responses to shifting seasonal patterns of water availability is critical to predicting how these ecosystems will respond to climate change. Changes in the relative abundances of C₃ and C₄ grasses can lead to long-term changes in grassland function because C₃ and C₄ grasslands differ in their phenology (Goodin & Henbrey, 1997; Piao et al., 2019), production potential (Winslow et al., 2003), forage quality (Barbehenn et al., 2004; Chamaillé-Jammes & Bond, 2010), and seasonal patterns of albedo (Richardson et al., 2012; Polley et al., 2013; Havrilla et al., 2022; Xie et al., 2022). These differences can alter land-atmosphere feedbacks, affecting carbon, energy, and water cycling (Cowling et al., 2007; Richardson et al., 2013; Xie et al., 2022), which becomes especially important when considering grasslands' role in the global carbon sink (Poulter et al., 2014; Ahlström et al., 2015; Le Quere et

al., 2017). Using the SAW Hypothesis as a framework, we provide direct evidence that shifts in the seasonal timing of water availability can affect ecosystem structure and function, particularly the functioning of C₃ and C₄ grasses, independent of temperature changes. Further, this study indicates that seasonal changes resulting from climate change may have outsized impacts on function because many ecosystem functions, particularly carbon cycling, are tightly linked with precipitation falling at certain times of the year (Milchunas et al., 1994; Huxman et al., 2004; Parton et al., 2012; Song et al., 2019; Hoover et al., 2022) and impacts from seasonal shifts may not be evident from annual trends. There is still a need for more studies to evaluate the role of seasonality on ecosystem function, particularly effects on C₃ and C₄ dynamics over multiple growing seasons. This study only considered portions of the carbon balance for this ecosystem and a more complete evaluation of the carbon cycling consequences of shifts in seasonal patterns of water availability is needed.

3.6 Tables

Table 3.1 Summary of the repeated measures mixed model ANOVA results for soil moisture, canopy greenness, and soil respiration with seasonality treatment, date, and their interaction as factors. Reported values include the degrees of freedom (numerator, denominator), the F-statistic, and the p value. Significant p values are bolded.

Variable	Soil moisture			Greenness			Soil respiration		
	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
Treatment	1, 18	3.08	0.096	1, 421	0.0078	0.93	1, 255	39.22	<0.0001
Date	27, 474	114.07	<0.0001	1, 478	8.28	0.0042	1, 246	53.26	<0.0001
Treatment x date	27, 474	28.01	<0.0001	1, 478	2.43	0.12	1, 246	48.43	<0.001

3.7 Figures

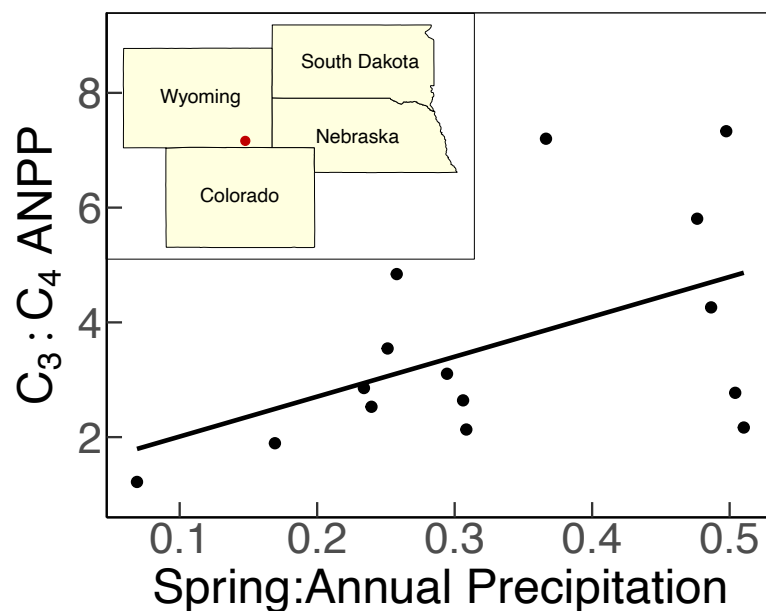


Figure 3.1. Linear regression between the ratio of $C_3:C_4$ grass aboveground net primary production (ANPP) and the proportion of spring precipitation (Mar. – May) to annual precipitation ($P=0.05$). Each point represents a year from 2004-2019. Long-term precipitation and productivity records taken from sites nearby (precipitation: Cheyenne Weather Forecast Office, (41.1516, -104.80622); productivity: USDA-ARS High Plains Research Station). Inset shows the location of the grassland site where these data were collected and where precipitation seasonality was manipulated.

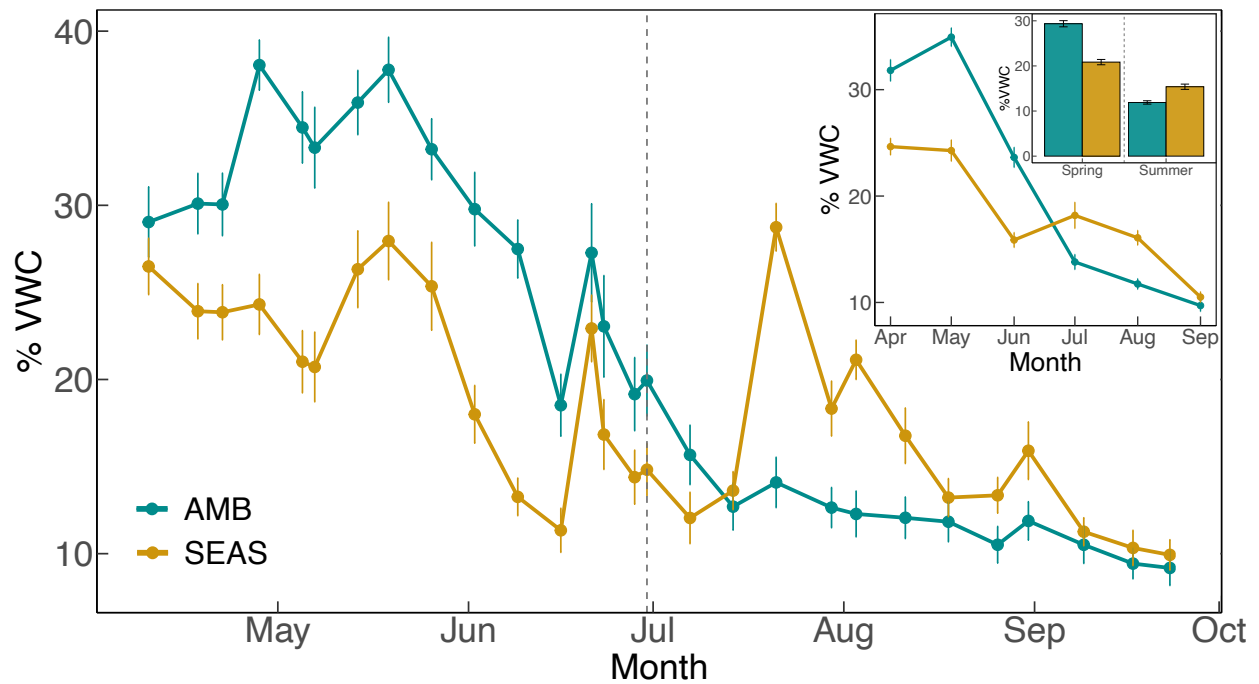


Figure 3.2. Seasonal patterns of soil moisture (%VWC) in response to altered timing of precipitation inputs. The hashed line at the end of June represents the timing for the removal of roofs. Filled circles represent mean daily soil moisture $\pm$ the standard error for each treatment (ambient (AMB) = orange, seasonally shifted (SEAS) = teal). The line graph inset shows monthly soil moisture means ($\pm$ SE) for each treatment. The inset bar graph shows average soil moisture for the early season (April 10 – June 30, $P < 0.001$ between treatments) and late season periods (July 1 – Sept. 23, $P < 0.0001$).

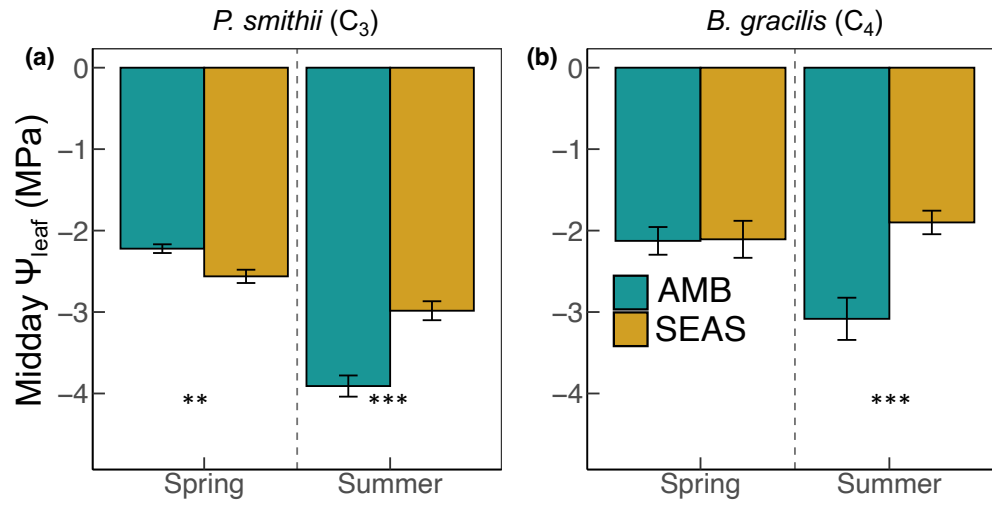


Figure 3.3. Mid-day leaf water potentials of dominant a) C₃ (*Pascopyrum smithii*) and b) C₄ (*Bouteloua gracilis*) grasses prior to and after roof removal. Bars represent average leaf water potentials $\pm$ standard error over different periods during the growing season (ambient (AMB) = orange, seasonally shifted (SEAS) = teal). Asterisks denote statistically significant differences in leaf water potential (** indicates $P < 0.001$, *** indicates $P < 0.0001$).

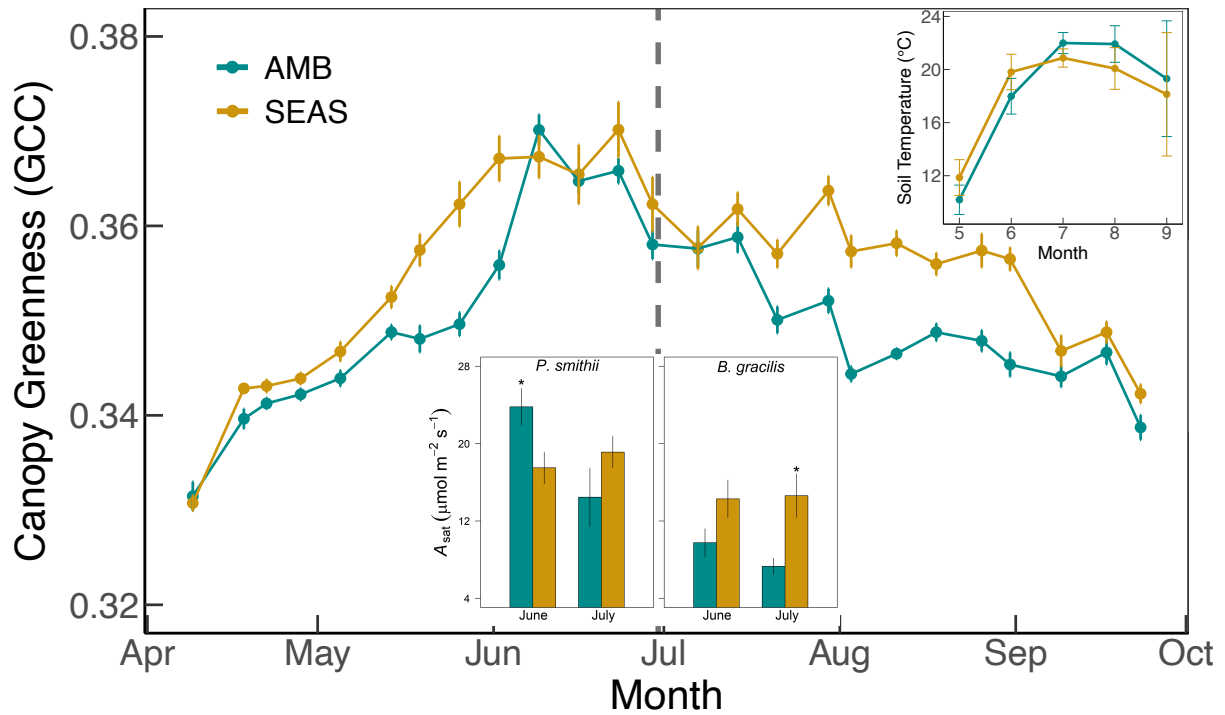


Figure 3.4. Growing season patterns in canopy greenness (green chromatic coordinate index (GCC), see text) for ambient (AMB) rainfall patterns and seasonally shifted patterns (SEAS) with a shift towards greater precipitation inputs in the summer months. The hashed line at the end of June represents the timing for the removal of roofs and beginning of water additions for the SEAS treatment. Filled circles represent average daily canopy greenness $\pm$ the standard error for each treatment. Center inset shows mean ($\pm$ standard deviation) A_{sat} , light-saturated net photosynthesis, values for *P. smithii* and *B. gracilis* between the treatments before and after shelter removal. Significance denoted by asterisks (* indicates $P < 0.05$). Upper right inset shows soil temperature ($^{\circ}\text{C}$) averaged monthly for the growing season.

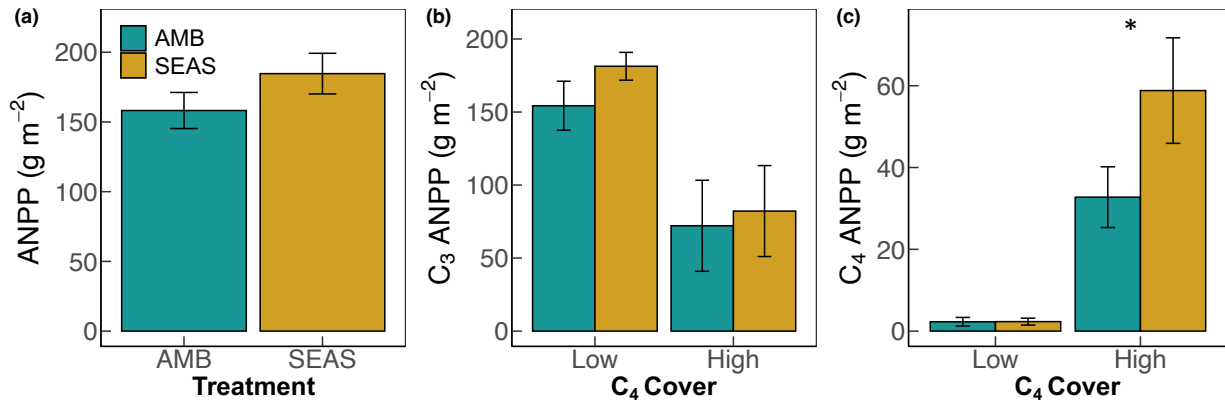


Figure 3.5. Response of (a) total, (b) C₃, and (c) C₄ aboveground net primary production (mean $\pm$ SE) to different patterns of seasonal water availability (ambient (AMB) = orange, seasonally shifted (SEAS) = teal). C₃ and C₄ ANPP categorized by %C₄ cover where low corresponds to < 5% and high > 25% cover. Asterisk denotes $P < 0.05$ ($P = 0.013$ for C₄ ANPP in plots with high C₄ cover).

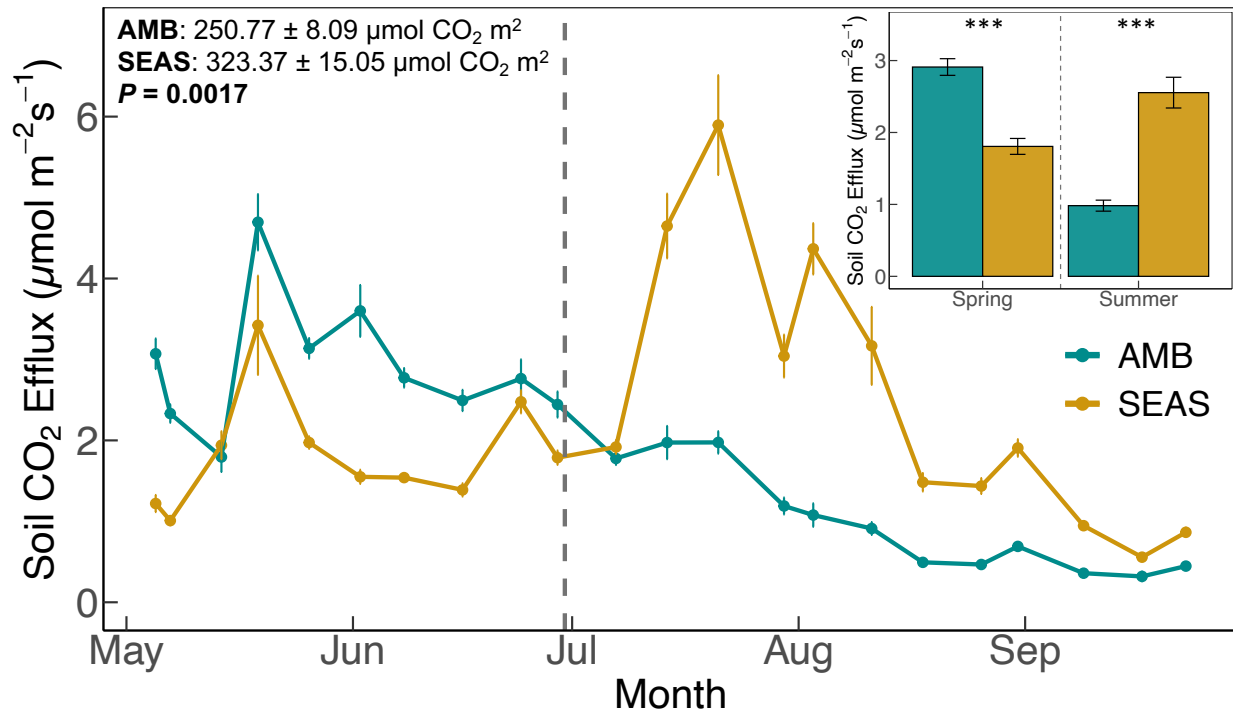


Figure 3.6. Differences in soil CO₂ efflux over the growing season between the different treatments. Mean $\pm$ standard error for each treatment (ambient (AMB) = orange, seasonally shifted (SEAS) = teal) by day. Hashed line represents timing of roof removal for the SEAS plots. Total soil CO₂ loss over the experimental period (May 5 – Sept. 23) as represented by area under the curve is shown in upper left; P-value shows significance from a two sample t-test. Inset depicts the mean $\pm$ standard error for the ten sampling dates before shelter removal (spring) and after (summer). Asterisks indicate significance (***) denotes $P < 0.0001$).

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CHAPTER 4: SIGNATURE OF AUTUMN DELUGES REVEALED DURING SPRING DROUGHT IN A SEMI-ARID GRASSLAND

4.1 Summary

Of the many dimensions of climate change forecasts, an increase in extremely large precipitation events (deluges) and shifts in seasonal patterns of water availability will both have important consequences for ecosystem function, particularly in water-limited regions. While previous work in the semi-arid shortgrass steppe in northeastern Colorado has demonstrated this ecosystem's strong sensitivity to deluges during the growing season, our understanding of ecosystem responses to deluges during the dormant season, which also shift seasonal patterns of water availability, is limited. Here, we imposed experimental 100 mm deluges (~ 30% of mean annual precipitation) in either September or October in a native C₄ dominated shortgrass steppe ecosystem to evaluate the impact of this post-growing season shift in water availability in the autumn as well as during the following growing season. Soil moisture for both deluge treatments remained elevated compared with ambient levels in the autumn and through April as spring precipitation was atypically low. Despite overall low levels of productivity due to spring drought, the previous autumn deluges led to significant increases in aboveground net primary production (ANPP), primarily driven by increases in C₄ grasses. C₃ ANPP was also enhanced, but it was largely due to an increase in the annual C₃ grass, *Vulpia octoflora*, in the October deluge treatment. While spring precipitation levels have historically been the primary determinants of ecosystem function in this semi-arid grassland, we posit that these compound climate extremes – an extremely wet autumn followed by a naturally-occurring spring drought – revealed the potential for meaningful carryover effects from autumn precipitation as well. With climate change increasing the likelihood of extreme events during all seasons, experiments

which create novel climatic conditions can provide new insights for understanding the controls of ecosystem functions in the future.

4.2 Introduction

For most terrestrial ecosystems, climate change is expected to alter both the availability of water (e.g., Jung et al., 2010; Collins et al., 2013) and the timing and nature of seasonal weather cycles (e.g., Menzel et al., 2006; White & Hastings, 2020), leading to shifts in seasonal patterns of water availability. The ecological consequences of these shifts are likely to be wide ranging (White & Hastings, 2020; Hajek & Knapp, 2022) and are still being discovered (e.g., Goren et al., 2023). While precipitation amounts and patterns play a primary role in determining water availability for ecosystem functioning, predicting how these will change in a warming world is challenging because of high regional variability in atmospheric circulation patterns and land surface feedbacks (Collins et al., 2013). However, a globally consistent prediction is for periods of extreme precipitation to become more common with a warming atmosphere (Fischer et al., 2013; Kunkel et al., 2013; Sillmann et al., 2015; Donat et al., 2016), and depending on when precipitation extremes occur, large precipitation events (deluges, defined as statistically extreme precipitation events) or periods of extreme precipitation deficits can alter overall seasonal patterns of water availability (Hajek & Knapp, 2022). Semi-arid and arid ecosystems are likely to be particularly sensitive to such an intensification of the hydrologic cycle because these ecosystems are chronically water-limited and large precipitation events can account for a substantial proportion of their annual precipitation total (Sala et al., 1992; Knapp et al., 2015). Given the importance of semi-arid ecosystems as drivers of variability in the global carbon cycle (Ahlström et al., 2015; Haverd et al., 2016; Poulter et al., 2014) and as economically important rangelands (Asner et al., 2004; Bengtsson et al., 2019), understanding how climate extremes and

shifts in seasonality will impact ecosystem functioning is critical for designing sustainable land management practices and informing ecological forecasts.

Sensitivity to extreme precipitation events, particularly deluges, has been well-documented in the semi-arid shortgrass steppe of North America (Heisler-White et al., 2008; Post & Knapp, 2019; 2020; 2021; Hoover et al., 2022). However, past research has been mostly limited to evaluating the ecological impacts of deluges during the growing season in these summer rainfall dominated grasslands (Hermance et al., 2015; Fig. A2.1). But periods of extreme precipitation can occur during the dormant season as well (Mahoney et al., 2015; Sillmann et al., 2015), and these are likely to increase as the frequency of deluges increases overall (Sillmann et al., 2015; Trenberth et al., 2015; Pall et al., 2017).

For example, in 2013 a period of extreme rainfall impacted the functioning of semi-arid grasslands located in the rain shadow of the Rocky Mountains (Colorado USA) during the autumn, typically a season with low precipitation inputs (Gochis et al., 2013; Concilio et al., 2015; Moore Powell, 2016). Immediately after this post-growing season deluge, which in some areas was equal in magnitude to mean annual precipitation (Concilio et al., 2015), a period of increased carbon uptake by previously senescing plants was evident but increases in ecosystem respiration resulted in an overall loss of carbon from ecosystems (Moore Powell, 2016). There were also significant carryover effects in the following growing season with carbon uptake and plant growth enhanced in the early spring of 2014 (Concilio et al., 2015, Moore Powell, 2016). Other studies (e.g., Prevéy & Seastadt, 2014) have also reported that dormant season precipitation, particularly snow (Loik et al., 2013; Li et al., 2020) can influence ecosystem structure and function the following spring in semi-arid ecosystems. However, these studies contrast with analyses of long-term productivity data from semi-arid grasslands which found no

evidence for carryover effects of dormant season precipitation on productivity the next growing season (Hoover et al., 2021).

Here, we experimentally assessed how autumn deluges can impact a grassland ecosystem, both in the autumn and during the following spring, in the water-limited shortgrass steppe of NE Colorado (Lauenroth & Burke, 2008). We predicted that deluges that occur post-growing season, similar to the natural event experienced in 2013, would have minimal impacts initially because of cooler temperatures coupled with plant senescence, but would augment soil moisture sufficiently to influence plant growth the following spring. So, we imposed two 100 mm deluge treatments – one in September (SEP) and one in October (OCT). Because these deluges represent ~40% of average growing season precipitation, we expected to see soil moisture carryover effects for both treatments, leading to enhanced spring green-up and plant growth. But, we hypothesized that the OCT treatment would have a greater impact because cooler temperatures would reduce evaporation losses, allowing greater soil moisture levels to persist into the next spring.

4.3 Methods

4.3.1 Site Description

Our study site was located in the North American shortgrass steppe at the USDA Central Plains Experimental Range in northeastern Colorado, USA (N 40.8422°, W 104.7156°). Mean annual temperature is 8.6°C (Lauenroth & Sala, 1992), and mean annual precipitation is 342 mm with ~20% of annual precipitation occurring during the autumn season (Sept. – Nov.; Hermance et al., 2015). Growing season precipitation (May – Aug.) accounts for majority (~75%) of the annual total. Long-term ANPP is ~74 g m⁻² with the majority of that comprised of perennial C₄ grasses (~75%, primarily *Bouteloua gracilis* and *Bouteloua dactyloides*) and the remainder

including perennial C₃ grasses, cool-season annual grasses, forbs, and cacti (Hoover et al., 2021). Soil texture at the study site is sandy clay loam (Sala et al., 1992), and although the site has a history of moderate cattle grazing, the area where this experiment was conducted had been protected from large ruminant grazers since 2011 (Post & Knapp, 2020).

4.3.2 Experimental Design

At the beginning of Sept. 2021, we established thirty 1-m² plots separated by at least 3 m. Plots were randomly assigned to one of two deluge treatments or the ambient group (AMB), which received only ambient precipitation. Both deluge treatments included the addition of 100 mm of water applied over six days during the autumn of 2021 – either in Sept. (SEP) or in Oct. (OCT). These two different deluge timing were used to denote early and late fall, to capture partial vs. complete senescence of grasses aboveground. Using long-term precipitation data from this site (Hoover & Derner, 2020), this amount of precipitation corresponds with two of the wettest autumn months on record (Sept. 1989 and 2013), each of which received approximately 100 mm of precipitation.

Water was applied between Sept. 15-20 and Oct. 20-25 for the SEP and OCT treatments, respectively, using a hand-held watering wand attached to a flow meter and water pump. Over the course of the week, 8 mm of water was added on the first day, 24 mm on the second, 18 mm on the third, and then 50 mm was applied on day 6. This water application pattern is similar to a 7-day high rainfall period observed in Sept. 1989, where rain accumulated for seven days with 50 mm recorded on the final day. To prevent overland flow off the plots, water was applied several times each day with only 4-8 liters of water added at a time. Added water was tested for nitrogen levels (American Agricultural Laboratory, Inc., Nebraska, USA) and was below US

EPA drinking water standards. Besides the deluge events, all plots received ambient precipitation.

4.3.3 Measured Responses

From Sept. 2021 – Aug. 2022, we monitored soil moisture, soil CO₂ efflux (soil respiration), and canopy greenness. From Sept. – Nov. 2021 and mid-Mar. – June 2022, measurements were made weekly, but during the winter, measurement frequency was more variable, ranging from biweekly to monthly intervals. This schedule allowed us to capture key phenological stages including autumn senescence and spring green-up with higher resolution. In addition, we also measured soil N availability during two separate sampling periods – the autumn and the spring – to assess both treatment and potential carryover of soil nitrate (NO₃⁻) and ammonium (NH₄⁺). Finally, above-ground net primary production (ANPP) was measured in mid-June to capture early-season plant growth responses.

Soil moisture (volumetric water content, % VWC) was measured using a 20 cm handheld soil moisture time-domain reflectometry probe (Campbell-Scientific Hydrosense II), which integrates soil moisture across the top 20 cm of soil. Because most of the root biomass in this ecosystem is within the upper 20 cm of soil (Milchunas & Lauenroth, 1989; Gill et al., 1999; Nelson et al., 2004), this measurement provides a reliable indicator of water available for the dominant grasses. We also used a Sentek Diviner probe (Diviner 2000, Sentek Pty Ltd.) on a subset of the plots to observe soil moisture dynamics to greater depths (0-60 cm with measurements at 10 cm increments).

We monitored canopy phenology with digital repeat photography to estimate canopy greenness (following the methods of Post & Knapp, 2020, Hoover et al., 2022). For each photograph, we placed an iPhone camera directly above a movable 50 x 50 cm frame positioned

in the corner of each plot; this image was then cropped to only include the interior area of the frame. The cropped photos were then analyzed with the R package EBImage (Pau et al., 2010), which calculates the average green chromatic coordinate (GCC) index (Filippa et al., 2016). The GCC index accounts for variation in image lighting by computing the greenness relative to the total brightness of each pixel as: $\text{green} / (\text{red} + \text{blue} + \text{green})$ (Filippa et al., 2016).

To measure soil CO₂ efflux, we installed permanent PVC collars (10 cm in diameter, n=5 per treatment) in bare areas between grasses at the beginning of September (2.4 cm belowground, 2 cm aboveground). If any plant growth occurred within the collars during the measurement period, it was removed (clipped to the base) prior to every measurement. We then used a 6400-09 soil flux chamber attached to an LI-6400XT (LiCor., Inc, Lincoln NE, USA) to measure soil respiration. Measurements were taken between 8:30hr – 12:30hr local time at ambient CO₂ concentration, humidity, and temperature

Nutrient availability was monitored using Plant Root Simulator probes (PRS, Western Ag Innovations Inc., Saskatoon, Canada) in both the autumn of 2021 and the spring of 2022. These 15 cm x 3 cm probes utilize either a cation or anion exchange membrane buried in the soil with minimal disturbance. For the autumn period, one set of probes (anion and cation) were inserted Sept. 9 and removed on Dec. 3, 2021 in 23 plots (n=8 for SEP and OCT, n=7 for AMB). Then, during the spring period, three sets of probes per plot were inserted on Mar. 16, 2022 and removed on June 22 in the same 23 plots. After removal, samples were washed using deionized water and sent to Western Ag Innovations for extraction. Probes were analyzed for NH₄⁺ and NO₃⁻ with a Technicon Autoanalyzer. Values were reported in $\mu\text{grams } 10 \text{ cm}^{-2} \text{ } 11 \text{ weeks}^{-1}$.

Finally, ANPP was estimated in mid-June (June 14-16) in all plots to capture early season growth and before potential mid- and late-summer rains. We harvested all aboveground

vegetation within two 0.1 m² subplots to ground height, sorted by functional group (C₃ grass, C₄ grass, forb, or annual C₃ grass). Vegetation was then dried at 60 °C for 48 hours before being weighed to the nearest 0.01g. Previous year's growth was easily distinguishable from current year growth and was excluded.

4.3.4 Statistical Analyses

All analyses were performed using R (version 4.1.3). For 20 cm soil moisture, GCC, and soil respiration data, we divided the experiment into two periods for analysis: the autumn treatment and the late winter-spring response periods. The treatment period includes all 2021 data (i.e., Sept. – Dec.), to focus on direct ecosystem responses to the autumn deluges. The response period spans from Mar. 15 – June 30, providing insight into how the autumn deluges affected early-season dynamics, and to detect potential carryover effects. For soil moisture, GCC, and soil respiration, we employed a repeated measures mixed model analysis of variance with treatment and sampling date as fixed effects, plot as a random effect, and an interaction term between treatment and date for the treatment and response periods separately (lme4 package, Bates et al., 2015). For ANPP and nutrient availability, we used a one-way ANOVA and Tukey post-hoc pairwise comparisons. Fall and spring measurements of nutrient availability were analyzed separately.

4.4 Results

4.4.1 Environmental Context

Data from a nearby NOAA weather station (NUNN 7 NNE, CO US, 40.7063, -104.7833) indicated that the entire experimental period (Sept. 2021 – June 2022) was drier than average (148 mm vs. 285 mm; Fig. A2.1). Autumn (Sept. – Nov.) precipitation totals in 2021 were ~32 mm (vs. 75 mm on average). Further, temperatures for the autumn period were higher

than normal (11.2°C vs. 8.4°C), with November temperatures particularly elevated. Early season precipitation (Mar. – June) amounted to ~85 mm, which is nearly 100 mm less than average (182 mm), and temperatures during this timeframe were in line with the long-term average. These patterns in precipitation and temperature were reflected in the soil moisture patterns for the AMB plots. With the exception of a small amount of precipitation fell during the late-spring period, soil moisture levels in the upper 60 cm were very low for majority of the experiment, and as a result, there was very little canopy green-up relative to normal springs (Fig. 4.1).

4.4.2 Treatment Period

Following each deluge, 20 cm soil moisture increased dramatically, and persisted through the year (Fig. 4.1, Fig. 4.2a). For the autumn treatment period (Sept. 10 – Dec. 10), average soil moisture ($\pm$ standard error) was: 13.68 $\pm$ 0.32% (AMB), 26.03 $\pm$ 0.69% (SEP), and 19.44 $\pm$ 0.86% (OCT). By the end of 2021, soil moisture levels in the SEP and OCT treatment converged but remained elevated compared with AMB plots (AMB = 11.68 $\pm$ 1.06 %, SEP 22.31 $\pm$ 1.17 %, OCT = 23.60 $\pm$ 2.96%). A repeated measured mixed model ANOVA showed significance for treatment, sampling date, and their interaction (Table 4.1). Deep soil moisture measurements indicate that soil moisture was elevated up to a depth of 40 cm from the deluges, and this deep soil moisture appears to persist through the treatment period for both deluge treatments (Fig. 4.1).

Despite both deluge treatments receiving the same amount of water, the SEP plots responded more strongly in both canopy greenness and soil respiration. In the SEP plots, canopy greenness showed a significant peak that lasted through October (Fig. 4.2b); this autumn green-up was driven by both C₃ grasses (primarily *Carex spp.*) and C₄ grasses (*B.gracilis* and *B. dactyloides*, personal observation). In contrast, there was only a modest increase in canopy

greenness immediately following the deluge for the OCT treatment, as most plants had completely senesced by this point. The repeated measures mixed model ANOVA for GCC shows significant effects for treatment, date, and their interaction (Table 4.1). Both the SEP and OCT treatments also showed increased rates of soil respiration following the simulated deluges (Fig. 4.2c). However, the maximum rate of soil CO₂ efflux following the SEP deluge was significantly higher than the OCT treatment (4.9 ± 1.2 vs 1.18 ± 0.11 $\mu\text{mol m}^{-2} \text{s}^{-1}$), suggesting enhanced CO₂ loss from the SEP treatment with the warmer temperatures (mean air temperature in Sept. was 17.9°C vs 9.8°C in Oct. in 2021). Further, elevated rates of soil respiration were observed for four weeks in the SEP treatment compared with only one week in the OCT treatment. Treatment, date, and their interaction were all significant in the repeated measured mixed model ANOVA for soil respiration (Table 4.1).

During the autumn, soil nutrient dynamics differed significantly between the AMB treatment and both deluge treatments (Fig. 4.3, Fig. A2.2). In particular, NO₃⁻ levels were much higher in both the SEP and OCT deluge treatments compared with the AMB values (AMB = 36.4 ± 12.6 , SEP = 111.9 ± 16.3 , OCT = 120.5 ± 14.1 $\mu\text{g 10 cm}^{-2} \text{ 11 weeks}^{-1}$), suggesting that the elevated soil moisture increased availability of this nutrient in the soil. There were no significant differences in NH₄⁺ levels between the treatments. Besides nitrogen, the deluges significantly increased availability for other several other nutrients (calcium, magnesium, sulfur, iron, and phosphorus; Fig. A2.2). Overall, soil nutrient availability responded similarly in the two deluge treatments; phosphorus was the only exception with elevated levels in the SEP vs. OCT treatment ($P < 0.0001$).

4.4.3 Response Period

Soil moisture levels in the deluge treatments remained elevated into the spring (Fig. 4.4) with mean soil moisture values between all treatments converging at the end of April. From that point, soil moisture patterns were similar among all treatments, but OCT deluge treatment had slightly lower values for the remainder of the season. Date and the interaction between treatment and date were significant in a repeated measures mixed model ANOVA for soil moisture during the response period (Mar. 15 – June 30; Table 4.1). Overall, 20 cm soil moisture values for the response period were higher in the SEP and OCT treatments (Table 4.2), and deeper soil moisture (30 – 40 cm) appeared to persist in the OCT and SEPT treatments throughout the early growing season even as the upper soils dried (Fig. 4.1).

Despite this carryover of soil moisture into the early spring, few differences emerged in ecosystem responses; patterns of canopy greenness, soil CO₂ efflux, and nutrient availability were similar for all treatments during the early growing season. There were no significant differences between the treatments for soil respiration (Table 4.1). Soil respiration patterns closely tracked soil moisture with increased rates after precipitation events (Fig. A2.4), but overall soil CO₂ efflux remained relatively low for the early season (Table 4.2). Similarly, GCC values were low for the early season in all treatments (see Fig. 4.1, Fig. A2.5); green-up was largely muted because of the spring dry conditions. The SEPT and OCT treatments had slightly higher GCC values than the AMB treatment at the beginning of the response period, but this difference quickly disappeared by early April. Overall, differences in phenology patterns were limited (Table 4.2, Fig. A2.5); however, a repeated measured mixed model ANOVA showed significant effects of date and the interaction of treatment and date (Table 4.1). Finally, nutrient availability did not differ between the treatments except for NO₃⁻ levels (Fig 4.3, Fig. A2.3).

These, however, were significantly higher in the AMB treatment compared with the SEP treatment (AMB = 41.48 ± 5.48 , SEP = 20.47 ± 3.36 $\mu\text{grams } 10 \text{ cm}^{-2} \text{ } 11 \text{ weeks}^{-1}$, $P = 0.0091$). The OCT plots did not differ from AMB or SEP treatments (OCT = 34.03 ± 4.19 $\mu\text{g } 10 \text{ cm}^{-2} \text{ } 11 \text{ weeks}^{-1}$). Further, these values were significantly lower than measured in the fall; in particular, the average difference between NO_3^- levels during the two time periods was hugely different between the two deluge treatments and the AMB treatment (AMB = 10.34 ± 9.04 , SEP = -93.45 ± 13.17 , OCT = -91.41 ± 17.44 $\mu\text{g } 10 \text{ cm}^{-2} \text{ } 11 \text{ weeks}^{-1}$; Figure A2.6).

Nonetheless, ANPP was significantly increased by both the SEP and OCT deluges (AMB = 26.92 ± 1.68 , SEP = 39.22 ± 1.84 , OCT = 41.46 ± 2.56 g m^{-2} , Fig. 4.5a). This additional productivity was primarily a result of increased C_4 ANPP, which accounted for $80.3 \pm 2\%$ of total productivity across all plots (Fig. 4.5b). C_3 ANPP was also enhanced in the SEP and OCT plots, but only the OCT treatment was significantly higher than the AMB treatment ($P = 0.003$, Fig. 5c). This enhancement in C_3 ANPP for the OCT treatment was primarily driven by an increase in a C_3 annual grass (*Vulpia octoflora*, Fig. 4.5c inset), which was not apparent in the SEP plots (annual ANPP in AMB = 0.06 ± 0.06 , SEP = 0.21 ± 0.17 , OCT = 5.67 ± 1.71 g m^{-2}).

4.5 Discussion

Shifts in the seasonal timing of water availability, induced by climate change, are expected to drive changes to ecosystem structure and function worldwide. Indeed, increases in the frequency of precipitation extremes are one of several causal mechanisms that can shift the seasonal availability of water in ecosystems (Hajek & Knapp, 2022). Here, we manipulated seasonal precipitation inputs in the shortgrass steppe of the US Great Plains by imposing deluges during the autumn (post-growing season) to assess immediate and potential carryover effects the following spring. Despite some evidence for carryover effects from autumn deluges in nearby

grasslands (Moore Powell, 2016, Concilio et al., 2015), most research in this region suggests that early season precipitation is the predominant driver of ecosystem function (e.g., productivity, Derner & Hart, 2007; Derner et al., 2008; Parton et al., 2012) with antecedent fall and dormant season precipitation relatively unimportant (Hoover et al., 2021). Indeed, the only signature of autumn precipitation detected from previous analyses of long-term productivity-precipitation records from the shortgrass steppe grassland was for C₃ annual grasses, which germinate in late winter into early spring (Dufek et al., 2018; Hoover et al., 2021). Thus, while there is some evidence for legacy effects based on previous year biomass in this grassland (Oesterheld et al., 2001; Sala et al., 2012), evidence for carryover effects from dormant season precipitation in this grassland is limited. Of course, one potential reason why the impact of autumn precipitation has been difficult to discern from long-term data is that historically, the autumn season has been relatively dry, whereas the spring and early summer months have the highest levels of precipitation (Lauenroth & Burke, 2008; Hermance et al., 2015; Fig. A2.1).

Results from our experimental autumn deluges suggest that increases in precipitation extremes post-growing season can affect ecosystem function during the following spring. Even though we did not observe meaningful differences in soil CO₂ efflux or canopy greenness in the following spring, we did measure enhanced ANPP in the two deluge treatments. This increase in ANPP was primarily driven by C₄ ANPP, which accounted for ~80% of overall ANPP, and this result is consistent with Concilio et al. (2015) following an extremely wet autumn in a nearby grassland. We also documented enhanced C₃ production, in particular, the OCT treatment increased C₃ production relative to the AMB treatment. This increase in C₃ ANPP was primarily driven by an increase in *Vulpia octoflora*, the only annual C₃ grass at this site. Previous work has shown that wet and warm winters facilitate the establishment of this annual grass (Dufek et al.,

2018), and it appears that the timing of the OCT deluge promoted germination and establishment of this species. An increase in this annual grass is relevant for land managers because *V. octoflora* has lower forage quality compared with perennial C₃ and C₄ grasses (Dufek et al., 2018). The limited response of other C₃ grasses (mostly perennial) is likely due to their low cover in this grassland; in a more mixed grassland, we would expect that C₃ grasses would be more responsive to additional autumn precipitation and increased soil moisture in the early spring (Winslow et al., 2003).

The increase in ANPP – C₄ ANPP in particular – was most likely a result of the elevated soil moisture levels in the SEP and OCT treatments in the spring. Even though we did not observe differences in GCC among the treatments, soil moisture plays an important role in early-season dynamics in the shortgrass steppe (Moore et al., 2015; Post et al., 2022). Since 2022 was exceptionally dry during the early season (85 mm vs. 182 mm on average for Mar. – June with Mar. – Apr. precipitation totals corresponding to less than the 5th percentile historically; Fig. A2.1), even limited amounts of soil moisture persisting from autumn deluges could enhance plant growth. We suspect that during a more typical spring, carryover of soil moisture is less important because more abundant precipitation during the early growing season is sufficient to initiate plant growth (Hoover et al., 2021; Post et al., 2022). Indeed, the lack of previous evidence for carryover effects of autumn precipitation is likely due to the historically uncommon co-occurrence of a very wet autumn followed by a dry spring. However, with climate change, the potential for precipitation extremes (wet and dry) at any time of year in this ecosystem is increasing (Sillmann et al., 2015), and thus, the likelihood of such novel combinations will increase.

Although we hypothesize that elevated soil moisture was the primary driver of enhanced ANPP in the deluge treatments, other factors may have also been relevant (Burke et al., 1997). We detected increased nutrient availability following the autumn deluges; in particular, there was a significant pulse of nitrate. While some degree of nitrogen loss is likely if plants are not able to utilize this pulse of nutrients (Dijkstra et al., 2012), these additional nutrients may have been taken up by plants, indicated by a huge decrease in NO_3^- availability between the autumn and spring for the SEP and OCT treatments (Fig. A2.6). In other words, even though plants had begun to senesce aboveground when experimental deluges were applied, root uptake of N may still have been possible (Milchunas & Lauenroth, 2001; Steinaker & Wilson, 2008). There is much evidence for phenological asynchrony with aboveground phenological stages differing from belowground activity (Steinaker & Wilson, 2008; Steinaker et al., 2010; Abramoff & Finzi, 2015; Schwieger et al., 2019).

Although not planned as part of this experiment, this grassland experience “compounded extremes” with the imposed fall deluge treatments preceding a natural extreme spring drought. Compound climate extremes are forecast to become more common with climate change (Zscheischler et al., 2018; Hoover et al., 2022). Early season ANPP in 2022 was extremely low (~36% of average total growing season ANPP; Hoover et al., 2021) due to low precipitation totals, and the plant canopy greened in the spring to less than 10% of normal (Fig. 4.1). While the autumn deluges imposed did not rescue this system from the extreme spring drought, production was significantly stimulated by these autumnal inputs. Given the likely increase in compounded extremes in the future, evaluating how such shifts in the seasonal dynamics can affect ecosystem function, particularly carbon cycling, is critical for land managers and decision makers (Hartman et al., 2020). Further, while our focus was on understanding autumn deluge

carryover impacts, it is also important to consider how seasonal changes in water availability may alter annual carbon cycling dynamics. We observed large increases in soil respiration in the SEP treatment, while the pulse of soil CO₂ efflux was dampened in OCT when temperatures were lower. This suggests that even relatively subtle differences in the timing of dormant season deluges can have outsized impacts on the carbon cycle. Overall, understanding how deluges and seasonal shifts in water availability will impact the carbon budget of these vast grasslands will require more comprehensive evaluation, but such an assessment is needed given the importance of dryland ecosystems to the global carbon cycle (Ahlström et al., 2015; Haverd et al., 2016; Poulter et al., 2014).

4.6 Tables

Table 4.1 Summary of the repeated measures mixed model ANOVA results for soil moisture, canopy greenness, and soil respiration with treatment, date, and their interaction as factors. Treatment period (Sept. – Dec.) is the upper section, and response period (Mar. – June) is below. Reported values include the degrees of freedom (numerator, denominator), the F-statistic, and the *P* value.

	Variable	Soil moisture			Canopy greenness			Soil respiration		
		<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>
Treatment Period	Treatment	2, 27	28.82	<0.0001	2, 27	5.32	0.01127	2, 12	30.68	<0.0001
	Date	15, 383	14.07	<0.0001	14, 377	505.18	<0.0001	14, 148	44.64	<0.0001
	Treatment x date	30, 383	43.39	<0.0001	28, 377	12.48	<0.0001	28, 148	11.67	<0.0001
Response Period	Treatment	2, 27	3.09	0.06169	2, 27	0.66	0.5242	2, 12	0.65	0.541
	Date	13, 342	29.65	<0.0001	15, 397	88.79	<0.0001	13, 136	60.49	<0.0001
	Treatment x date	26, 342	5.40	<0.0001	30, 397	2.59	<0.0001	26, 136	0.96	0.5266

Table 4.2. Mean ($\pm$ SE) for 20 cm soil moisture (% volumetric water content, VWC), canopy greenness (GCC), and soil respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$) during the response period (Mar. 15 – June 30). Treatments refer to: AMB = ambient precipitation, SEP = September deluge, and OCT = October deluge.

Treatment	Soil Moisture	Canopy Greenness	Soil Respiration
AMB	19.06 $\pm$ 0.42	0.335 $\pm$ 0.0003	0.82 $\pm$ 0.09
SEP	23.71 $\pm$ 0.46	0.335 $\pm$ 0.0004	0.84 $\pm$ 0.08
OCT	21.43 $\pm$ 0.70	0.335 $\pm$ 0.0003	0.75 $\pm$ 0.08

4.7 Figures

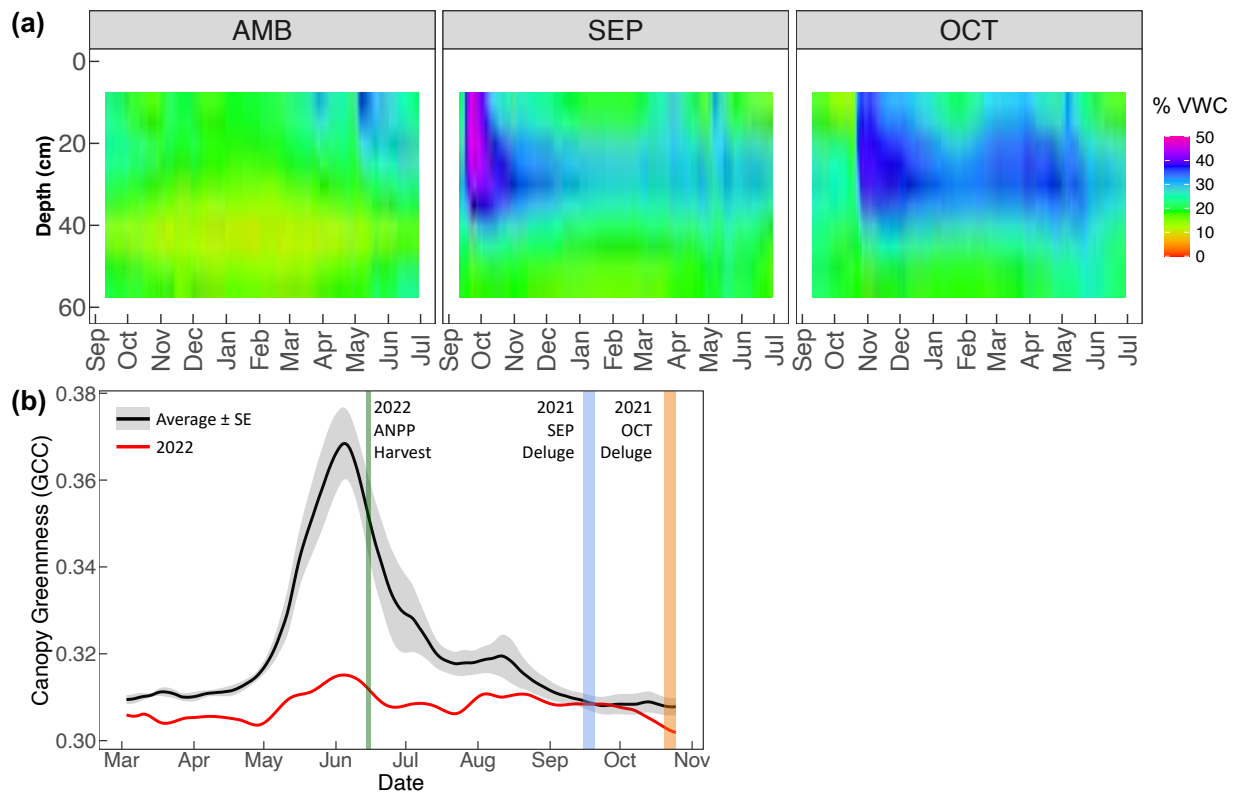


Figure 4.1. (a) Representative soil moisture (volumetric water content, %VWC) with depth and time for the three precipitation treatments from mid-Sept. 2021 through June 2022. Arrows on SEP and OCT graphs indicate timing of 100 mm deluge during the autumn (SEP = September deluge, OCT = October deluge, AMB = ambient precipitation). Soil moisture measurements were integrated to 5 cm resolution and the daily timescale using linear interpolation (R package *imputeTS*; Moritz & Bartz-Beielstein, 2017). (b) Phenological dynamics for March – October at the Central Plains Experimental Range in northeastern Colorado. The black line shows the average smoothed 3-day 90th percentile green chromatic coordinate index (GCC) $\pm$ standard error from 2016-2021, and the red line shows 2022 for the PhenoCam site: cperagm (40.8402, -104.7672). Data were retrieved using the R package *phenocamr* (Hufkens et al., 2018). The green bar denotes the timing of summer aboveground net primary production harvest in 2022, and the blue and orange bars denote the timing of the autumn deluges applied in 2021. Although patterns and values are similar, absolute values differ between this site and observed GCC values from those observed and the experiment (Seyednasrollah et al., 2019).

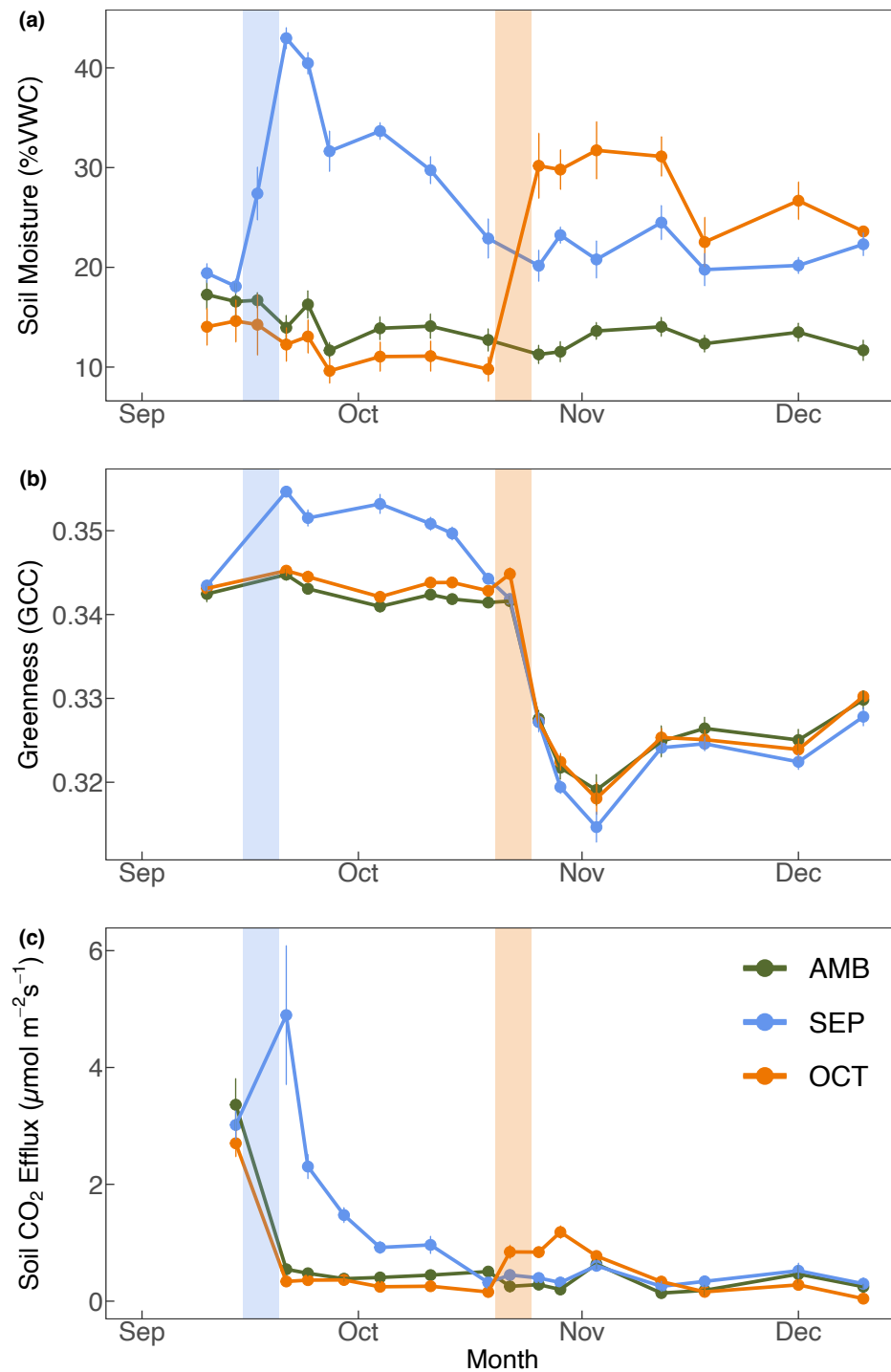


Figure 4.2. Treatment effects on (a) 20 cm soil moisture (volumetric water content, %VWC), (b) canopy greenness (green chromatic coordinate index, GCC), (c) soil CO₂ efflux (μmol m⁻²s⁻¹). Points represent mean treatment value ± standard error for each sampling date. Shaded regions show the timing of each deluge period (blue=Sept. deluge, orange=Oct. deluge). Treatment period represents Sept. – Dec. 2021.

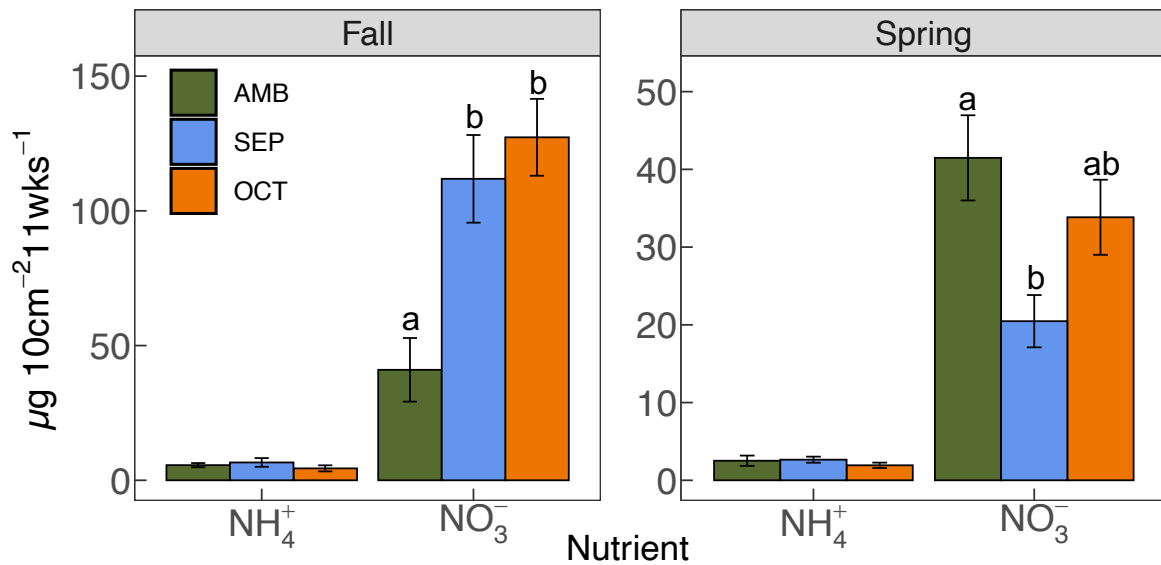


Figure 4.3. Ammonium (NH_4^+) and nitrate (NO_3^-) mean ($\pm$ SE) values during the fall (Sept. 9 – Dec. 3, 2021) and spring (Mar. 16 – June 22, 2022) periods reported in $\mu\text{g } 10\text{ cm}^{-2} 11\text{ weeks}^{-1}$. Letters denote significant differences between treatments ($P < 0.05$); y-axes differ between the two periods.

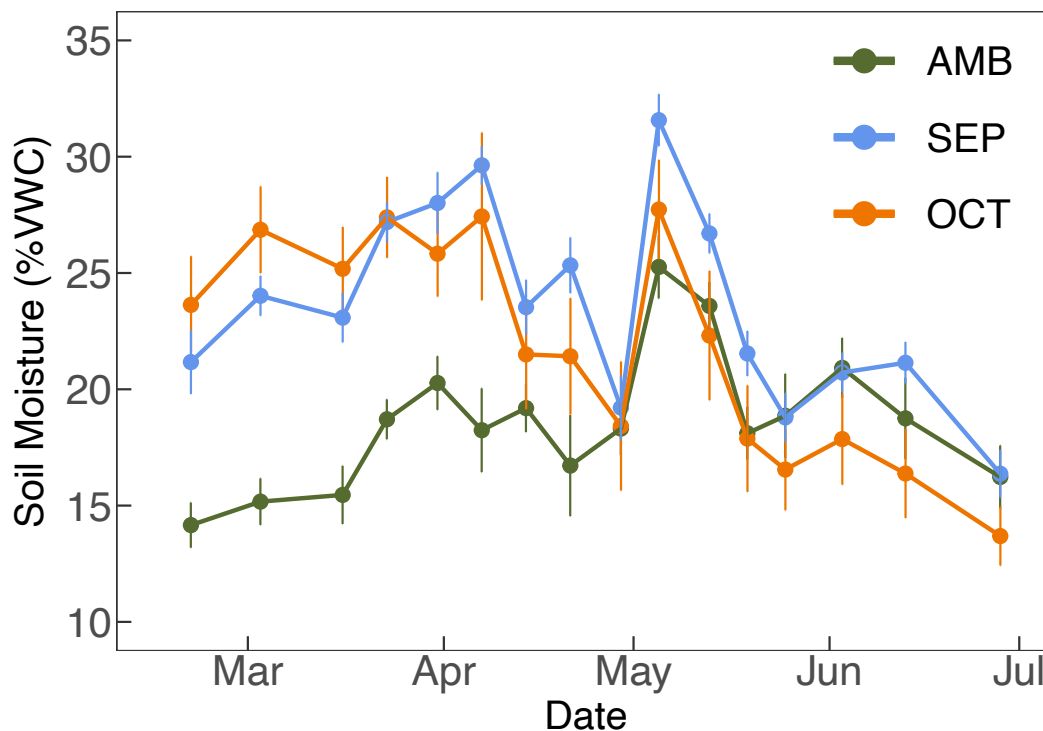


Figure 4.4. Mean ($\pm$ SE) soil moisture (volumetric water content, %VWC) integrated over the top 20 cm during the late-winter and spring period (Feb. 15 – June 30). Lines represent each treatment: AMB = ambient precipitation (green line), SEP = Sept. deluge (blue line), and OCT = Oct. deluge (orange line).

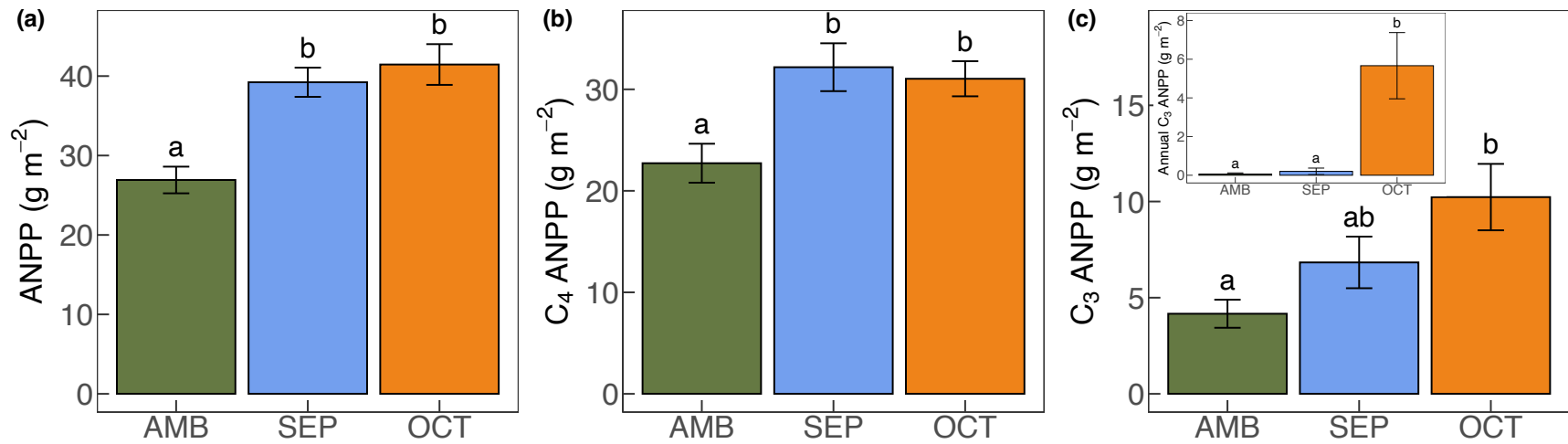


Figure 4.5. Carryover effects of (a) total, (b) C_4 , and (c) C_3 aboveground net primary production (ANPP; mean $\pm$ SE) from autumn deluges in the shortgrass steppe. Inset shows the ANPP of the annual C_3 grass, *Vulpia octoflora*. Letters denote significance between treatments ($P < 0.05$).

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CHAPTER 5: CONCLUSIONS

Seasonal patterns of water availability are shifting globally as a result of climate change (Collins et al., 2013; Greve et al., 2018; Konapala et al., 2020; Padrón et al., 2020). Dryland ecosystems are likely to be particularly sensitive to these seasonal shifts because the timing of water availability modulates both the dynamics of ecosystem functioning (Noy Meir 1973; Knapp et al., 2008; Parton et al., 2012; Zeppel et al., 2014; Petrie et al., 2016) as well as the relative abundances and productivity of different functional groups (i.e., C₃ vs. C₄ grasses, shrubs vs. grasses; Winslow et al., 2003; Germino & Reinhardt, 2014; Gremer et al., 2018; Renne et al., 2019; Xie et al., 2022). For semi-arid North American Great Plains grasslands in particular, understanding how shifts in the seasonal timing of water will alter the distribution of C₃ and C₄ grasses and their functioning has important implications from land management and carbon cycle perspectives (Barbehenn et al., 2004; Chamaillé-Jammes & Bond, 2010; Bengtsson et al., 2019; Piao et al., 2019). Yet, climate change impacts at the seasonal timescale are often underappreciated in ecological research – despite their clear importance. This was the underlying motivation for the research reported in this dissertation. Herein I explored several mechanisms that can cause water availability to shift seasonally in the US Great Plains and report results from two field experiments that directly manipulated seasonal patterns of precipitation and soil moisture.

5.1 Research Summary

My research demonstrated that shifts in the seasonal availability of water may occur through a variety of mechanisms and that these shifts will almost certainly affect grassland structure and function. For my first study (Chapter 2), I conducted a review exploring different

mechanisms by which climate change may alter the seasonal patterns of water availability and outlined the potential consequences, with the US Great Plains as a case study. Using long-term weather data from NOAA weather stations across a north-south transect across the central US Great Plains, I considered several potential mechanisms driving these seasonal shifts: (1) changes in precipitation patterns (i.e., wetter springs, drier summers), (2) increases in the frequency of extreme hydrologic years that alter the seasonal distribution of precipitation (Knapp et al., 2020), and (3) increases in evapotranspiration driven by warming temperatures (Padrón et al., 2020). With these mechanisms, I found that the Great Plains region is most vulnerable to changes driven by warming (consistent with findings from Hufkens et al., 2016; Seager et al., 2018) with regional vulnerability to extreme drought years. While the ecological consequences of these shifts are likely to be wide ranging, I identified a need for explicit evaluation of ecosystem responses to seasonal changes in water availability through experiments.

Building off this analysis, I designed a field experiment to evaluate how shifts in the seasonality of rainfall affects ecosystem structure (C_3 vs. C_4 grasses) and function (carbon cycling) in a northern mixed grass prairie in southeastern Wyoming (Chapter 3). Without changing total precipitation amount, I reduced spring rainfall (April – June 2021) in treatment plots and returned the excluded amount of rainfall throughout the summer (July- September), effectively shifting spring rainfall to summer rainfall. This shift in rainfall seasonality created divergent patterns of soil moisture, influencing patterns of soil respiration and canopy greenness. I also observed significant increases in C_4 aboveground net primary production (ANPP) in plots with higher cover of C_4 in response to the additional summer rainfall. However, in contrast to our expectations, I did not record significant differences in total or C_3 ANPP, likely due to the extremely wet spring conditions prior to the construction of the rainout shelters. Nonetheless,

this study provides evidence that alterations in seasonal timing of precipitation can shift the relative abundances of C₃ vs C₄ species in this grassland.

I then examined the carryover effects of an extremely wet dormant season in the shortgrass steppe in northeastern Colorado (Chapter 4). While most studies from this grassland suggest that the primary driver of ecosystem functioning, including ANPP, is early season (spring) precipitation (Derner & Hart, 2007; Derner et al., 2008; Parton et al., 2012), observational studies from a nearby grassland have shown that extreme events in the autumn can increase carbon uptake in the following growing season (Concilio et al., 2015; Moore Powell, 2016). To assess this carryover effect experimentally in this native C₄-dominated grassland, I applied a single deluge (100 mm) in either mid-September or late October, an amount that corresponds to two of the wettest autumn months on record. Immediately after these deluges, I observed increased rates of soil CO₂ efflux and enhanced greenness, with stronger ecosystem responses to the September deluge. More importantly, the increase in soil moisture persisted through April and likely contributed to higher levels of ANPP observed in the deluge treatments in the following spring. The increases in ANPP were primarily driven by C₄ grasses; however, C₃ ANPP was also enhanced, largely due to increases in an annual C₃ grass in the October deluge treatment. I hypothesize that I was able to document carryover effects because of a naturally occurring drought in the spring, underscoring the responsiveness of this semi-arid grassland to changes in seasonal precipitation extremes.

5.2 Implications and Future Directions

Overall, my dissertation research demonstrates the importance of seasonal patterns of water availability in modulating grassland composition and function, highlighting the relevance in understanding how climate change may shift these patterns. Despite the pervasive influence of

seasonality on ecological processes, it has historically been a relatively unappreciated aspect of most ecological studies because of the greater data requirements (throughout the year and across many years) and the resulting complexity of including seasonal variables into ecological models (White & Hastings, 2020). Further, while there has been growing attention on the consequences of alterations in seasonal temperatures (i.e. earlier start of spring, longer growing season, warming winters) for plant growth and ecosystem function (Xia et al., 2014; Zheng et al., 2018; Wang et al., 2020; Xu et al. 2020), seasonal shifts in water availability may actually be more revealing in water-limited regions, such as the semi-arid grasslands of the North American Great Plains.

For both of my field studies, I observed significant differences in the relative productivity of several functional groups with changes in the seasonal water balance. Within a single growing season, I provided experimental evidence in support of the Winslow et al. (2003) seasonal availability of water algorithm designed to better understand the distribution and productivity of C₃ and C₄ grasses globally (Chapter 3), and I documented increased production of C₄ and C₃ grasses from carryover dormant season moisture (Chapter 4). Many models do not account for these interannual fluctuations in ecosystem structure (Xie et al., 2022), but differences between functional groups in terms of forage quality (Barbehenn et al., 2004; Chamaillé-Jammes & Bond, 2010), phenology (Goodin & Henbrey, 1997; Piao et al., 2019), and feedbacks into the atmosphere (Richardson et al., 2013) can be substantial. Given that semi-arid ecosystems play a large role in regulating the interannual variability in the global carbon sink (Poulter et al., 2014; Ahlström et al., 2015; Haverd et al., 2016), including these responses in earth system models would likely improve ecological forecasting of the carbon cycle. Additionally, from a management perspective, understanding seasonal legacy effects (Bastos et al., 2020) would

improve forage production predictions and thus decision making for ranchers (Hartman et al., 2020).

It's important to highlight that I was able to document differences in the relative productivities and abundances of these various functional groups within a single year. Thus, over longer periods of time, there may be larger shifts in the structures of ecosystems and communities in response to changes in the seasonal availability of water (Gremer et al., 2018; Knapp et al., 2020). For example, even without changes in the total amount or patterns of precipitation, increased rates of evapotranspiration with warming temperatures will reduce water availability, especially in the warmest months (Chapter 2). Understanding these long-term, directional patterns may reveal additional consequences. Further, my experiments were focused on understanding the responsiveness of semi-arid grasslands to isolated changes at the seasonal timescale; future work should consider multiple drivers with greater temporal coverage to provide insight into the long-term structural effects from shifts in seasonal water availability. While experiments allow exploration of novel climatic conditions in a controlled setting, climate change is already presenting opportunities to explore the relationships between seasonality and ecosystem processes. Thus, leveraging existing networks, such as the AmeriFlux or Long Term Ecological Research networks, to investigate these questions will help guide the most relevant field experiments.

Finally, there are several other potential ecosystem consequences of shifting seasonal patterns of water availability. My experiments focused primarily on understanding aboveground responses, but in these semi-arid grasslands, most of the carbon is belowground (Milchunas & Lauenroth, 2001). Thus, to develop a more complete understanding of carbon cycling responses, it is imperative to account for how plant roots respond to seasonal shifts in soil moisture.

Previous work in the shortgrass steppe has already demonstrated substantial shifts in belowground net primary production in response to differing precipitation patterns (Milchunas et al., 2005; Post & Knapp, 2020). It's also worth noting that my work focused on grasslands dominated by native grasses, but changes in the seasonal timing of water availability may also create an opportunity for exotic invasive plants as these species often have different phenology from native species, allowing them to utilize resources when native species are not able (Fridley 2012; Concilio et al., 2017). These sorts of compositional changes may have significant consequences both economically and ecologically (Simberloff et al, 2013; Cuthbert et al., 2022).

In conclusion, shifts in the seasonal availability of water due to climate change has the potential to significantly alter ecosystem processes, especially in water-limited dryland regions, such as semi-arid grasslands. My dissertation research confirmed the importance of this aspect of climate change and provided experimental evidence that seasonal shifts in water availability can drive compositional changes, primarily C_3 and C_4 dynamics, as well as alter carbon cycling in a single year. My hope is that this work will promote additional studies explicitly evaluating the ecosystem consequences of shifting seasonality of both temperature and water and improve the decision-making process for land managers and policy makers tasked with safeguarding the future of these grasslands.

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APPENDIX 1

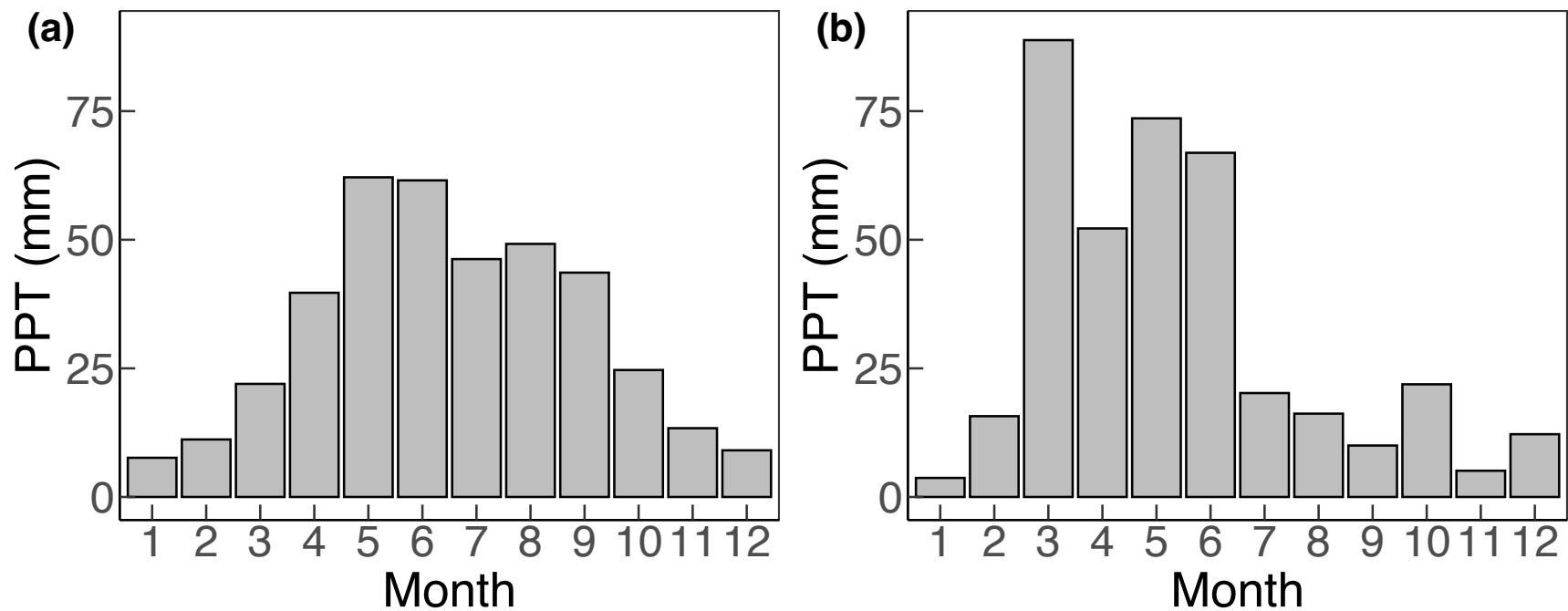


Figure A1.1. Monthly precipitation totals (in mm) based on (a) long-term normals (1991-2020; PRISM Climate Group, 2022) and (b) observations from a nearby weather station for 2021 (the experiment year; Cheyenne Weather Forecast Office, (41.1516, -104.80622)).

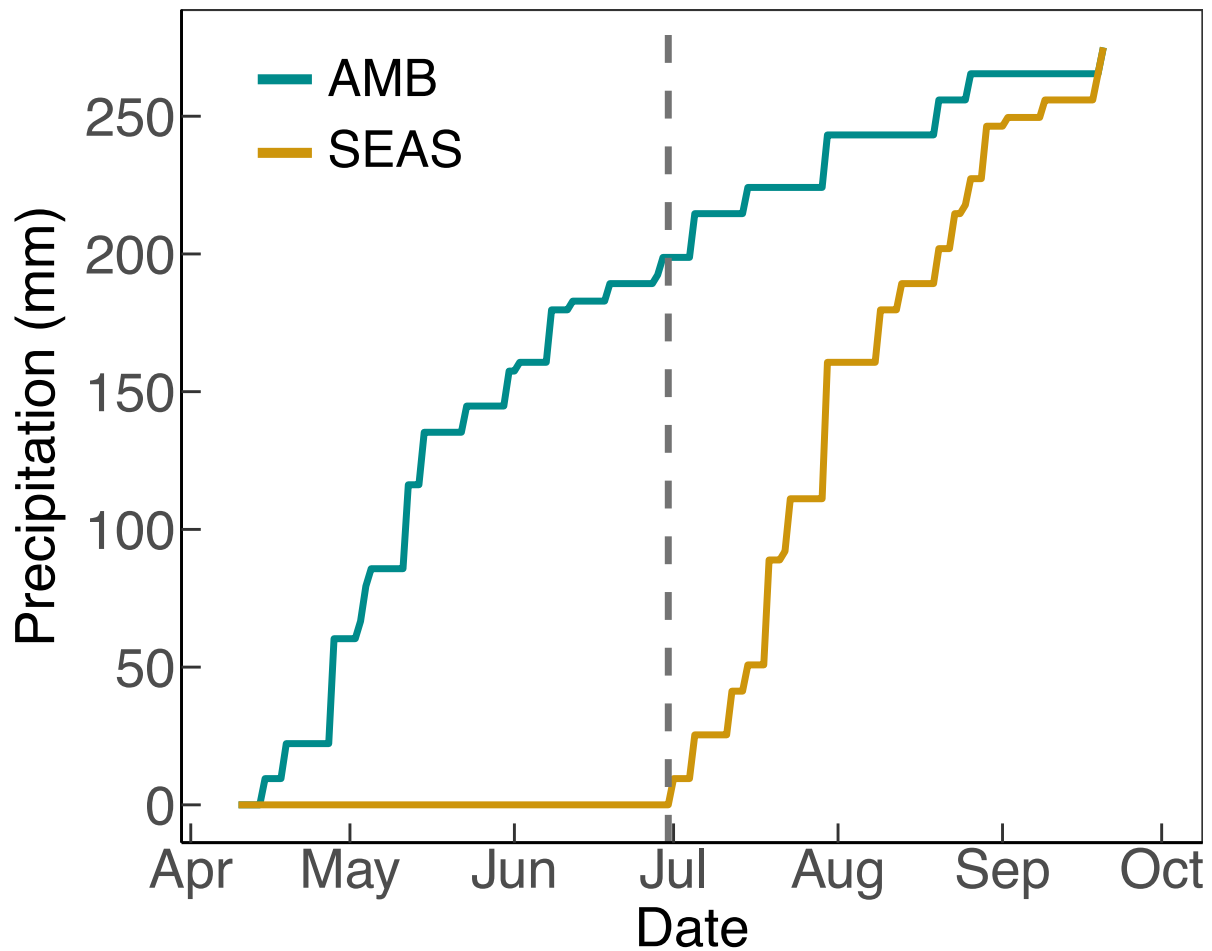


Figure A1.2. Daily precipitation patterns (in mm) for the ambient (AMB) and seasonally shifted (SEAS) treatments. AMB only includes naturally occurring precipitation, while the SEAS treatment includes both manually added water and ambient precipitation. The dashed line at the end of June represents the timing of roof removal for the SEAS treatment. Excluded precipitation was applied to the SEAS plots in a similar pattern as it fell during the early growing season, such that the SEAS treatment experienced a similar number of events of corresponding magnitude during the later part of the growing season.

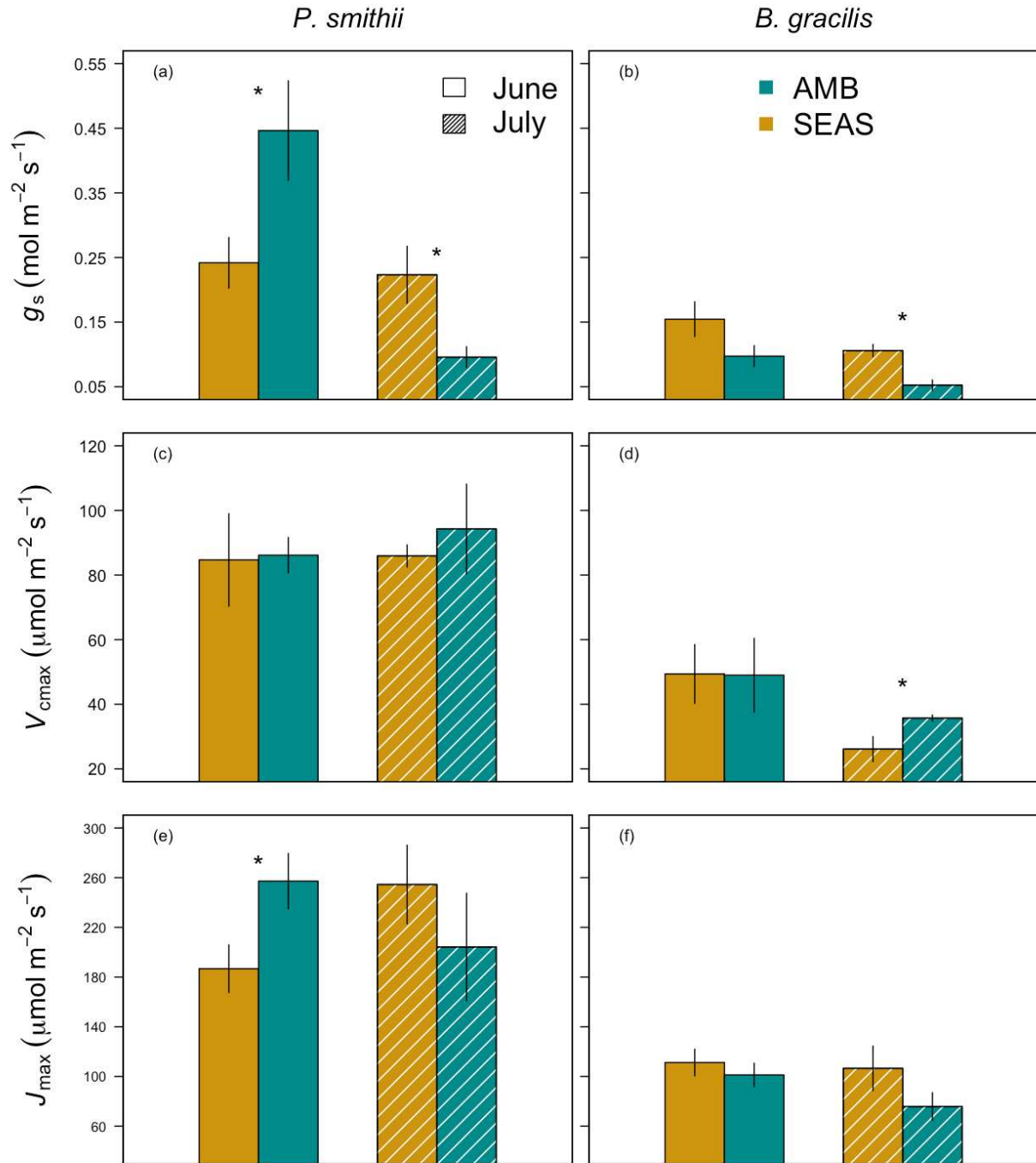


Figure A1.3. Mean ($\pm$ standard error) values for leaf gas-exchange and photosynthetic parameters for *P. smithii* (C₃ grass; a, c, e) and *B. gracilis* (C₄ grass; b, d, f) in June and July. Colors correspond to treatment (orange = ambient (AMB), teal = seasonally shifted (SEAS)). Variables descriptions: g_s , stomatal conductance to water vapor; V_{cmax} , maximum rate of Rubisco carboxylation; J_{max} , maximum rate of electron transport for RuBP regeneration.

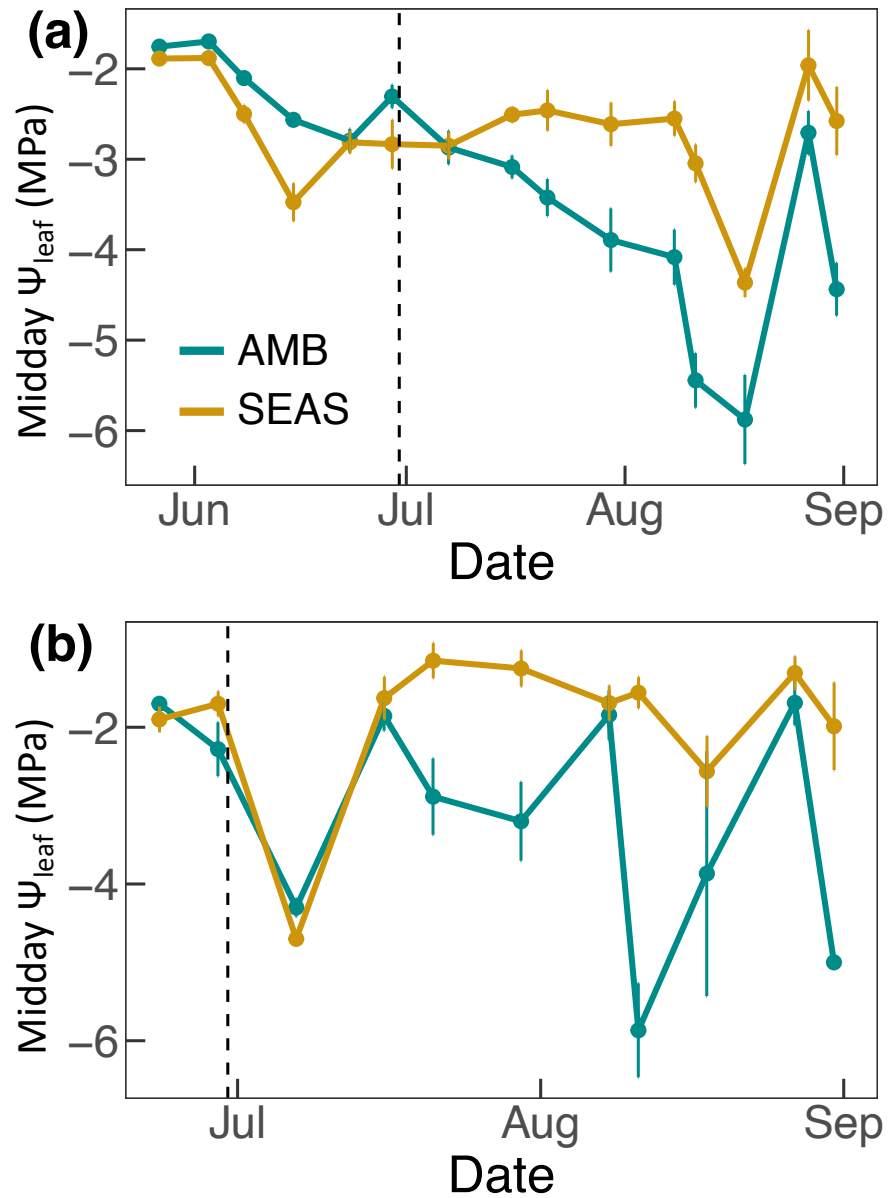


Figure A1.4. Mean ($\pm$ standard error) mid-day leaf water potentials of dominant (a) C₃ (*Pascopyrum smithii*) and (b) C₄ (*Bouteloua gracilis*) grasses throughout the growing season. Dashed line represents timing of roof removal.

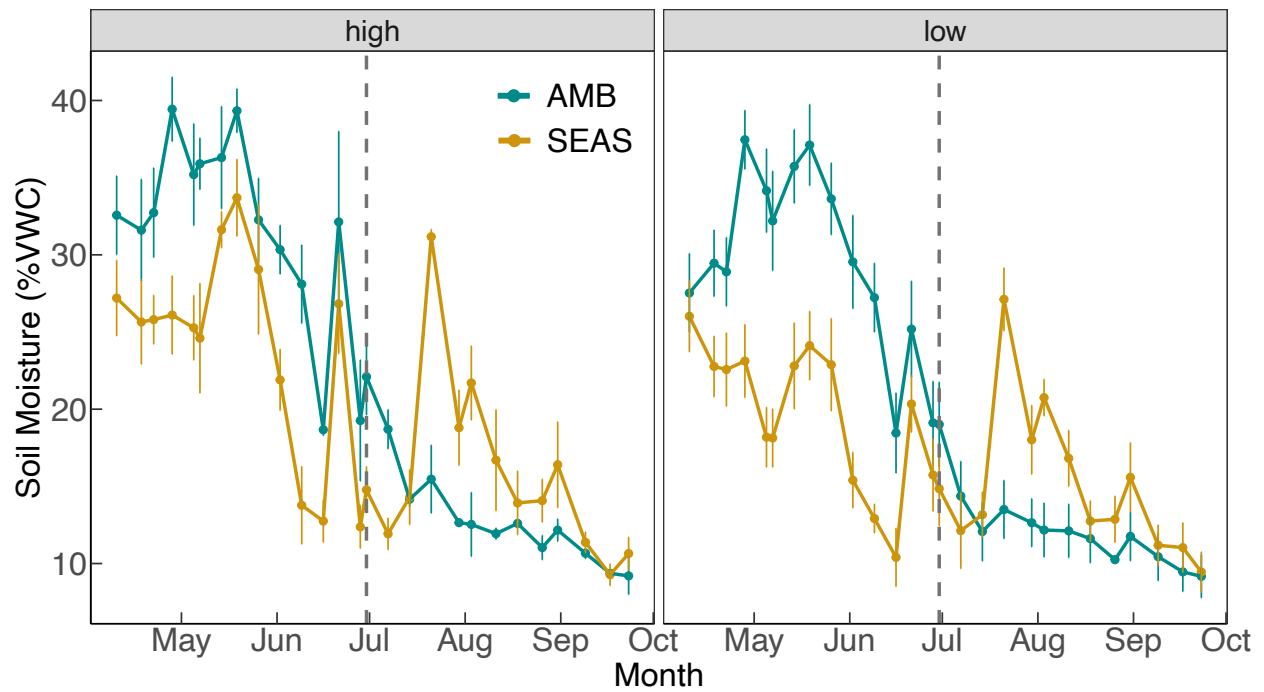


Figure A1.5. Mean ($\pm$ standard error) 20 cm soil moisture for each treatment categorized by %C₄ cover where low corresponds to < 5% and high > 25% cover. Dashed line represents timing of roof removal.

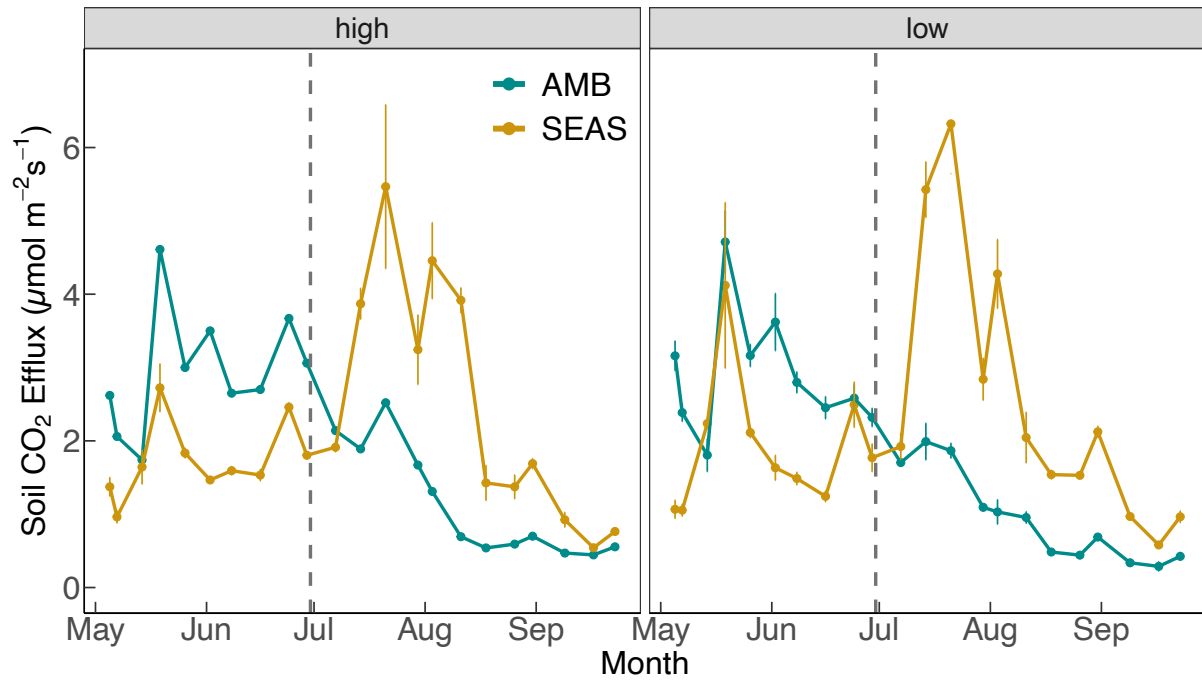


Figure A1.6. Mean ($\pm$ standard error) 20 cm soil moisture for each treatment categorized by %C₄ cover where low corresponds to < 5% and high > 25% cover. Dashed line represents timing of roof removal.



Figure A1.7. Photograph showing shelter design to exclude precipitation. Rainout shelters were $2.44 \text{ m} \times 3.05 \text{ m}$ and made of clear corrugated polycarbonate (Suntuf, Palram Americas).

APPENDIX 2

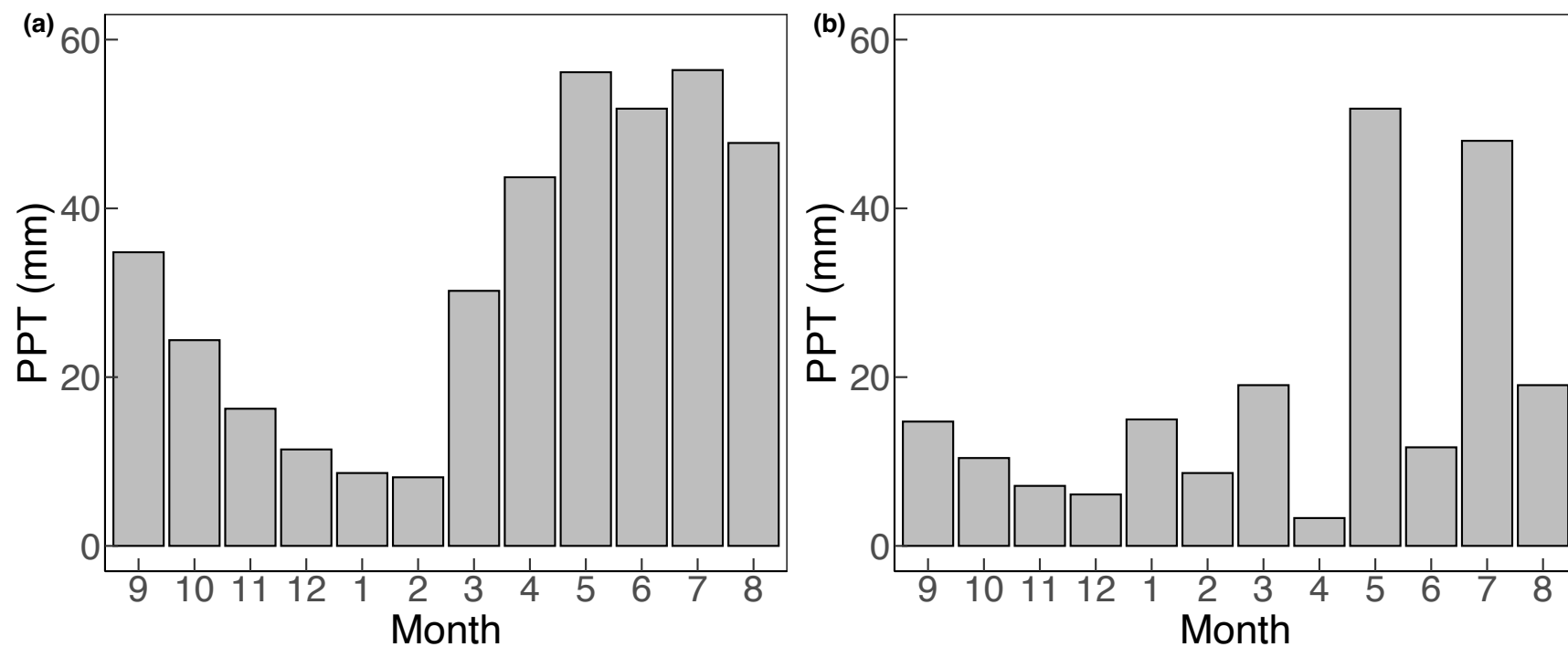


Figure A2.1. Monthly precipitation totals (in mm) based on (a) long-term normals and (b) observations from for Sept. 2021 – Aug. 2022 to encompass the entire experimental period. Both datasets are taken from a nearby weather station (NUNN 7 NNE, CO US, 40.7063, -104.7833).

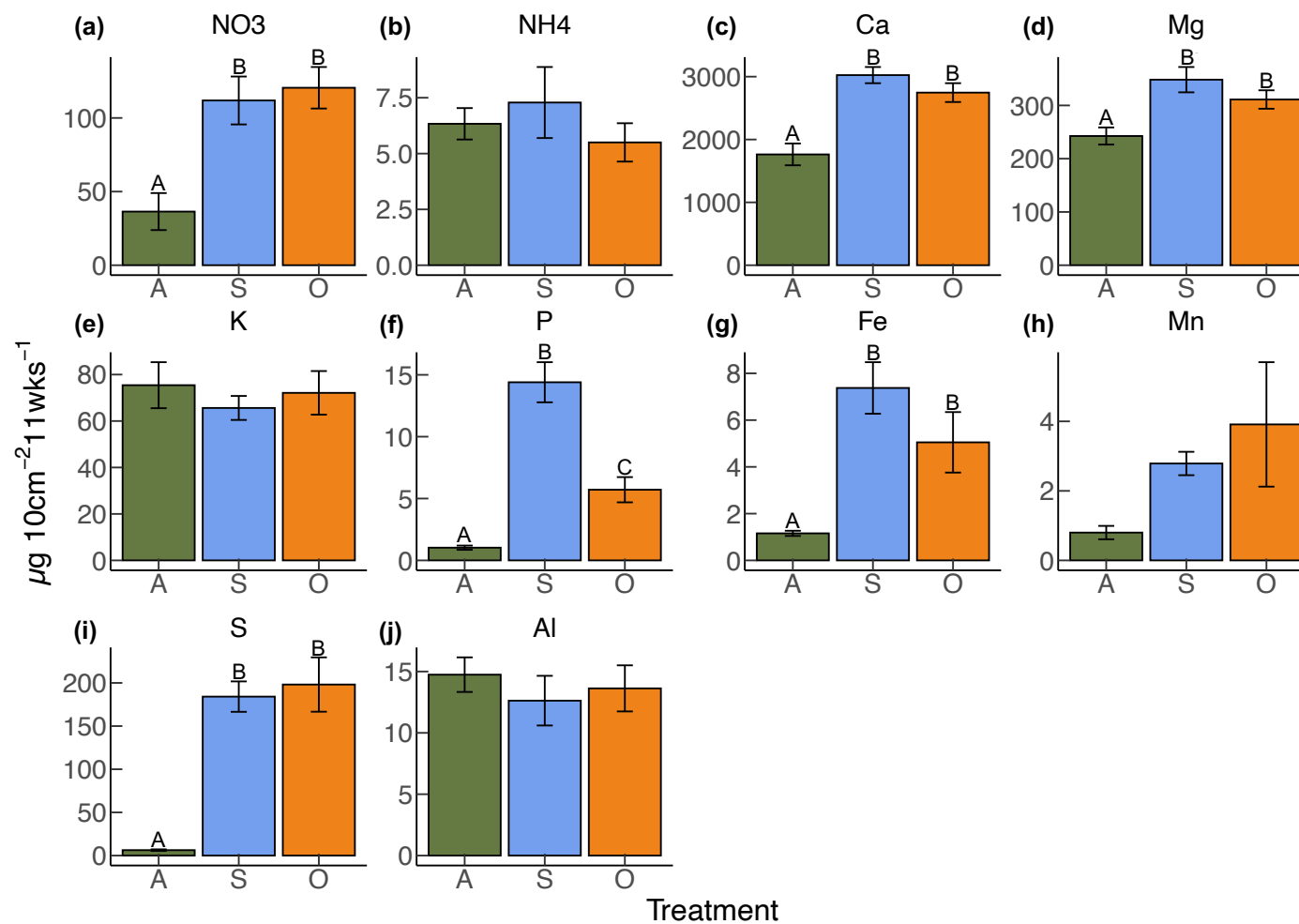


Figure A2.2. Mean ($\pm$ SE) values in $\mu\text{g } 10\text{ cm}^{-2} \text{ 11 weeks}^{-1}$ for (a) nitrate, (b) ammonium, (c) calcium, (d) magnesium, (e) potassium, (f) phosphorus, (g) iron, (h) manganese, (i) sulfur, and (j) aluminum during the fall (Sept. 9 – Dec. 3, 2021). Letters denote significant differences between treatments ($P < 0.05$).

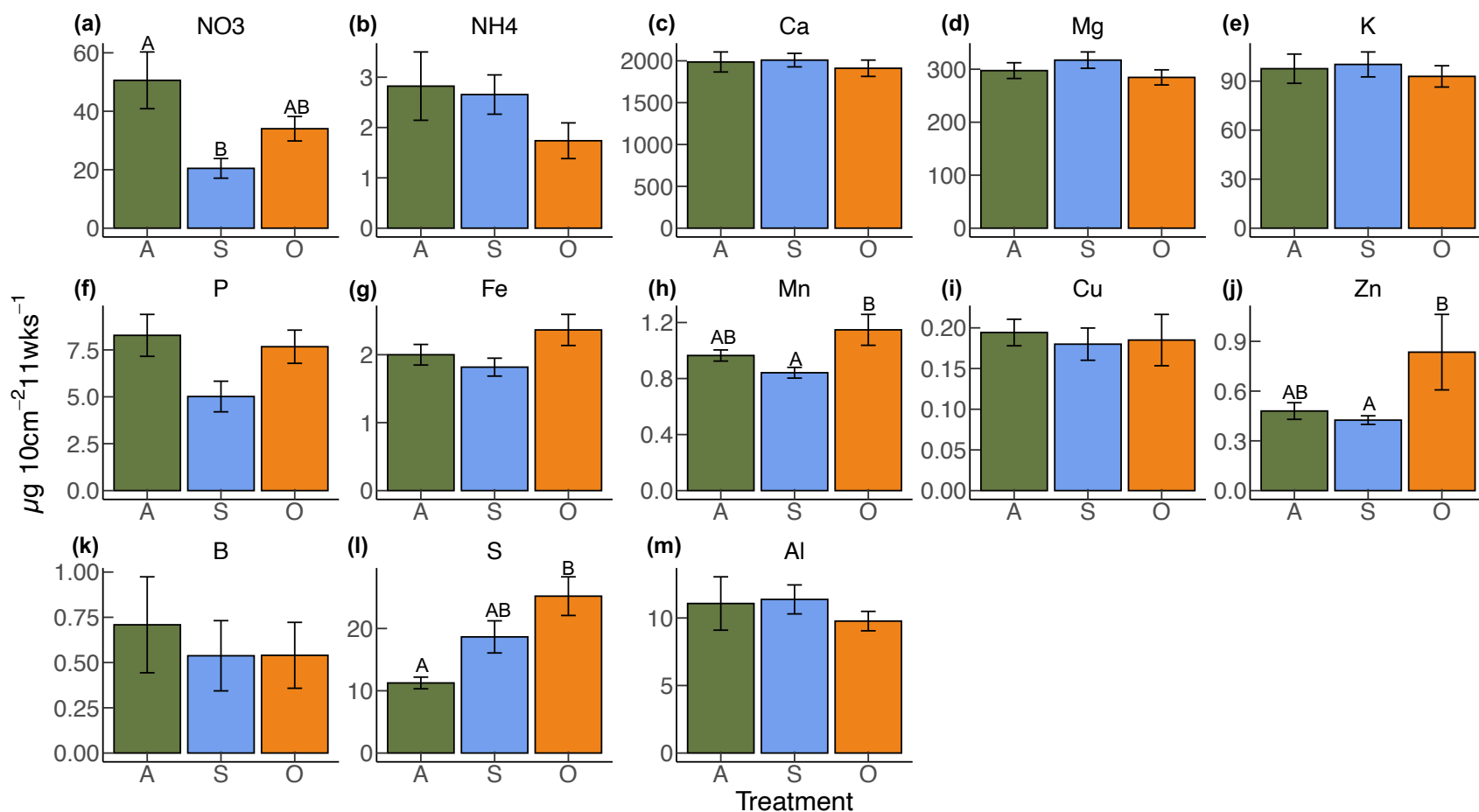


Figure A2.3. Mean ($\pm$ SE) values in $\mu\text{g } 10\text{ cm}^{-2} \text{ 11 weeks}^{-1}$ for (a) nitrate, (b) ammonium, (c) calcium, (d) magnesium, (e) potassium, (f) phosphorus, (g) iron, (h) manganese, (i) copper, (j) zinc, (k) boron, (l) sulfur, and (m) aluminum during the spring (Mar. 16 – June 22, 2022). Letters denote significant differences between treatments ($P < 0.05$).

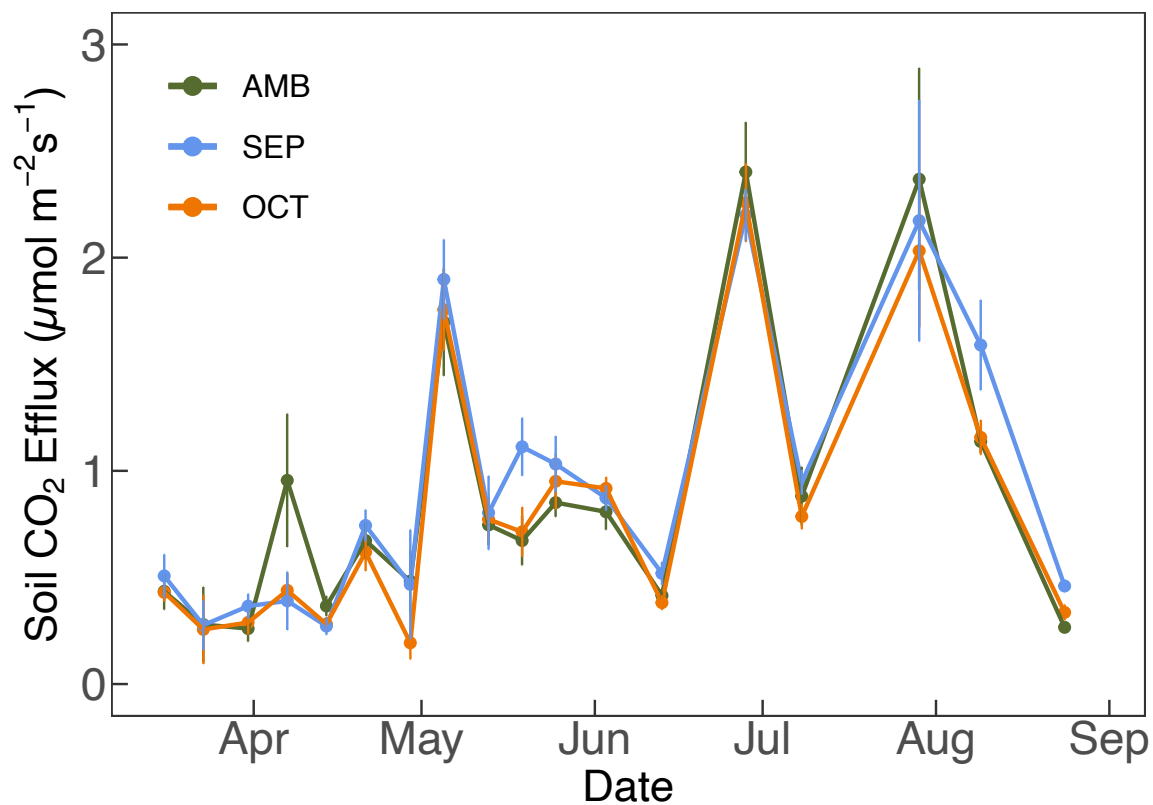


Figure A2.4. Mean ($\pm$ SE) soil CO₂ efflux ($\mu\text{mol m}^{-2}\text{s}^{-1}$) during the response period (Mar. 15 – June 30). Lines represent each treatment: AMB = ambient precipitation (green line), SEP = Sept. deluge (blue line), and OCT = Oct. deluge (orange line).

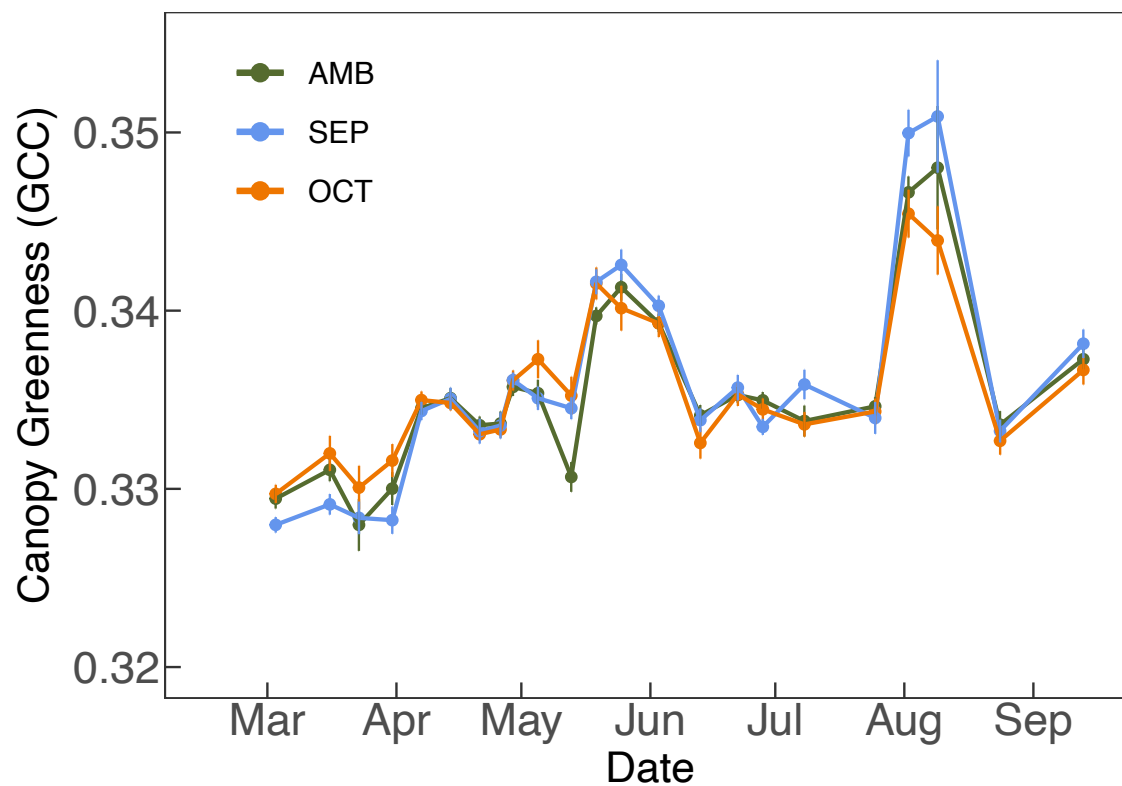


Figure A2.5. Mean ($\pm$ SE) canopy greenness (green chromatic coordinate index, GCC) during the response period (Mar. 15 – June 30). Lines represent each treatment: AMB = ambient precipitation (green line), SEP = Sept. deluge (blue line), and OCT = Oct. deluge (orange line).

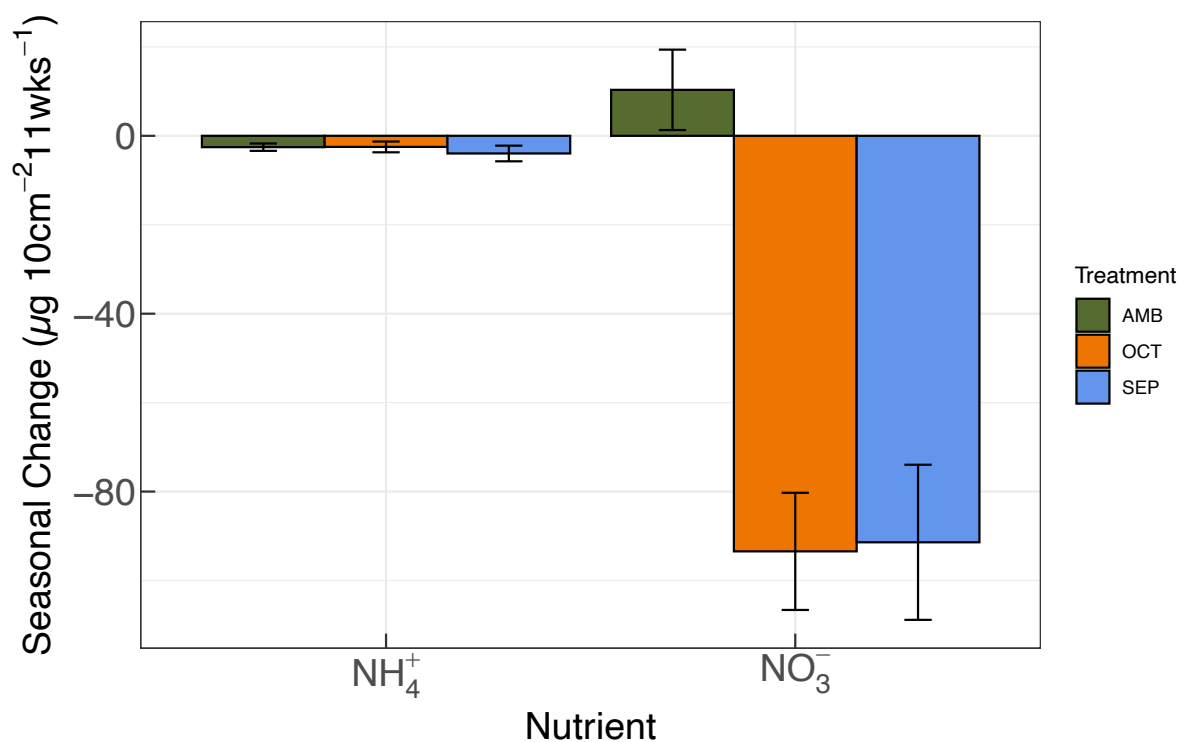


Figure A2.6. Mean difference ($\pm$ SE) in ammonium (NH_4^+) and nitrate (NO_3^-) availability between the spring and autumn for the different treatments (AMB = ambient precipitation (green bars), SEP = Sept. deluge (blue bars), and OCT = Oct. deluge (orange bars)).