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The Effect of the Foresummer Drought on Carbon Exchange in Subalpine Meadows

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Abstract

Climate in subalpine meadows of the Rocky Mountains can be characterized by an early (foresummer) drought that occurs after snowmelt (May) and lasts until the start of the summer monsoon season (July). Climate change models predict an increase in the length and severity of this dry period due to earlier snowmelt dates, rising air temperatures, and shifts in the start and/or intensity of the North American monsoon. However, it is unknown how changes in the severity of this early season dry period will affect ecosystem carbon exchange. To address the importance of early season drought, we combined a watering manipulation with 11 years of ecosystem carbon exchange data across an elevational gradient at the Rocky Mountain Biological Laboratory in Gothic, Colorado. Long-term trends reveal that earlier snowmelt dates lead to a decrease in net ecosystem productivity (NEP), in part because of the positive effect on early growing season drought conditions. Manipulating the strength of the foresummer drought by watering revealed that the timing of growing season precipitation is more important than the total amount for determining cumulative NEP. The strength of the foresummer drought did not significantly impact ecosystem respiration rates, but plants that experienced a strong foresummer drought exhibited more water stress, and lower instantaneous rates of NEP, even during the rainy season. Our results highlight the central role of the foresummer drought in determining rates of carbon exchange throughout the growing season, and the potential for an increasingly negative balance of carbon in subalpine meadows under future climate change.

Key words: climate change; sualpine; NEP; foresummer drought; elevation gradient; watering manipulation.

Introduction

Hydroclimate plays an important role in ecosystem carbon exchange in western North America (Schwalm and others 2012). In these arid systems, soil moisture positively affects the rates of photosynthesis by plants (Farquhar and Sharkey 1982), as well as respiration by soil microbes (Ryan and Law 2005). Global climate change predictions point to shifts in atmospheric circulation patterns over mid-

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latitudes that may alter the timing and duration of both winter snowstorms and summer monsoon events in western North America (IPCC 2013). We know that the timing of precipitation events can affect rates of carbon exchange (Huxman and others 2004), but how these changing precipitation regimes may influence terrestrial carbon fluxes and storage is not well understood. The timing of the arrival and duration of the North American monsoon may be particularly important, as monsoonal moisture contributes much of the summer precipitation that falls in the western United States (Adams and Comrie 1997; Higgins and others 1997). Additionally, moisture from the previous growing season can play an important role in carbon fluxes (Potts and others 2006; Patrick and others 2007).

Recent global climate model ensembles (for example, CMIP5) predict a shift toward lower snowfall, earlier snowmelt, and faster runoff in western North America(Higgins and Shi 2001), becoming widespread by the late Twenty-first century (Diffenbaugh and others 2013). Additionally, some studies project a redistribution of precipitation in the summer monsoon regime toward reduced spring precipitation and increased late monsoon precipitation (Rauscher and others 2008; Biasutti and Sobel 2009; Seth and others 2011). Together, these changes would increase the length of time between snowmelt and the occurrence of the summer monsoon, or the 'foresummer' part of the growing season. Currently, the intermountain west experiences a foresummer drought of variable strength (magnitude and duration) (see supplemental Figure 3). Low snowpack years with faster melting snow would expose plants to potentially longer and drier periods before the onset of monsoonal moisture. With the added predictions of increasingly warmer temperatures, this foresummer period could become more drought-like. A valuable question therefore is how important is the magnitude and duration of this 'foresummer' period for carbon flux in western North America?

The Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado (elevation 2900 m), experienced a strong foresummer drought in 2012. This drought was caused by a dramatic shift in the jet stream, low snowpack, and high temperatures (Kellner and Niyogi 2014). The shift in the jet stream led to reductions in snowpack in the mid-latitudes as well as a reduction of summer rainfall before the July Monsoons (supplemental Table 1, supplemental Figure 3). The 2011–2012 winter had a low snowpack and the second earliest snowmelt date recorded in RMBL history. Total snow accumulation during winter in 2011–2012 was 640 cm, which is about half as much as average (1117 cm). The date of first bare ground was April 23rd, about a month earlier than average (May 21). The early season also experienced higher than average temperatures (supplemental Table 1), which dried out soil due to increases in evaporation. Total growing season precipitation in 2012 was 34% of average. Winter precipitation averages are for the time between 1974 and 2011 (available at www.rmbl.org). In short, in 2012, this area experienced many of the climate characteristics that are identified by future climate projections.

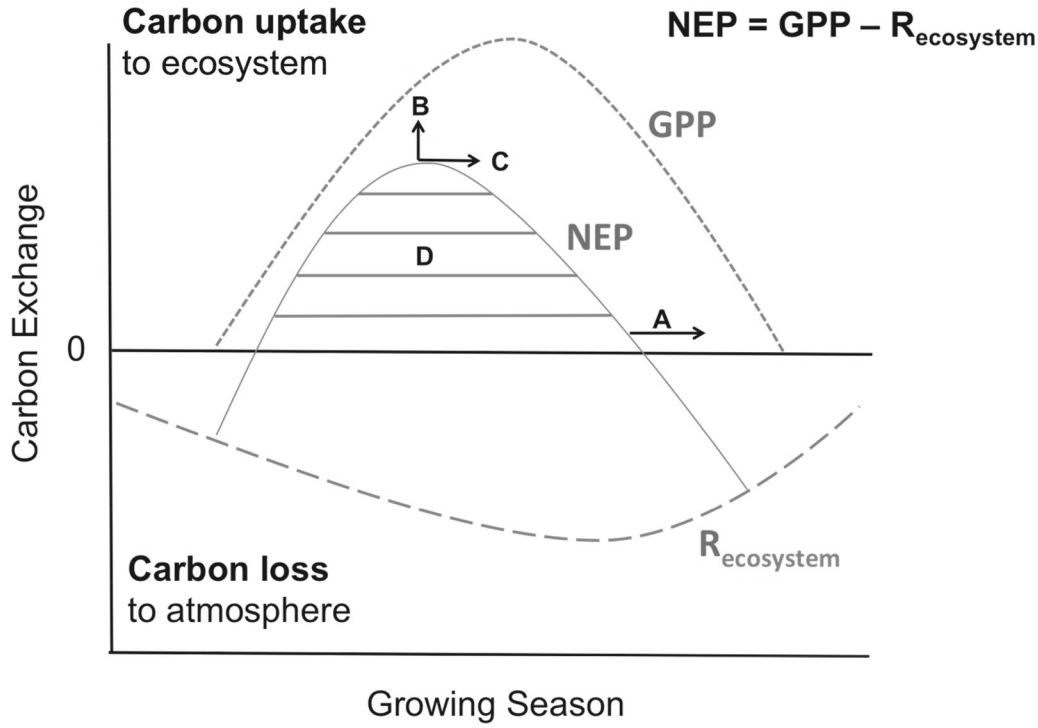
To understand how variability in the timing and strength of the foresummer drought affects ecosystem carbon exchange and plant water status, we conducted a watering experiment during the summer of 2012. The watering manipulation was designed such that the un-watered ''control'' group served as the most severe drought treatment, and three other treatment groups incrementally decreased the length and severity of the dry period. We also re-measured the same plots the following year (2013) to determine if antecedent soil moisture affected rates of carbon exchange. To assess the generality of this experiment, we then compared our findings with 11 years of carbon flux monitoring along a nearby elevational gradient that spans from Sagebrush-steppe to high subalpine meadows below tree line (2475–3380 m.a.s.l.) (Johnston and others 2001). The 11 years between 2003 and 2013 experienced a wide range of foresummer soil moisture conditions between severe drought and wetter than average (−3 to +1 on the Palmer drought index) (Colorado Climate Center 2013).

Comparing and synthesizing results from a manipulation and a long-term gradient experiment allows for the identification of robust trends in ecosystem responses to climate change (Dunne and others 2003). Our manipulative experiment allows for a direct analysis of the effect of changes in foresummer soil moisture on NEP independent of yearly climate (for example, snowmelt date, temperature, solar radiation) or changes in community composition. Indeed, such climate change manipulations have recently been argued for in the literature (Thompson and others 2014). Combining a manipulative experiment with observations from a long-term gradient experiment allows for the analysis of ecosystem response to natural variations and facilitates the identification of thresholds and tipping points (Dunne and others 2004; Kreyling and others 2013).

Box 1 Net ecosystem productivity (NEP) is metric which includes aboveground and belowground inputs to ecosystem carbon flux.

NEP is the gross primary productivity (GPP) of plants minus respiration by autotrophs and heterotrophs ($R_{ecosystem}$) (Begon and others 2009). NEP is often equated to NEE, or the inverse of NEE (Kirschbaum and others 2001). At night when plants are respiring NEP would be the same as $R_{ecosystem}$ (Reay and others 2007). NEP is measured as a rate—in this case it is measured in units of μ moles of CO_2 per m^2/s. This rate can be positive or negative, depending on whether or not the plants and soil together are taking in CO_2 or releasing it into the atmosphere.

The NEP curve over the course of the growing season can be predicted to change in a number of ways in response to experimental or natural variation in available moisture. Plants could remain active later in the season (*A*). The rate of peak uptake could increase (*B*) or shift in time (*C*). Any of these changes have the potential to influence cumulative NEP (*D*).



Together, experimental and natural variations in soil moisture allow us to address a number of questions about carbon dynamics in this system:

(1) *How does the length and severity of the foresummer drought affect carbon fluxes in the subalpine zone*? In this system, plant productivity has been shown to be primarily limited by water availability (Lamanna 2012). Thus, we would predict that changes in soil moisture availability could lead to a number of changes to the seasonal NEP curve (Box 1). The length of the growing season could be extended (Box 1 part A), and the rate of peak uptake could increase (Box 1 part B) or shift in time (Box 1 part C); these changes could influence cumulative NEP (Box 1 part D).

(2) *Do results from the watering manipulation match those found through natural variation in precipitation, and do these relationships change over an elevational gradient*? Given the primary role of soil moisture in plant productivity, we would expect the relationship between carbon fluxes and soil moisture to be similar regardless of manual manipulation or natural variation. We also expect that higher elevation sites, which have a longer duration of snow cover and a shorter growing season, may be less affected by foresummer drought because they will experience less water stress due to high water availability in the soil.

(3) *Are there year-to-year time lags in precipitation effects on carbon flux*? We re-visited the watering manipulation the following growing season to assess antecedent watering effects (Potts and others 2006). Specifically, we assessed if the watering treatment in 2012 influenced soil nutrients and

carbon exchange the following year, and if the water-balance of the previous year influenced carbon exchange along the gradient.

Methods

Experimental Design and Site Description

The watering manipulation took place in the East River valley on the site of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado (30°57′N, 106°59′W, 2900 m elevation). Subalpine meadows in this location are characterized by dry, rocky soil, and long-lived perennial plants. The site of the experiment is dominated by herbaceous plants and grasses, within a mosaic of shrubs and aspen-spruce forest (Langenheim 1962), although the slope in which the experiment took place was naturally free of trees and shrubs. ''Ecosystem'' level measurements for this experiment refer only to herbaceous and graminoid-dominated meadows. Conclusions should not be drawn about how communities with shrubs respond to watering manipulation, as shrubs have distinct functional responses to drought. Plots were south to southwest facing with a slope of about 15°. A list of species on all plots and their abundances is given in supplemental Table 2.

Five 1.0 m × 1.0 m plots for each of four treatments were randomly established in a grid at least 5 m distance apart from one another. There was no significant difference in soil moisture (supplemental Figure 1) or soil carbon (supplemental Figure 2) between plots at the beginning of the growing season. Since 2012 was a severe drought year for this system, the non-manipulated group experienced the longest drought duration. Water was applied to the ''May'' treatment group from May 15–May 31 (total 87.1 L—1.74 cm per plot), to the ''June'' treatment group from May 15–June 30 (total 155.2 L—3.10 cm per plot), and to the ''July'' treatment group from May 15 to July 31 (total 314.2 L—6.28 cm per plot), thereby creating three different drought scenarios. Water was applied to plots approximately five times a week at dusk using a watering can or backpack sprayer. Water that was treated with chlorine was exposed to the air for least 48 h to allow chlorine to evaporate. The amount of water applied to each treatment was calculated by taking the maximum precipitation from that month in the last 30 years and adding this amount in addition to the natural precipitation that fell during the experiment (Long term weather data collected at RMBL by the EPA Castnet station GTH161, latitude 38.95627, longitude-106.98587, elevation 2915 m.a.s.l.). A majority of watering experiments add or remove water equal to extremes from the recent past (Beier and others 2012). However, studies that add water equal to the recent extremes do not provide the same 'wet' conditions as a naturally occurring extremely wet year because humidity and atmospheric pressure are not altered (Beier and others 2012). Further, added water to an experimental plot is absorbed by the dry soil surrounding the watering treatment. Thus, we chose a volume of water equal to the maximum monthly precipitation in the recent past instead of the mean in an attempt to provide an upper limit for watering while not delivering a watering regime that exceeded recent experience.

Table 1. Latitudes, Longitudes, and Elevations of the Study Sites

Gradient sites		
Elevation (m)	N latitude	W longitude
2475	38°39′16″	106°51′40″
2700	38°52′55″	106°58′46″
2815	38°53′50″	106°58′43″
3165	38°57′38″	107°01′53″
3380	38°58′10″	107°02′28″

Elevational Gradient

We assembled 11 years (2003–2013) of ecosystem carbon flux data along an elevational gradient that ranged between 2460 and 3380 m above sea level and spanned a geographic distance of 39 km. The elevational gradient contains five long-term study sites that run from dry, shrub-dominated sagebrush-steppe (2475 m.a.s.l.) through the subalpine zone, to just below tree line (3380 m.a.s.l) (Table 1). These long-term study sites were established by Enquist in 2003 and have been sampled every year since. The gradient spans the Washington Gulch and East River valleys near RMBL, Crested Butte, and Almont, CO, USA. Each study site is approximately 50 m^2 and is characterized by similar local slope, aspect, and vegetation physiognomy. All sites consist of a mixture of shrubs, grasses, and forbs. There is substantial turnover of plant species between sites with very few of the 120 species sampled occurring in more than two of the sites (Bryant and others 2008). Additionally, shrub cover across the gradient decreases from a high of

33% at the lowest elevation site to 0% at the highest. A species list for each site is given by Bryant and others (2008). Every year, five 1.3 m × 1.3 m plots were established haphazardly along the local slope of each study site, with at least five meters distance between plots.

Equipment and Measurements: Carbon Flux

Carbon flux was measured as instantaneous daytime peak uptake (ca. 10:00) and nighttime peak respiration (ca. 22:00) (see Saleska and others 1999 for diurnal measurements). Ambient CO_2 was measured by a Li-Cor 7500 infra red gas analyzer for 30 s, and then the tent was put in place over the plot and the CO_2 concentration within the tent was measured for 90 s (Jasoni and others 2005). Daytime measurements were only taken under cloudless conditions. The tent was designed to let in 75% of photosynthetically active radiation (tent fabric by Shelter Systems). Air inside the tent was well mixed by fans, and the tent chamber was sealed using a long, canvas skirt along the base of the tent that was covered with a heavy chain. The volume of the tent used along the gradient was 2.197 m^3, whereas a smaller tent of volume 0.125 m^3 was used in the watering experiment. Although the tent flux method (described by Jasoni and others 2005) does not offer the same spatial coverage as continuous flux measurements such as eddy flux towers, the portable design does allow the ability to rapidly assess carbon flux at a wider variety of locations within and across experimentally manipulated and control plots (Arnone and Obrist 2003).

Each watering treatment plot had a ¼ m buffer outside of the tent flux area that was treated with water, and used for soil cores, soil efflux, and plant water potential measurements. Soil efflux was measured at the same time as NEP using a Li-Cor 6400 portable photosynthesis machine with the soil chamber. The soil chamber fits inside a PVC soil collar, which was placed in the plot at least two weeks prior to the first measurement. PVC soil collars had four holes (~2 cm diameter) drilled into the side. The holes were oriented so that they were under the soil and about 2 cm of PVC remained above ground. Soil efflux was measured in two places in each plot along the gradient and one place per plot for the manipulation. Measurements for the watering experiment were taken every other week due to equipment and weather constraints. Carbon flux measurements along the elevational gradient were taken 4 weeks after snowmelt and then again at peak season (approximately 4 weeks after the first measurement, or when the majority of plants reached maximum height). Measurements for the watering manipulation were taken once every 2 weeks for the 2012 and 2013 growing seasons. Carbon flux data were calculated the same way for the gradient and manipulation. To determine NEP, we fit a linear model to the carbon flux measurements following Jasoni and others (2005).

Environmental Variables

Weather stations provided information on temperature, precipitation, and in some cases solar radiation. Soil moisture and temperature at 10 cm depth were logged once an hour by HOBO data loggers (Onset). Soil moisture was also measured gravimetrically, and percent soil carbon was analyzed using 10-cm soil cores. The Palmer Drought Index (PDI) values were taken from the Colorado Climate Center, and are resolved to the Central Mountains region (http://ccc.atmos.colostate.edu/wendy/spi/reg24.out). A PDI value of −2 is a moderate drought; PDI of −3 is a severe drought, PDI of 1 is a 'wet' year and PDI of 0 is a 'normal' year in terms of soil moisture (details can be found at http://ccc.atmos.colostate.edu).

Water Potential

When plants absorb less water from the environment through their roots than is transpired from their leaves water potential (Ψ) decreases and drought stress develops (Tezara and others 1999). Pre-dawn (minimum) shootwater potential (Ψ_{PD}) measurements were made with a pump-up pressure chamber (PMS Instrument Company, Corvallis, OR, USA). Measurements were taken at two time points June 27th and July 19th. At least one stem of the most abundant individual, *Viguiera multiflora* was sampled in the buffer zone of each of 20 plots during each time period. The buffer zone of the plot received the same watering treatment, but was located just outside of the flux measurement area.

Biomass

A multivariate model was used to estimate the aboveground biomass on each plot. This model was based on destructively harvesting biomass across the elevational gradient. This model uses the height and percent cover of herbs, shrubs, and grasses on each plot to calculate the total aboveground biomass in grams of carbon ($R^2 = 0.78$, $P < 0.0001$) (Lamanna 2012).

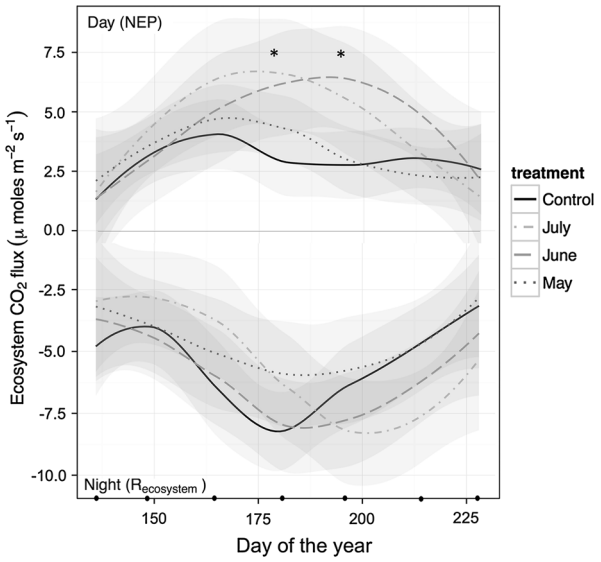


Figure 1. Daytime (*top*) and nighttime (*bottom*) ecosystem CO_2 flux rates for each treatment over the growing season. *Black dots* along the *x*-axis show measurement times. *Shadows* represent 95% confidence intervals for the five replicates of each treatment. *Lines* are smoothed using a loess function. *Daytime*—the plots watered through June or July have significantly greater rate of daytime CO_2 flux (NEP) during the peak season than the plots watered only in May and the control plots (ANOVA, $df = 1$, $F = 4.956$, $P = 0.0398$), denoted by *asterisks*. There is no difference between treatment groups during the last time period (ANOVA, $df = 3$, $F = 0.344$, $P = 0.79$), suggesting that treatment did not influence the length of the growing season. *Nighttime*—there were no significant differences between treatment groups for nighttime ecosystem respiration (ANOVA, $df = 1$, $F = 0.096$, $P = 0.76$).

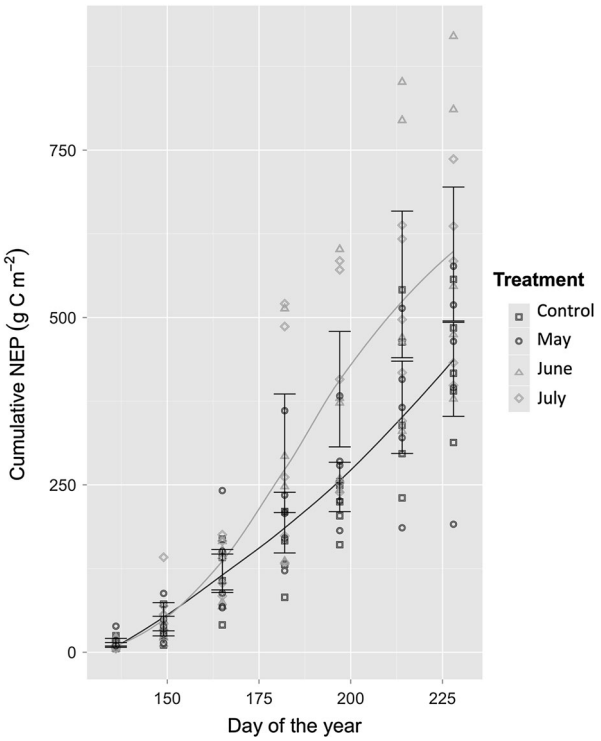


Figure 2. Cumulative NEP throughout the growing season. Data points are plot-level values with watering treatments indicated by *shape*. Plots watered through June are shown in *gray* and plots not watered through June are shown in *black*. *Bars* represent bootstrapped 95% confidence intervals.

Results

Watering Experiment

NEP

Measurements of peak daytime net ecosystem productivity (NEP—defined in Box 1) followed a peaked distribution over the course of the growing season whereby the compensation point was reached simultaneously by all treatment groups, but the peak rate of uptake was reached early in the season, around mid-June, for May and control groups. In contrast, June and July treatment groups experienced a significantly later peak rate of uptake around early July, and a greater peak rate of uptake (ANOVA, $df = 1$, $F = 4.956$, $P = 0.0398$) (Figure 1). Differences in the rate of peak carbon uptake between groups contributed to differences in cumulative NEP, visually depicted in Box 1 part 'D'. Together, all plots watered through June had significantly higher cumulative daytime NEP values than those plots that were not watered or watered only in May (ANOVA, $df = 1$, $F = 4.507$, $P = 0.048$; Figure 2—last time period). Although watering treatments led to significant differences in the rate of ecosystem carbon uptake during the daytime, they did not lead to significant differences at night. There were no differences in peak season $R_{ecosystem}$ (ANOVA, $df = 1$, $F = 0.096$, $P = 0.76$), or cumulative differences in $R_{ecosystem}$ between groups (ANOVA, $df = 1$, $F = 2.254$, $P = 0.151$). Note that $R_{ecosystem}$ is equivalent to nighttime NEP (defined in Box 1).

NPP

There are two ways to estimate the contribution of plants to the uptake of carbon (NPP) in this system: using gas exchange measurements or by calculating the overall green plant biomass accumulation at the end of the growing season. The first method requires subtracting the rate of daytime soil respiration from the rate of ecosystem productivity (NEP). This was done by measuring rates of soil efflux using a Li-Cor 6400 with a soil chamber

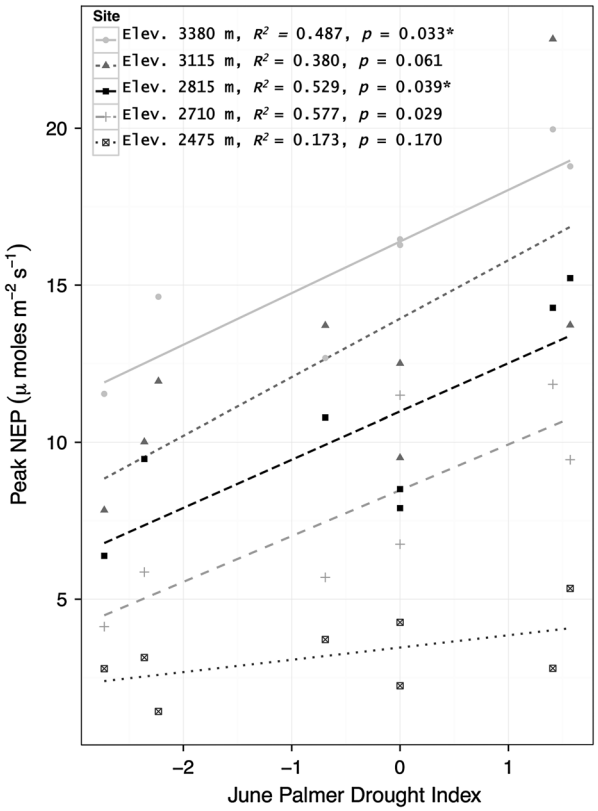


Figure 3. Peak NEP is positively correlated with the foresummer drought. Each *line* represents a site and each data point represents the average of five plots per site in a given year. The relationship between NEP and June Palmer drought index (PDI) is significant (or nearly significantly) in all four sites along the subalpine gradient (p values <0.05 are given an *asterisk*). The lowest site does not show a significant relationship between drought and the rate of peak carbon uptake. This figure was revised from Lamanna (2012).

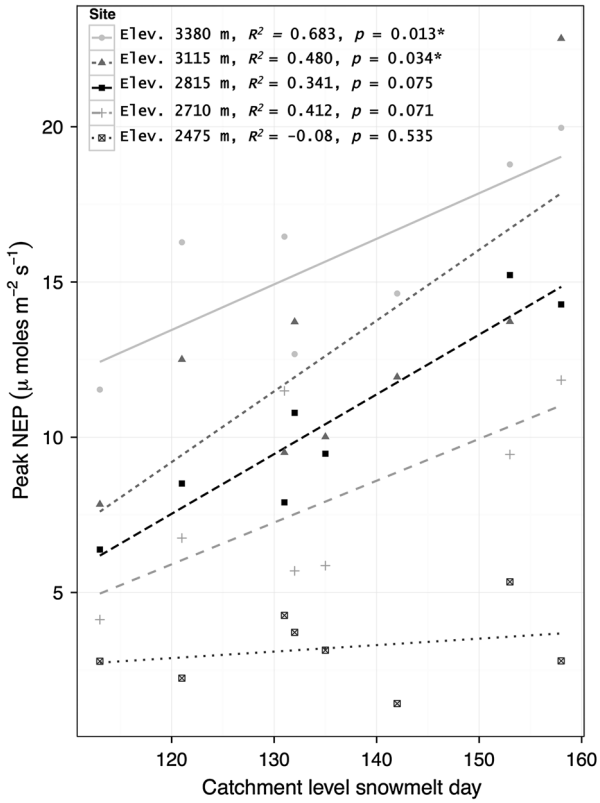


Figure 4. Peak NEP vs. catchment-level snowmelt date depicted as the day of the year. Each *line* represents a site and each data point represents the average of five plots per site in a given year. Peak season NEP decreases with earlier snowmelt dates, except at the lowest elevation (sagebrush-steppe) site, which shows no relationship between NEP and snowmelt date (p values <0.05 are given an *asterisk*).

attachment. The soil efflux measurements were scaled to the amount of bare ground in each plot in order to obtain a rough estimate of plot-level heterotrophic respiration at the time of the measurement. This value was subtracted from the NEP value obtained during the same measurement period in order to obtain an, admittedly very approximate, estimate of NPP. This method revealed that plots watered through June had a significantly greater rate of NPP than those that were not (ANOVA $df = 1$, $F = 1.328$, $P = 0.0484$; supplemental Figure 4). The latter method requires estimating biomass with a model that uses the height and cover of plants to estimate biomass based on harvesting biomass in previous years. It is notable that this method only estimates aboveground biomass and is only possible because there are no woody plants present on these plots. The plant biomass method corroborates this result, albeit at a slightly smaller significance level (ANOVA, $df = 1$, $F = 3.61189$, $P = 0.0569$).

Soil

The watering treatment significantly increased the soil moisture of currently watered plots (that is, watering made plots significantly more wet, supplemental Figure 1, ANOVA, $P < 0.05$). Watering did not affect soil temperature during the day (ANOVA, $df = 3$, $F = 0.416$, $P = 0.742$), or during the night (ANOVA, $df = 3$, $F = 0.002$, $P = 0.953$). Treatment did not lead to significant differences in soil respiration during the day (supplemental Figure 5, ANOVA, $df = 3$, $F = 0.917$, $P = 0.438$), although nighttime soil respiration increased with watering treatment (ANOVA, $df = 3$, $F = 3.584$, $P = 0.0156$). Watering treatments applied in 2012 did not significantly affect soil moisture at the start

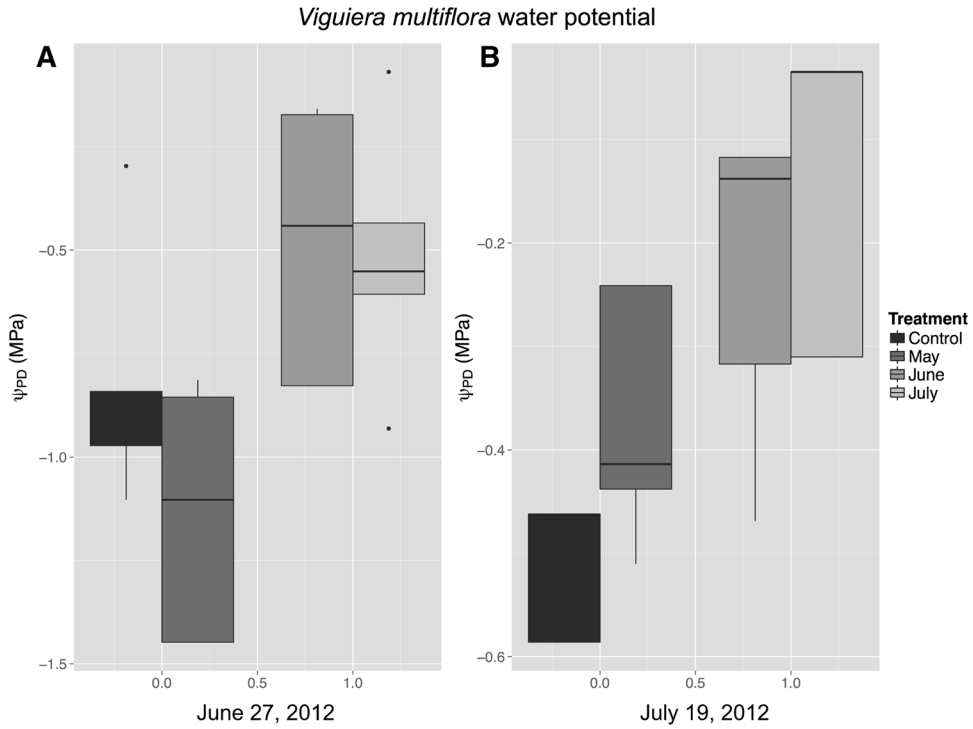


Figure 5. Boxplot of daily minimum (pre-dawn) water potential (Ψ_{PD}) measurements for the most common species, *Viguiera multiflora*, in each watering treatment. The upper and lower hinges of the box correspond to the first and third quartiles. The upper/lower whisker extends from the hinge to the highest/lowest value that is within 1.5 times the distance between the first and third quartiles. Data beyond the whisker are plotted as *points*. The *black line* indicates the median. More negative Ψ_{PD} values indicate more water stress. Measurements were taken using a pump-up chamber (PMS Instrument Company) on June 26th and July 19th, 2012. Tukey's highly significant difference test reveals that all watering treatment groups are significantly different from one another except for June and July ($P < 0.05$). Plants that were watered through the foresummer drought (June) experienced less water stress than those plants that were not. Plants that experienced the strong foresummer drought (control and May groups) experienced low water potential values even in July during the summer rain.

of the following growing season (May 13, 2013, ANOVA, $df = 3$, $F = 1.796$, $P = 0.191$), or rates of daytime or nighttime soil respiration at the start of the following growing season (May 13, 2013, ANOVA, daytime: $df = 3$, $F = 1.289$, $P = 0.312$, nighttime: $df = 3$, $F = 0.623$, $P = 0.61$).

Soil organic carbon was not statistically different between treatments (supplemental Figure 2), but during the last time period, the May and Control groups had more soil organic carbon than the June and July groups at a nearly significant level (ANOVA, $df = 1$, $F = 3.893$, $P = 0.064$). This result suggests that the increased respiration rates in the watered plots may have resulted in decreased soil carbon at the end of the growing season. Slight differences in soil carbon carried over to the beginning of the 2013 growing season. On May 13, 2013, there was more soil organic carbon in the May and Control plots than in the June and July plots (ANOVA, $df = 1$, $F = 3.86$, $P = 0.065$).

Water Potential

We found that there were significant differences in Ψ_{PD} among treatment groups in the common plant, *Viguiera multiflora,* on June 27 (one-way ANOVA, $df = 3$, $F = 32.12$, $P < 0.001$; Figure 5) and July 19th (one-way ANOVA, $df = 3$, $F = 23.98$, $P < 0.001$; Figure 5). Those plots that were watered more recently typically exhibited less negative Ψ_{PD}. A post-hoc Tukey's Highly Significant Difference test shows that all pairs of treatments (except June–July) are significantly different from one another in both time periods ($P < 0.05$).

Elevational Gradient

Relationship Between Palmer Drought Index and Peak NEP

All five sites along the gradient showed a positive relationship between the June Palmer drought

index (PDI) and peak season NEP. Increased drought conditions in June lowered peak season NEP. Linear regression reveals that the relationship is significant in three of the five sites (Figure 3). All elevations appeared to respond similarly to drought, except the lowest elevation site, which did not have a strong relationship to the June PDI (Figure 3). In a linear model with sites as factors, the June PDI explains 78% of the variance in peak season NEP (df = 9 and 28, F = 15.33, $P < 0.0001$, *Adj.* R^2 = 0.777), with more negative PDI values having lower rates of peak NEP. Additionally, lower PDI values lead to lower peak plant height (df = 9 and 174, F = 13.62, $P < 0.0001$, *Adj.* R^2 = 0.383) and lower overall aboveground biomass (df = 9 and 130, F = 2.106, P = 0.033, *Adj.* R^2 = 0.067). There was no relationship between the June PDI of the previous year or the previous water-year's precipitation and the present year's peak NEP.

Relationship Between Snowmelt Date and NEP

We found a significant positive relationship between catchment-level melt date and peak NEP, indicating that years with earlier melt dates had a lower rate of NEP at peak season (Figure 4). The lowest elevation site did not show a significant relationship with melt date (linear regression, *Adj.* R^2 = 0, P = 0.55). This is perhaps not surprising as this site is not consistently snow covered throughout the winter and spring. In a linear model with site included as a factor, snowmelt date *alone* explains 71% of the variance in peak season NEP (df = 9 and 28, F = 10.91, $P < 0.0001$, *Adj.* R^2 = 0.707), with earlier melt dates having lower rates of peak NEP. Additionally, earlier snowmelt dates lead to lower peak plant height (df = 9 and 174, F = 16.8, $P < 0.0001$, *Adj.* R^2 = 0.437) and lower overall aboveground biomass (df = 9 and 130, F = 3.425, $P < 0.0001$, *Adj.* R^2 = 0.136). Snowmelt date and June PDI are positively correlated with one another (linear regression, df = 1 and 257, F = 140, $P < 0.001$, *adj.* R^2 = 0.35; Figure S10).

Discussion

Watering Treatment Led to Changes in the Growing Season NEP Curve

Given that soil moisture affects ecosystem productivity, we predicted a number of potential ways that the NEP curve could have changed with watering treatment (Box 1). For example, a decrease in the length of the foresummer drought could (A) extend the effective growing season later into the summer; (B) alter the rate of peak uptake; or (C) alter the timing of peak uptake, any of which could lead to changes in (D) the total cumulative NEP (Box 1). Although there appeared to be no extension of the effective growing season (all treatment groups showed a similar decline in NEP by the end of August), there was an increase in the seasonal peak carbon uptake as well as a shift in seasonal peak uptake to later in the season (Figure 1A) resulting in significantly higher carbon uptake for plots watered through June and July than those that were un-watered or only watered in May (Figure 2). It is notable that the early growing season (May) is dominated by small plants such as *Erythronium grandiflorum* and *Claytonia lanceolata*. Thus, the response to watering early in the season may not be the same as the response to watering later in the season, as the changing species composition may have different soil moisture needs and the magnitude of carbon flux is necessarily smaller.

In general, an increase in cumulative NEP in watered plots could be attributed to three mechanisms: (i) an increase in aboveground carbon uptake due to an increase in total leaf area (biomass); (ii) an increase in above ground carbon uptake due to increased photosynthetic rates per unit leaf area; or (iii) a decrease in heterotrophic respiration. Our results are consistent with case (i). In general, biomass in the June and July watered plots was greater than the un-watered plots (supplemental Figure 4). The watering treatment did not lead to changes in soil respiration during the day (supplemental Figure 5), but it did lead to significant differences in nighttime soil respiration with more watered plots having a greater rate of soil respiration (supplemental Figure 7). However, changes in nighttime soil respiration were not enough to create statistically significant differences in $R_{ecosystem}$ between treatments (Figure 1). Differences in growing-season cumulative carbon uptake appear to be due primarily to changes in NPP due to increases in aboveground plant biomass (supplemental Figure 4).

It is important to note that inferences about carbon balance over the course of the year should be made with caution, especially given that rates of carbon exchange were only measured once every 2 weeks. For example, we attribute changes in NEP primarily to aboveground components over the course of the growing season, but small changes in rates of heterotrophic respiration can become important at longer timescales. Even though changes in heterotrophic respiration with treatment are smaller in magnitude, and not statistically different in this study, they have the potential to influence

respiration rates for the rest of the year, which could become important when considering the effects of changes in soil moisture on a yearly carbon budget.

The watering manipulation showed that stronger droughts resulted in less carbon uptake. Interestingly, the response was not linear as biomass accumulation was not proportional to the amount of added water. In fact, it appeared that there was a critical threshold around June where plants that were not watered through June were stunted (accumulated less biomass) and water stressed (showed critical levels of Ψ_{PD} in July). For example, the common herbaceous plant *Viguiera multiflora* showed significantly less water stress on plots that were watered through June (Figure 5). It is notable that the July watering treatment received roughly twice the amount of water as the June treatment, but this did not lead to significant differences in NEP, biomass, or plant moisture stress between these groups. When drawing conclusions it is important to remember that the results from watering manipulations may not parallel natural variations in precipitation as precipitation events also increase humidity and cloud cover, while manual water additions do not (Beier and others 2012).

Long-Term Patterns Along an Elevational Gradient

Analysis of an 11-year dataset along an elevational gradient in the subalpine revealed that the strength of the naturally varying foresummer drought does influence the peak rate of carbon exchange (Figure 3). As June conditions become drier, NEP declines during the 'peak' season: usually around July or approximately 8 weeks after local snowmelt when vegetation is tallest, the summer rains have started, and soil moisture is relatively high. With sites as factors in a linear model, June drought explained 77% of the variance in peak season NEP. Based on the watering manipulation we might assume that this is primarily due to soil moisture stress inhibiting aboveground biomass accumulation. It is important to note that the watering manipulation results are specific to a drought year, but the same relationship between the June Palmer Drought Index and peak season NEP holds true in years without drought(for example, a 'wet' June, PDI $>$ 1, still has a higher peak NEP than a 'normal year,' PDI = 0).

Along the gradient we also found that catchment-level snowmelt date strongly influenced peak season NEP—explaining 71% of the variance when controlling for site. Years with earlier melt dates had lower peak season NEP. It is conceivable that the longer growing season caused by early melt dates would promote greater carbon storage (Euskirchen and others 2006), especially in high altitude sites with short growing seasons. However, this appears not to be the case as we have found that earlier melt dates lead to decreases in the rate of peak NEP as well as leading to plants of shorter stature and lower overall biomass accumulation. Again, we point to decreases in early season soil moisture as a potential critical factor limiting carbon uptake in years with longer growing seasons. A similar phenomenon has also been found in a nearby subalpine forest near Nederland, Colorado (Moore and others 2008; Hu and others 2010). Further, it has been found that although growing season length can place an upper limit on aboveground NPP, soil moisture affects where ecosystem aboveground NPP falls below that limit in the subalpine zone (Berdanier and Klein 2011).

Snowmelt date and June PDI co-vary so that early snowmelt dates increase the strength of the foresummer drought (supplemental Figure 8). However, these variables are also likely related to other physiologically important variables such as air temperature and the amount of winter snowfall. It is also known that snowmelt date can affect the phenology of some species in this system, independent of temperature or daylight length (Dunne and others 2003; Iler and others 2013b). Future work in this area would benefit from disentangling the relative effects of these variables through temperature and snowmelt date manipulations.

Antecedent Effects

The watering manipulation plots were re-measured the following growing season. No differences were found between plots that experienced different watering treatments the year before in NEP (supplemental Figure 9a), $R_{ecosystem}$ (supplemental Figure 9b), soil respiration, or soil moisture. At the end of the growing season, soil carbon was slightly higher in the 'May' and 'Control' plots than in the 'June' and 'July' plots at a marginally significant level (see supplemental Figure 2). It is possible that decreases in soil respiration left more organic soil carbon in the drier plots at the beginning of the following growing season. Similarly, no relationship was found between amounts of total rainfall during the previous water-year, or the previous year's June PDI and the current year's peak NEP at any site along the elevational gradient. Antecedent precipitation has been found to influence the phenology and flowering of certain species in this

area (Iler and Inouye 2013; Iler and others 2013a); however, we did not find evidence that Antecedent precipitation plays a major role in the overall rate of community level carbon exchange.

Implications for the Future

Despite the fact that subalpine meadows may experience increases in temperature and longer growing seasons in the future, they are not likely to increase their growing season carbon uptake. This conclusion is in agreement with the work of many others (for example, Angert and others 2005; Hu and others 2010; Berdanier and Klein 2011; Schwalm and others 2012). Angert and co-authors found that although there was accelerating springtime CO_2 uptake in northern hemisphere terrestrial vegetation in general between 1985 and 2002, there was lower net CO_2 uptake during the summer, canceling out the increased springtime rates. Lower summer uptake was concluded to be the result of hotter and drier summers in the mid- and high-latitudes (Angert and others 2005). Schwalm and co-authors confirm that the western North American carbon sink declines during periods of drought (Schwalm and others 2012). Hu and co-authors find that longer growing seasons lead to decreases in CO_2 uptake in subalpine forests, also pointing to dry soils as a mechanism (Hu and others 2010).

The results of our study also fit into a larger ongoing story about subalpine meadow carbon dynamics based on a warming experiment at the Rocky Mountain Biological Laboratory. A long-term overhead heating experiment was started there in 1991 to understand how increases in surface temperature would affect community dynamics (Harte and Shaw 1995; Price and Waser 1998), as well as carbon dynamics (Saleska and others 1999; Harte and others 2006). Increases in surface temperature lead to advanced snowmelt dates and phenology as well as decreases in soil moisture. Initial results showed that warming significantly reduced carbon uptake on dry meadow plots at peak season. Plants on warmed plots performed photosynthesis for shorter periods of the day than non-warmed plots, presumably because of moisture stress. Decreases in carbon uptake were not attributed to changes in soil respiration (Saleska and others 1999). Furthermore, the effect of year-to-year variation in precipitation on NEP in the warming experiment was significantly larger than the effect of warming (Lamanna 2012). The results of our study show that soil drying can significantly decrease daytime carbon uptake at peak season as well, but in this case we know that this effect is independent of snowmelt date or air temperature.

An important conclusion from these studies is that the declining health and fitness of some subalpine herbs and grasses could leave meadows vulnerable to changes in plant community composition, or woody plant encroachment from lower elevations (Harte and Shaw 1995; Harte and others 2006; Poore and others 2009). However, barring changes in litter composition from community shifts (Saleska and others 2002) or plant acclimation to warming conditions, the general prediction is for an increasingly negative carbon budget in subalpine meadows with climate change.

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REFERENCES

Adams DK, Comrie AC. 1997. The North American monsoon. Bull Am Meteorol Soc 78:2197–213.

Angert A, Biraud S, Bonfils C, Henning CC, Buermann W, Pinzon J, Tucker CJ, Fung I. 2005. Drier summers cancel out the CO_2 uptake enhancement induced by warmer springs. Proc Natl Acad Sci USA 102:10823–7.

Arnone JA, Obrist D. 2003. A large daylight geodesic dome for quantification of whole-ecosystem CO_2 and water vapour fluxes in arid shrublands. J Arid Environ 55:629–43.

Begon M, Townsend C, Harper J. 2009. Ecology: from individuals to ecosystems. New York: Wiley.

Beier C, Beierkuhnlein C, Wohlgemuth T, Penuelas J, Emmett B, Körner C, de Boeck H, Christensen JH, Leuzinger S, Janssens IA, Hansen K, Arnone J. 2012. Precipitation manipulation experiments—challenges and recommendations for the future. Ecol Lett 15:899–911.

Berdanier AB, Klein JA. 2011. Growing season length and soil moisture interactively constrain high elevation aboveground net primary production. Ecosystems 14:963–74.

Biasutti M, Sobel AH. 2009. Delayed Sahel rainfall and global seasonal cycle in a warmer climate. Geophys Res Lett 36:L23707.

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL. 2008. Microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. PNAS 105:11505–11.

Diffenbaugh NS, Scherer M, Ashfaq M. 2013. Response of snow-dependent hydrologic extremes to continued global warming. Nat Clim Chang 3:379–84.

Dunne JA, Harte J, Taylor KJ. 2003. Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. Ecol Monogr 73:69–86.

Dunne JA, Saleska SR, Fischer ML, Harte J. 2004. Integrating experimental and gradient methods in ecological climate change research. Ecology 85:904–16.

Euskirchen ES, McGuire AD, Kicklighter DW, Zhuang Q, Clein JS, Dargaville RJ, Dye DG, Kimball JS, McDonald KC, Melillo JM, Romanovsky VE, Smith NV. 2006. Importance of recent shifts in soil thermal dynamics on growing season length, productivity, and carbon sequestration in terrestrial high-latitude ecosystems. Glob Chang Biol 12:731–50.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33:317–45.

Harte J, Saleska S, Shih T. 2006. Shifts in plant dominance control carbon-cycle responses to experimental warming and widespread drought. Environ Res Lett 1:014001.

Harte J, Shaw R. 1995. Experiment shifting dominance within a montane vegetation community: results of a climate-warming experiment. Science 267:876–80.

Higgins RW, Shi W. 2001. Intercomparison of the principal modes of interannual and intraseasonal variability of the North American monsoon system. J Clim 14:403–17.

Higgins RW, Yao Y, Wang XL. 1997. Influence of the North American monsoon system on the U.S. Summer precipitation regime. J Clim 10:2600–22.

Hu J, Moore DJP, Burns SP, Monson RK. 2010. Longer growing seasons lead to less carbon sequestration by a subalpine forest. Glob Chang Biol 16:771–83.

Huxman TE, Snyder KA, Tissue D, Leffler AJ, Ogle K, Pockman WT, Sandquist DR, Potts DL, Schwinning S. 2004. Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141:254–68.

Iler AM, Høye TT, Inouye DW, Schmidt NM. 2013a. Long-term trends mask variation in the direction and magnitude of short-term phenological shifts. Am J Bot 100:1398–406.

Iler AM, Høye TT, Inouye DW, Schmidt NM. 2013b. Nonlinear flowering responses to climate: are species approaching their limits of phenological change? Philos Trans R Soc Lond B Biol Sci 368:20120489.

Iler AM, Inouye DW. 2013. Effects of climate change on mast-flowering cues in a clonal montane herb, *Veratrum tenuipetalum* (Melanthiaceae). Am J Bot 100:519–25.

IPCC. 2013. Working group I contribution to the IPCC fifth assessment report on climate change 2013.

Jasoni RL, Smith SD, Arnone JA. 2005. Net ecosystem CO_2 exchange in Mojave Desert shrublands during the eighth year of exposure to elevated CO_2. Glob Chang Biol 11:749–56.

Johnston B, Huckaby L, Hughes T, Pecor J. 2001. Ecological types of the Upper Gunnison Basin. Serv Tech Rep R2-RR-2001.

Kellner O, Niyogi D. 2014. Assessing drought vulnerability of agricultural production systems in context of the 2012 drought. J Anim Sci 92:2811–22.

Kirschbaum M, Eamus D, Gifford R, Roxburgh S, Sands PJ. 2001. Definitions of some ecological terms commonly used in carbon accounting. Coop Reserach Cent Carbon Accounting, Canberra 2001:2–5.

Kreyling J, Jentsch A, Beier C. 2013. Beyond realism in climate change experiments: gradient approaches identify thresholds and tipping points. Ecol Lett 17:125-e1.

Lamanna CA. 2012. The structure and function of subalpine ecosystems in the face of climate change. http://arizona.openrepository.com/arizona/bitstream/10150/228495/1/azu_etd_12118_sip1_m.pdf.

Langenheim JH. 1962. Vegetation and environmental patterns in the Crested Butte Area, Gunnison County, Colorado. Ecol Soc Am 32:249–85.

Patrick L, Cable J, Potts D, Ignace D, Barron-Gafford G, Griffith A, Alpert H, Van Gestel N, Robertson T, Huxman TE, Zak J, Loik ME, Tissue D. 2007. Effects of an increase in summer precipitation on leaf, soil, and ecosystem fluxes of CO_2 and H_2O in a sotol grassland in Big Bend National Park, Texas. Oecologia 151:704–18.

Poore RE, Lamanna CA, Ebersole JJ, Enquist BJ. 2009. Controls on radial growth of mountain big sagebrush and implications for climate change. West North Am Nat 69:556–62.

Potts DL, Huxman TE, Cable JM, English NB, Ignace DD, Eilts JA, Mason MJ, Weltzin JF, Williams DG. 2006. Antecedent moisture and seasonal precipitation influence the response of canopy-scale carbon and water exchange to rainfall pulses in a semi-arid grassland. New Phytol 170:849–60.

Price MV, Waser NM. 1998. Effects of experimental warming on plant reproductive phenology in a subalpine meadow. Ecology 79:1261–71.

Rauscher SA, Giorgi F, Seth A, Diffenbaugh NS. 2008. Extension and Intensification of the Meso-American mid-summer drought in the twenty-first century. Clim Dyn 31:551–71.

Reay DS, Hewitt CN, Smith KA, Grace J. 2007. Greenhouse gas sinks. Oxfordshire: CABI.

Ryan MG, Law BE. 2005. Interpreting, measuring, and modelling soil respiration. Biogeochemistry 73:3–27.

Saleska SR, Harte J, Torn MS. 1999. The effect of experimental ecosystem warming on CO_2 fluxes in a montane meadow. Glob Chang Biol 5:125–41.

Saleska SR, Shaw MR, Fischer ML, Dunne JA, Still CJ, Holman ML, Harte J. 2002. Plant community composition mediates both large transient decline and predicted long-term recovery of soil carbon under climate warming. Global Biogeochem Cycles 16:3-1.

Schwalm CR, Williams CA, Schaefer K, Baldocchi D, Black TA, Goldstein AH, Law BE, Oechel WC, Paw UKT, Scott RL. 2012. Reduction in carbon uptake during turn of the century drought in western North America. Nat Geosci 5:551–6.

Seth A, Rauscher SA, Rojas M, Giannini A, Camargo S. 2011. Enhanced spring convective barrier for monsoons in a warmer world? Clim Change 104:403–14.

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401:914–17.

Thompson RM, Beardall J, Beringer J, Grace M, Sardina P. 2014. Moving beyond methods: the need for a diverse programme in climate change research. Ecol Lett 17:125.