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Seasonality of soil moisture mediates responses of ecosystem phenology to elevated CO_2 and warming in a semi-arid grassland

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Summary

1. Vegetation greenness, detected using digital photography, is useful for monitoring phenology of plant growth, carbon uptake and water loss at the ecosystem level. Assessing ecosystem phenology by greenness is especially useful in spatially extensive, water-limited ecosystems such as the grasslands of the western United States, where productivity is moisture dependent and may become increasingly vulnerable to future climate change.

2. We used repeat photography and a novel means of quantifying greenness in digital photographs to assess how the individual and combined effects of warming and elevated CO_2 impact ecosystem phenology (greenness and plant cover) in a semi-arid grassland over an 8-year period.

3. Climate variability within and among years was the proximate driver of ecosystem phenology. Individual and combined effects of warming and elevated CO_2 were significant at times, but mediated by variation in both intra- and interannual precipitation. Specifically, warming generally enhanced plant cover and greenness early in the growing season but often had a negative effect during the middle of the summer, offsetting the early season positive effects. The individual effects of elevated CO_2 on plant cover and greenness were generally neutral.

4. Opposing seasonal variations in the effects of warming and less so elevated CO_2 cancelled each other out over an entire growing season, leading to no net effect of treatments on annual accumulation of greenness. The main effect of elevated CO_2 dampened quickly, but warming continued to affect plant cover and plot greenness throughout the experiment. The combination of warming and elevated CO_2 had a generally positive effect on greenness, especially early in the growing season and in later years of the experiment, enhanced annual greenness accumulation. However, interannual precipitation variation had larger effect on greenness, with two to three times greater greenness in wet years than in dry years.

5. *Synthesis*. Seasonal variation in timing and amount of precipitation governs grassland phenology, greenness and the potential for carbon uptake. Our results indicate that concurrent changes in precipitation regimes mediate vegetation responses to warming and elevated atmospheric CO_2 in semi-arid grasslands. Even small changes in vegetation phenology and greenness in response to warming and rising atmospheric CO_2 concentrations, such as those we report here, can have large consequences for the future of grasslands.

Key-words: climate change, greenness, image analysis, northern Great Plains, phenology, short grass steppe

Introduction

Climate change impacts have been well documented across many ecosystems, and as atmospheric CO_2 concentrations are predicted to rise to 550 $\mu L\ L^{-1}$ by the end of this century, they are expected to generate surface warming of 1.9–4.4 °C globally (Easterling 2000; Christensen *et al.* 2007; Solomon *et al.* 2009). The magnitude and relative ecological effects of elevated temperatures, atmospheric CO_2 and regional estimates of precipitation all vary geographically (Weltzin *et al.* 2003; Seager *et al.* 2007; Loarie *et al.* 2009). In the northern Great Plains, temperatures have already increased by 0.8–0.9 °C, relative to the 1970s baseline, and are continuing to warm at a rate of 0.1 °C per decade (Kunkel *et al.* 2013). Some regional models predict an overall increase in

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precipitation across the northern Great Plains (Easterling 2000; Christensen *et al.* 2007; Knapp *et al.* 2008), as well as a potential increase in the frequency and severity of drought (Easterling 2000; Karl & Trenberth 2003; Seager *et al.* 2007; Knapp *et al.* 2008) and generally less predictability in the timing and amount of precipitation (Karl & Trenberth 2003; Woodhouse 2003; Kunkel *et al.* 2013). Warming is expected to further reduce soil moisture, exacerbating the effects of dry periods (Knapp *et al.* 2008; Shen *et al.* 2008; Woodhouse *et al.* 2010). In water-limited semi-arid grasslands, such as those found in the northern Great Plains, the seasonal distribution of precipitation drives all aspects of ecosystem function (Hovenden, Newton & Wills 2014) and concurrent changes in temperature and atmospheric CO_2 are particularly important due to their indirect effects on water availability. Arid and semi-arid ecosystems constitute over 30% of Earth's terrestrial surface and provide critical ecosystem services, including forage production, carbon storage and wildlife habitat, so the effect of climate change in these ecosystems is of practical concern.

Semi-arid grasslands are a mixture of grasses, forbs and shrubs, which vary in their responses to climate change (Fay *et al.* 2002; Morgan *et al.* 2007; Nippert *et al.* 2009; Pendall *et al.* 2011; Dieleman *et al.* 2012). Generally, plants can exhibit large responses to elevated CO_2 and warming in the short term (Morgan *et al.* 2004, 2011; Nowak, Ellsworth & Smith 2004), but there is also evidence of acclimation to both elevated CO_2 (Rogers & Humphries 2000; Nowak, Ellsworth & Smith 2004; Gunderson *et al.* 2010; Smith & Dukes 2012) and warming (Luo 2007; Milcu *et al.* 2012). Furthermore, there is some evidence of potential feedbacks between plant responses and the atmosphere that include altered photosynthesis and respiration rates that can influence ecosystem carbon emissions as well as uptake (Luo 2007; Morgan *et al.* 2007; Chapin *et al.* 2008; Nippert *et al.* 2009; Jiang, Zhang & Lang 2011; Milcu *et al.* 2012). The variation in the strength and direction of plant responses to climate change necessitates the use of both multifactorial experiments and long-term observations that capture interannual variation in climate to better predict ecosystem function under future climate scenarios.

Ecosystem phenology (the timing of ecosystem activity) is an important metric for tracking the effects of climate change (Cleland *et al.* 2006; Sherry *et al.* 2006; Smith, Knapp & Collins 2009; Richardson *et al.* 2010). Ecosystem phenology has most often been quantified via remote sensing of greenness indices, such as the normalized difference vegetation index (NDVI), which can detect the dates of green-up in the spring and senescence in the fall with high resolution (Lucht 2002; Tanja *et al.* 2003; Pettorelli *et al.* 2005; Crimmins & Crimmins 2008; Richardson *et al.* 2009). Satellite detection of greenness also allows for estimation of net and gross carbon uptake (Paruelo *et al.* 1997; Potter 2003) because the timing of annual photosynthetic activity and carbon uptake is directly related to plant greenness (Piao *et al.* 2006; Richardson *et al.* 2009). As climate changes, plants can shift their phenology, altering species interactions and community dynamics (Edwards & Richardson 2004; Winder & Schindler 2004; Suttle, Thomsen & Power 2007; Tylianakis *et al.* 2008) which drive the annual cycle in carbon uptake (Weltzin *et al.* 2003; Richardson *et al.* 2012). With changes in seasonal timing of photosynthetic activity, primary productivity can be expected to respond simply as a result of the change in the number days available for carbon uptake and plant growth. Therefore, changes in vegetation greenness closely approximate the effects of climate change on carbon uptake (Gamon *et al.* 1995; Kurc & Benton 2010). Green cover may also be a good predictor of plant cover, annual net primary production (ANPP) and total biomass (Paruelo *et al.* 1997; Flombaum & Sala 2007; Kurc & Benton 2010) and can be estimated without destructive harvest, making it an inexpensive and fast method for ANPP estimation.

Satellite remote sensing and high-resolution repeat photography provide opportunities to increase the frequency and accuracy of phenological observations (White & Nemani 2006; Crimmins & Crimmins 2008; Richardson *et al.* 2009). In particular, near-surface remote sensing of phenology using digital cameras provides the means to increase temporal and spatial resolution, and because digital photographs contain colour and shape-specific information, it is also used to estimate plant cover (Booth & Cox 2008), biomass (Paruelo *et al.* 1997; Luscier *et al.* 2006), leaf area (Gamon *et al.* 1995; Przeszlowska, Trlica & Weltz 2006) and greenness (Kurc & Benton 2010). We used repeat photography to examine how elevated CO_2 and temperature affect ecosystem phenology (encompassing both plant cover and plot greenness) over an 8-year period, including several dry and wet years. Other studies report that elevated CO_2 can improve plant water-use efficiency (Drake, Gonzalez-Meler & Long 1997; Morgan *et al.* 2011) and therefore reduce consumptive plant water use, but warming can have the opposite effect. Based on previous research, we expected greening to occur earlier in warmed plots (Cleland *et al.* 2007; Richardson *et al.* 2007; Dieleman *et al.* 2012). However, we expected warming to have a negative effect on greenness later in the growing season as a result of drying soils (Harte *et al.* 1995) and exacerbated thermal stress (Wan *et al.* 2005). We expected elevated CO_2 to enhance soil moisture (Pendall *et al.* 2003; Morgan *et al.* 2004, 2011) and, as a result, increase greenness. When combined, we expected elevated CO_2 and warming to have an overall positive effect on ecosystem phenology.

Materials and methods

PRAIRIE HEATING AND CO_2 ENRICHMENT EXPERIMENTAL DESIGN

The Prairie Heating and CO_2 Enrichment (PHACE) experiment was initiated in 2005 at the USDA-ARS High Plains Grasslands Research Station, located west of Cheyenne, WY, USA. The vegetation at PHACE is a northern mixed-grass prairie and is dominated by C_3 graminoids (55% of the plant community, predominately *Pascopyrum smithii* and *Hesperostipa comata*). C_4 grasses make up 25% of the plant community (predominately blue grama *Bouteloua gracilis*) and 20% of the plant community consists of sedges, forbs and small shrubs (mostly *Artemisia frigida*). Annual precipitation at the site averages 384 mm, mean winter air temperature is −2.5 °C, and mean summer air temperature is 17.5 °C. The site was regularly grazed since 1974, but was fenced to prevent livestock grazing in 2005.

In 2005, 20 circular 3.4-m-diameter plots were established with a 60-cm-deep impermeable barrier to prevent lateral inflow. Elevated atmospheric CO_2 was applied starting in April 2006 and accomplished using mini-FACE (The Free Air CO_2 Enrichment) technology (*c*. 385 ppmv ambient and 600 ppmv elevated; Miglietta *et al.* 1997) using 3.3-m-diameter FACE rings. Elevated CO_2 was applied in early spring and turned off in late autumn annually (Table S1 in Supporting Information). A differential daytime/night-time warming (1.5/3 °C) treatment was applied using infrared heaters (Harte *et al.* 1995) in full factorial design, with five replicates for each of the four combinations (ct, ambient CO_2 and ambient temperature; cT, ambient CO_2 and elevated temperature; Ct, elevated CO_2 and ambient temperature; and CT, elevated CO_2 and elevated temperature). Elevated CO_2 treatments were applied in early spring of 2006, and warming was started in April of 2007 and was maintained continuously until the end of July 2013. All plots were irrigated by hand in 2006 to facilitate establishment of an associated experiment (20 mm × 8 irrigation dates, the equivalent of 160 mm of additional growing season precipitation). The site, experimental set-up and treatment performance are described in detail in Morgan *et al.* (2011). Total precipitation was measured directly in the form of rain collected at the PHACE site. During winter months, snow precipitation was estimated by averaging from five nearest NOAA meteorological stations, including the Cheyenne Airport located approximately 10 km away from the PHACE site (http://www.ncdc.noaa.gov/cdo-web/) and all snow data are presented as water equivalent. During snow-free months, PHACE site rainfall data gaps were filled using rainfall data from the USDA High Plains Grassland Research Station (HPGRS) meteorological station next to the PHACE site. Growing season temperature was calculated as the mean temperature from March through September annually. Wintertime precipitation includes total precipitation from November through March, spring precipitation includes total precipitation from April through June, and summer precipitation includes total precipitation from July through September. The 30-year mean data were downloaded from the NOAA Cheyenne Airport meteorological station (http://www.ncdc.noaa.gov/IPS/lcd/lcd.html). Soil volumetric water content (VWC) was measured from 5 to 15 cm soil depth using EnviroSMART probes (Sentek Sensor Technologies, Stepney, SA, Australia) and logged hourly.

BIOMASS QUANTIFICATION

A metal wire grid divided into twenty-four 25 × 25 cm quadrats (1.5 m^2 total) was placed over each plot within a metre of the ring centre, and vegetation in every other quadrat (12 total; 0.75 m^2 total) was clipped to the crown in late July at the time of peak biomass. Harvest location within the wire grid was alternated each year. All harvested material was sorted to species, dried for 3 days at 60 °C and weighed.

IMAGE ANALYSIS – GREENNESS

Repeat photographs of each experimental plot were taken in the same location within each plot biweekly from end of March until end of October from 2007 to 2012 and March–July 2013 using a 2-m tall camera stand with a 1-m^2 ground frame (Booth *et al.* 2004). In 2008, we were only able to take photographs once per month. We developed a novel method to quantify the percentage of green in each plot over time. We first converted each image into a data matrix using MATLAB R2011a (The Math-Works, Natick, MA, USA) and the *imread()* command. Each element in the matrix represented a pixel with a value for red, green and blue weight of the pixel between 0 and 255. These values were converted to an hue, saturation, value (HSV) scale for classification using the *rgb2hsv()* command, which creates a value for hue, saturation and value between 0 and 1 for each pixel. We chose to use the HSV scale for detection of green instead of the red, green, blue (RGB) scale because it provides a simpler method for detecting the colour green. In the RGB scale, the colour of a pixel is created from the interrelationship of all the RGB values, and a pixel's G (green) value alone does not determine the appearance of green for the pixel. Thus, determining the acceptable range of RGB values for each parameter to count pixels that appear green requires a more complicated set of rules than is required for the HSV scale. In addition, the HSV scale is not as sensitive as the RGB greenness index to changes in brightness associated with taking photographs under variable cloud cover (Yabusaki *et al.* 2014). In the MATLAB HSV scale, the greenest hues have a value of approximately 0.25, in contrast to brown hues with a value of approximately 0.09. The saturation HSV captures how much of the hue exists in the colour. In pixels with a high saturation, the visible appearance of the pixel will be the hue, while pixels with a low saturation will appear grey. The HSV value parameter describes the brightness of the pixel. High values indicate that the pixel is dark and low values indicate a light pixel, such that a pixel with a value of 0 is white and a pixel with a value of 1 is black.

Using the HSV scale, we defined the upper and lower boundaries of HSV values for 'green' for a subset of images and applied these boundaries to the other images. All other pixels were classified as not green. This approach defined the HSV range for green to be 0.16–0.50 for hue, 0.075–1.0 for saturation and 0.09–0.91 for value. The percentage of greenness for each image was the number of pixels identified as 'green' divided by the total number of pixels in the image (see Appendix S1 for MATLAB code). For images taken after the annual July biomass harvest, the harvested portions of the images were omitted from quantification of greenness. The percentage of greenness in these images did not include pixels that fell into the harvested sections.

IMAGE ANALYSIS – VEGETATION COVER

To estimate ground cover by species from repeat photographs taken 2007–2012, we used Sample Point (Booth, Cox & Berryman 2006), a free software package that superimposes a grid of crosshairs over each image to facilitate manual user classification of 225 focal image pixels. Using this method, we quantified the cover of litter, soil, the dominant perennial graminoids *P. smithii*, *H. comata*, *Carex duriuscula* (C_3 sedge), the dominant C_4 graminoid *B. gracilis*, the sub-shrub *A. frigida* and the perennial C_3 forb *Sphaeralcea coccinea*. Plants that did not fall into the above categories were identified as 'unknown'. Because biomass was clipped annually in July, we present cover by species and functional group, combining C_3 graminoids, C_4 graminoids, forbs and sub-shrubs for the entire photograph area through July of each year and from August until October, the area-scaled non-harvested portions of the plot.

DATA ANALYSIS

To estimate seasonal and annual greenness accumulation, we integrated the area under the greenness-by-date curve seasonally from

March–May for spring growth, June–August for summer growth, September–October for autumn growth and March–October for annual accumulation. The experiment was shut down in July 2013, and we did not quantify annual greenness accumulation in 2013. We assessed the singular and interacting effects of elevated CO_2 and warming on seasonal variation in greenness by date using a repeated-measures MANOVA. The effects of year, elevated CO_2 (ambient 'ct' and elevated 'Ct'), warming (ambient 'ct' and warmed 'cT') and their interaction (elevated CO_2 and warming 'CT') on accumulated seasonal and integrated annual greenness were also assessed using a two-way ANOVA, with block as a random effect. Because greenness varied substantially between years ($F_{1,6} = 114.3$, $P < 0.0001$), we followed up this ANOVA with two-way analyses within each year, with CO_2 and warming as the main effects. In 2006, only the elevated CO_2 treatment was in place and we used a one-way ANOVA to test for the effect of CO_2 on seasonal and annual greenness accumulation. The relationship between the treatment response ratios (calculated as the greenness under PHACE treatment divided by greenness under the ambient condition) and soil VWC was examined using a general linear model with a normal distribution of error terms. We also used a general linear model with a normal distribution of error terms to examine the relationship between plant cover and greenness. Because above-ground biomass was harvested in mid-July annually, we tested whether the relationship between greenness accumulation up to mid-July and above-ground biomass differed among years. All statistical analyses were performed using JMP PRO 11 software (SAS Institute, Cary, NC, USA).

Results

The PHACE site experienced two relatively cool years (2009 and 2011) and two warm dry years (2006 and 2012), relative to the 30-year mean of 8.3 °C (Table 1). 2006 and 2012 were the driest of the 8-year study and were exceptionally dry (215 and 223 mm of precipitation, respectively), relative to the 30-year mean of 378 mm, while 2009 and 2011 were relatively wet years. However, low summer precipitation in 2006 was supplemented with 160 mm to bring the annual precipitation up to 375 mm. Along with differences in total annual precipitation, the seasonal timing of precipitation also varied between years (Table 1). The PHACE experimental treatments achieved target microclimate conditions (Morgan *et al.* 2011; Blumenthal *et al.* 2013). Warming reduced and elevated CO_2 increased soil water content, and under the combination of warming and elevated CO_2, soil moisture did not differ from ambient plots during the growing season (Pendall *et al.* 2013).

ECOSYSTEM PHENOLOGY – GREENNESS

Greenness varied seasonally and between years (Fig. 1), as did the effects of elevated CO_2 and warming on greenness (Table 2). Seasonal warming effects were commonly dependent on date as indicated by the significant warming × date interactions (Table 2), with enhancement of greenness in the spring and suppression in summer (Fig. 2). In contrast, the effects of elevated CO_2 alone were only significant in 2006 and marginally so in 2007 and there were no significant interactions between elevated CO_2 and date, suggesting that despite apparent variation in magnitude, the effects of elevated CO_2 on greenness were consistent in their direction across the growing season (Table 2). When warming was combined with elevated CO_2, greenness matched or exceeded the effects of warming alone (Fig. 1). The effects of elevated CO_2 tracked the effects of warming in the relatively wetter years of 2009, 2010 and 2011, whereas greenness under elevated CO_2 and warming exceeded that of warming alone during the drier years of 2007, 2008, 2012 and 2013 (Fig. 1).

When integrated across the spring, summer and autumn, seasonally accumulated greenness was greater in elevated CO_2 plots early and in the middle of the growing season in the first year of CO_2 enrichment (Fig. 2a, see Table S2), but the positive effect of elevated CO_2 diminished over time and was not significant again until 2013, when it was very dry in the spring (Table S2). The effects of warming were marginally positive in the spring in all years, with the exception of the dry spring in 2008 (Fig. 2a, Table S2). The seasonal responses of greenness to elevated CO_2 and warming influenced annual accumulation (Fig. 2b). Relative to ambient plots, the isolated effect of elevated CO_2 on annual greenness accumulation was generally positive in the first 3 years of the CO_2 enrichment experiment but was negligible by 2009 (Fig. 2b, Table S2). However, elevated CO_2 significantly increased annual greenness accumulation only in 2006 (Table S2). In contrast, the isolated effect of warming was generally smaller but also more variable from year to year and had no significant effect on annual greenness accumulation in any year (Fig. 2b, Table S2). The combined effect of warming

Table 1. Prairie Heating and CO_2 Enrichment site annual temperature and precipitation data from 2006 through 2013. In 2006, 160 mm of irrigation was added to all plots to yield annual precipitation equivalent to 375 mm (irrigation in addition to the annual precipitation of 215 mm). The experiment ended in June 2013

	30-year mean	2006	2007	2008	2009	2010	2011	2012	2013
Mean annual temperature (°C)	8.3	8.55	7.6	7.7	6.3	7.6	6.4	8.8	7.7
Growing season temperature (°C)	13.3	13.7	13.3	11.6	12	12.2	11.7	15.1	12
October–March (mm)	68	80	91	115	68	134	100	82	66
April–June (mm)	176	59	114	142	238	225	177	78	105
July–September (mm)	132	77	176	212	135	49	145	89	281
Annual precipitation (mm)	378	*375*	434	410	495	377	425	223	478

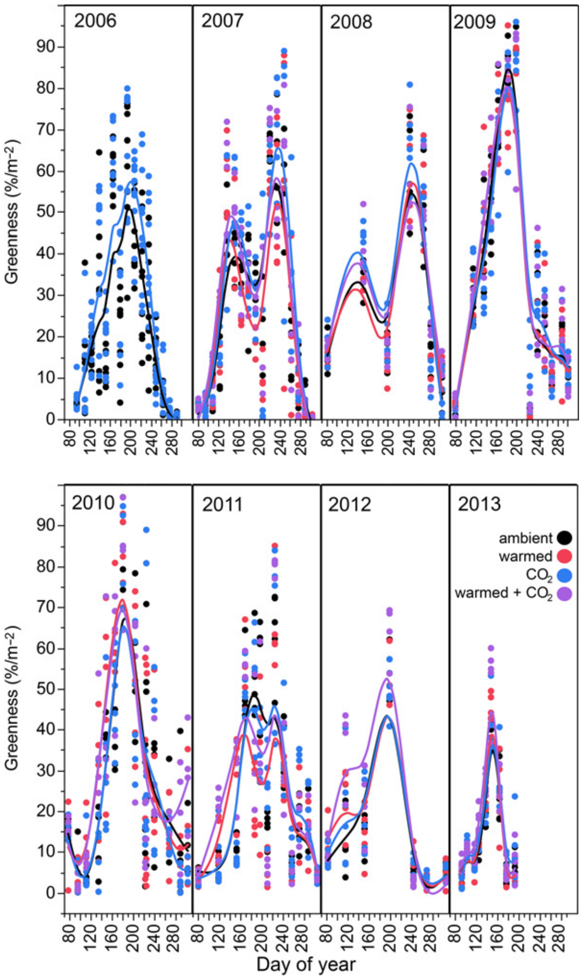

Fig. 1. Effects of elevated CO_2 and warming on daily greenness (number of green pixels) from ambient plots from 2006 to 2013, quantified from digital photographs of the plots taken biweekly between March and November annually. In 2006, only the effects elevated CO_2 are shown. In 2013, the Prairie Heating and CO_2 Enrichment experiment was terminated in July and we present data only for the March–July time period. Ambient plots are filled in black, warmed plots red, elevated CO_2 plots blue and elevated CO_2 and warmed plots purple. The data were smoothed using a cubic spline, with a lambda of 0.05, and the fit line reflects this spline smoothing function.

and elevated CO_2 on greenness accumulation was neutral for most of the experiment but became significantly positive relative to the ambient plots in the last 2 years of the study (Fig. 2b).

The effects of elevated CO_2 and warming treatments on greenness accumulation were much lower than the overall effect of interannual variation in greenness, which was two to three times higher in wet years (mean 472.1 ± 19.7 SE in 2007 and 467.3 ± 4.8 SE in 2009) than in dry years (mean 115.4 ± 5.3 SE in 2012 and 211.7 ± 4.6 in 2008; Fig. 1). There was a generally positive relationship between plot greenness and precipitation from the previous 2-week period ($R^2 = 0.27$, $P < 0.0001$) that did not vary among treatments when aggregated across all the sampling dates ($F = 0.3$, d.f. = 3, $P = 0.8$). The relationship between VWC and greenness depended on season ($\chi^2 = 28.9$, d.f. = 2, $P < 0.0001$) and varied among treatments ($\chi^2 = 13.6$, d.f. = 2, $P = 0.001$), with a significant interaction between season and treatment ($F = 6.7$, d.f. = 2, $P = 0.001$) and VWC and treatment ($\chi^2 = 7.1$, d.f. = 2, $P < 0.03$). The relationship between greenness and VWC was positive and strongest in the summer months ($R^2 = 0.3$, $P < 0.0001$) and weakest in the autumn ($R^2 = 0.003$, $P = 0.4$), but the effects of the PHACE treatments on greenness were strongest in the spring, when greenness increased with increasing VWC under warming, but not under elevated CO_2 (Fig. 3).

ECOSYSTEM PHENOLOGY – VEGETATION COVER

Across years, there was a strong positive relationship between average plant live cover and greenness ($R^2 = 0.75$, $P < 0.0001$, Fig. 4a) as well as a positive saturating relationship between greenness accumulated before the harvest date and harvested plant biomass ($\chi^2 = 10.5$, $P = 0.001$, Fig. 4b). However, the strength of the positive relationship between greenness and biomass varied among years ($\chi^2 = 155$, $P < 0.0001$) that was driven largely by the strongly positive relationship between greenness accumulation and biomass in the wet years 2009 ($R^2 = 0.67$, $P = <0.0001$) and 2013 ($R^2 = 0.63$, $P = <0.0001$), a marginally significant positive relationship in the relatively dry year 2012 ($R^2 = 0.2$, $P = 0.05$), but not in other years. In addition, the relationship between greenness and plant cover varied seasonally ($\chi^2 = 155$, $P = 0.003$) and was stronger in the summer and autumn and weakest in the spring. There was no relationship between biomass and greenness in 2006 ($R^2 = 0.004$, $P = 0.8$), but because water was added to all plots during this year, we excluded these data from the overall analysis.

Plant cover across all plots and treatments was low in 2007 and 2008, covering on average 34% of the plots, intermediate in 2010 and 2011, covering an average of 40%, and relatively high in 2009 and 2012, covering more than 50% of the plots. However, the relative contribution of different functional groups to total plant cover varied across years (Fig. S1) and the relative influence of elevated CO_2 and warming also differed seasonally as well as among years and plant functional groups (Table S3). There was frequently an interaction between warming and date, indicative of benefits early and detriments later in the season, at least for grasses (Table S3). Across the 8-year experiment, C_3 graminoids made up on average 40% of the plot cover, the C_4 grass *B. gracilis* covered 38%, and forbs and the shrub *A. frigida* on average covered the remaining 22%.

Discussion

Our novel greenness index provided an ecosystem phenology measure that was sensitive to climate change manipulation treatments and was related to variations in plant biomass and cover. In addition, repeat measurements across the growing season allowed us to capture not only the seasonal variation but also how the effects of the climate change treatments

Table 2. A repeated-measures manova of the effects of warming and elevated CO$_2$, date and their interaction on per cent greenness across each growing season. In 2006, only the elevated CO$_2$ treatments were in place and we present the results of a one-way manova. We report the *F*-test statistics, nominator and denominator degrees of freedom, and *P*-values

Year	Source	*F*-test statistic	NumDF, DenDF	*P*-value
2006	Date	432.7	14, 4	**0.0001**
	Elevated CO$_2$	0.34	1, 17	**0.03**
	Elevated CO$_2$ × Date	11.02	14, 4	0.14
2007	Date	2955.6	16, 1	*0.06*
	Warming	0.02	1, 16	0.6
	Elevated CO$_2$	0.2	1, 16	*0.08*
	Warming × Elevated CO$_2$	0.006	1, 16	0.8
	Warming × Date	110.1	1, 16	0.3
	Elevated CO$_2$ × Date	18.3	16, 1	0.6
	Warming × Elevated CO$_2$ × Date	36.9	16, 1	0.5
2008	Date	51.6	6, 11	**<0.0001**
	Warming	0.03	1, 16	0.5
	Elevated CO$_2$	0.23	1, 16	*0.07*
	Warming × Elevated CO$_2$	0.002	1, 16	0.85
	Warming × Date	2.3	6, 11	**0.02**
	Elevated CO$_2$ × Date	0.8	6, 11	0.3
	Warming × Elevated CO$_2$ × Date	0.4	6, 11	0.6
2009	Date	1215	12, 5	**<0.0001**
	Warming	0.22	1, 16	*0.08*
	Elevated CO$_2$	0.001	1, 16	0.9
	Warming × Elevated CO$_2$	0.2	1, 16	*0.09*
	Warming × Date	12.7	12, 5	**0.04**
	Elevated CO$_2$ × Date	0.9	12, 5	0.9
	Warming × Elevated CO$_2$ × Date	1.9	12, 5	0.7
2010	Date	454	13, 4	**0.0001**
	Warming	0.1	1, 16	0.2
	Elevated CO$_2$	0.003	1, 16	0.8
	Warming × Elevated CO$_2$	0.008	1, 16	0.7
	Warming × Date	11.3	13, 4	0.1
	Elevated CO$_2$ × Date	11.7	13, 4	0.1
	Warming × Elevated CO$_2$ × Date	1.4	13, 4	0.9
2011	Date	442.1	12, 5	**<0.0001**
	Warming	0.1	1, 16	0.2
	Elevated CO$_2$	0.2	1, 16	0.1
	Warming × Elevated CO$_2$	0.08	1, 16	0.3
	Warming × Date	42.5	12, 5	**0.003**
	Elevated CO$_2$ × Date	3.2	12, 5	0.4
	Warming × Elevated CO$_2$ × Date	11.5	12, 5	**0.05**
2012	Date	83.1	6, 10	**<0.0001**
	Warming	0.4	1, 15	**0.04**
	Elevated CO$_2$	0.1	1, 15	0.2
	Warming × Elevated CO$_2$	0.09	1, 15	0.3
	Warming × Date	5.1	6, 10	**0.002**
	Elevated CO$_2$ × Date	1.2	6, 10	0.2
	Warming × Elevated CO$_2$ × Date	0.8	6, 10	0.3
2013	Date	137	8, 9	**<0.0001**
	Warming	0.2	1, 16	*0.07*
	Elevated CO$_2$	0.4	1, 16	**0.02**
	Warming × Elevated CO$_2$	0.06	1, 16	0.3
	Warming × Date	2.3	8, 9	*0.09*
	Elevated CO$_2$ × Date	1.1	8, 9	0.5
	Warming × Elevated CO$_2$ × Date	1.8	8, 9	0.2

P-values in bold are significant at $\alpha = 0.05$; italicized *P*-values are marginally significant at $\alpha = 0.1$

varied across eight growing seasons. Though the strength of the relationship between biomass and greenness varied between wet and dry years, the consistency of the relationship indicates that our novel approach of using digital photographs is a promising tool to quickly assess phenology, green plant cover and, indirectly, plant biomass, in grassland ecosystems. However, because the relationship between biomass and greenness depended on seasonal and interannual variation precipitation, we caution against single time point assessments that do not capture this variation. The long-term nature of our study provided the opportunity to understand not only mean responses to climate manipulations but also how soil moisture mediated these responses. The results of our 8-year climate manipulation experiment show that the effects of elevated CO$_2$ and warming on ecosystem phenology (greenness and plant cover) vary in magnitude, and because the direction of the responses changed across a growing season, their cumulative effects were often negligible. Precipitation and soil moisture varied widely during the study period and influenced ecosystem phenology both directly (greenness was two to three times greater in wet years than in dry years) and indirectly by mediating the magnitude and direction of the climate manipulation effects.

EFFECTS OF PHACE TREATMENTS ON ECOSYSTEM PHENOLOGY

Not only can interannual variation in precipitation have large consequences for plant growth (Groisman & Knight 2008; Fay *et al.* 2011; Morgan *et al.* 2011), but it can also dictate vegetation responses to global change treatments (Naumburg *et al.* 2003; Albert *et al.* 2011; Fay *et al.* 2011). Our findings only partially support the general expectation that elevated CO$_2$ should enhance grassland plant growth, and the overall effects of elevated CO$_2$ on greenness in our study were relatively small and transient. Though the greenness response to elevated CO$_2$ was relatively large in the first year of the experiment, it diminished quickly, providing some evidence that plants may be acclimating to elevated CO$_2$ conditions in our plots, as others have shown (Sage, Sharkey & Seemann 1989; Drake, Gonzalez-Meler & Long 1997; Newingham

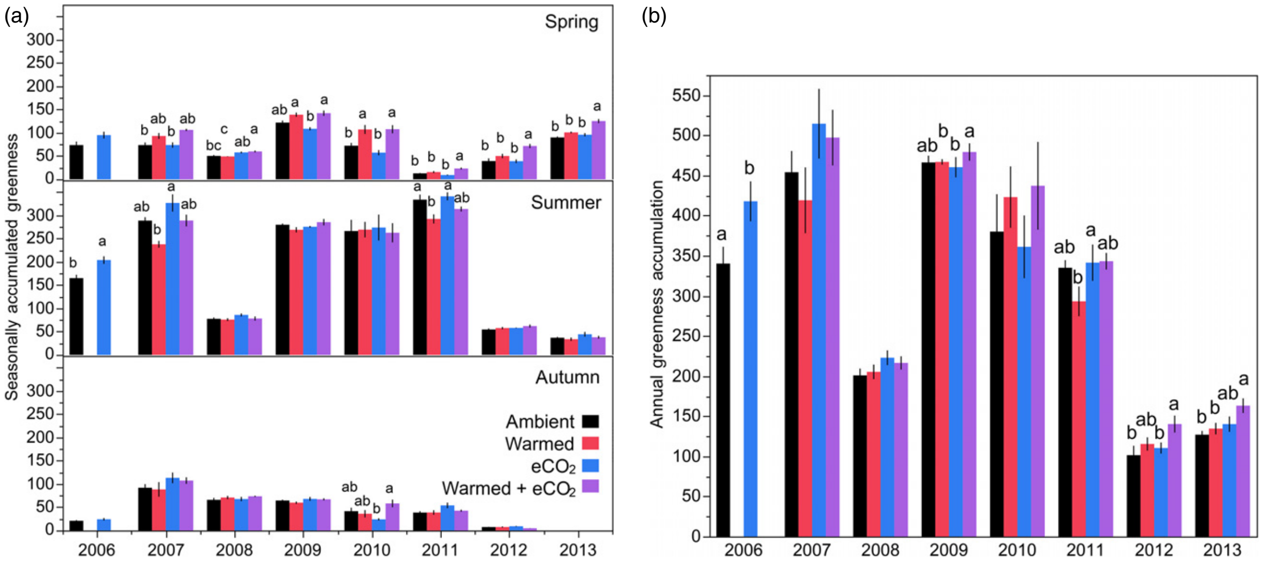

Fig. 2. The isolated and combined effects of elevated CO_2 and warming on greenness accumulation seasonally (Panel a) from March–May, June–August, September–October, and annually (Panel b) from 2006 to 2013. Data from 2006 show only the effects of elevated CO_2. In 2013, the Prairie Heating and CO_2 Enrichment experiment was terminated in July and we present data only for the March–May and June–July dates. The results from a one-way ANOVA, with Student's t *post hoc* means separation test ($\alpha = 0.05$), are presented. Different letters indicate statistically significant differences at α=0.05, and all other comparisons are not statistically significantly different. Error bars are ± 1 SE. Ambient plots are filled in black, warmed treatments red, elevated CO_2 treatments blue and the combination of warming and elevated CO_2 treatments purple.

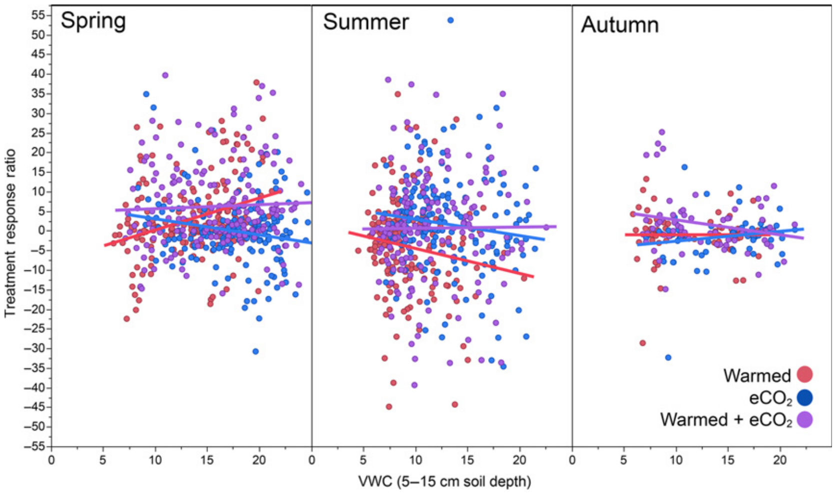

Fig. 3. The relationship between the effects of the Prairie Heating and CO_2 Enrichment treatments on greenness (calculated as the response ratio of the climate change treatment divided by the ambient control) and volumetric soil water content (5–15 cm soil depth) during the spring, summer and autumn months. Warmed plots are red, elevated CO_2 plots blue and elevated CO_2 and warmed plots purple.

et al. 2013). However, it is also possible that the water-saving effects of elevated CO_2 are less apparent during a number of relatively wet years in the middle of the experiment or that lower initial biomass in the elevated CO_2 plots reduced the size of treatment effects (Morgan *et al.* 2011). Furthermore, other factors, such as changes in plant community composition (Langley & Megonigal 2010; Fay *et al.* 2011; Zelikova *et al.* 2014), changes in allocation patterns from shoots to roots (Suter *et al.* 2002) and nitrogen limitation, can all suppress the stimulatory effects of elevated CO_2 (Luo *et al.* 2004; Reich *et al.* 2006; Bradford *et al.* 2012), though there is currently no evidence for this in the PHACE experiment.

In contrast to the transient effects of elevated CO_2, the effects of warming on ecosystem phenology were more consistent. We found some support for our general expectations that warming would reduce greenness during drier periods as a result of decreased water availability (Paruelo, Sala & Beltrán 2000; Zhao & Running 2010; Dieleman *et al.* 2012), which strongly limits plant growth at this site. In addition, the effects of warming were substantially positive during wet periods, in agreement with other findings that precipitation can mediate the effects of warming on phenology and the length of the growing season (Rustad *et al.* 2001; Fay *et al.* 2011; Xia & Wan 2012). Indeed, soil VWC mediated the treatment effects on greenness, especially during summer months, when an additional unit of water yielded the greatest increase in greenness across all plots. However, during the spring months when water was not limiting, warming had the

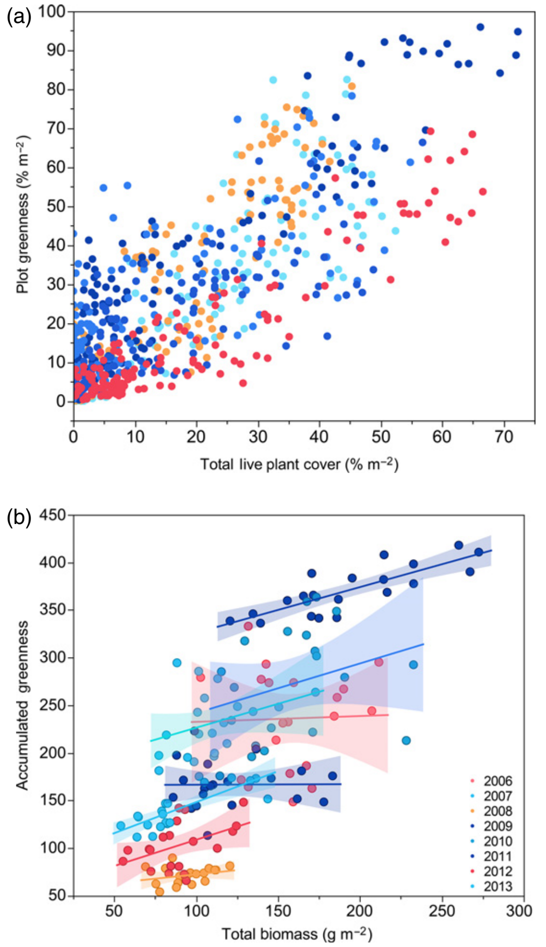

Fig. 4. The relationships between (a) greenness and live plant cover, averaged across treatments within each sample date and (b) total harvested green biomass (harvested in July annually) per plot and accumulated plot greenness quantified from digital plot photographs taken at the time of the biomass harvest. Points are filled in to reflect variation in annual precipitation, with driest years filled in shades of red and the wettest year filled in dark blue. Precipitation is also described in Table 1.

largest impact on greenness. In the spring, photosynthetic uptake is generally temperature limited (Lucht 2002; Tanja *et al.* 2003) and warming enhanced plant growth and greenness when conditions were more optimal in this semi-arid grassland. In the middle of the growing season, the effects of warming were either negligible or negative. Though longer growing seasons can be associated with increased net ecosystem productivity (Nemani *et al.* 2003; but see Piao *et al.* 2008; Hu *et al.* 2010), the positive effect of warming on greenness early in the growing season was often offset by a generally negative effect later, and when integrated across the entire year, we saw no overall effect of warming on greenness. Thus, warming and to a lesser extent elevated CO_2 are likely to alter the seasonal distribution more than the total amount of greenness in this ecosystem. Though the effects were seasonally variable, we found that warming continued to affect ecosystem phenology, while the effects of elevated CO_2 diminished after the first 2 years of the experiment. Our results support other studies that have found that in the long-term, plants continue to respond to warming (Rustad *et al.* 2001; Wang, Rich & Price 2003; Fay *et al.* 2011) but have the capacity to acclimate to elevated CO_2 (Sage, Sharkey & Seemann 1989; Drake, Gonzalez-Meler & Long 1997; Pendall *et al.* 2003; Morgan *et al.* 2004). However, we must also consider that the timing of beginning the field elevated CO_2 and warming treatments were offset by one growing season, giving the elevated CO_2 plots an extra year under experimental manipulation. In addition, the elevated CO_2 treatments were started in a dry year and warming in a wet year. These factors may influence the temporal dynamics of ecosystem phenology responses to the experimental climate manipulation, including acclimation.

The combination of warming and elevated CO_2 most closely approximates future climate regimes for the northern Great Plains. When applied in combination, the effect of warming and elevated CO_2 on ecosystem phenology varied seasonally and often reflected the effect of the dominant factor, most often the effect of warming. This led to more greenness under ‘future’ than ‘present’ climate conditions in the spring, and less frequently, in autumn months, similar to the positive influence of increasing soil temperature on green above-ground biomass in the spring and autumn months in the tallgrass prairie (Wan *et al.* 2005). Interestingly, our results contrast with those reported in a recent meta-analysis that examined multifactorial experiments where both CO_2 and temperature were elevated and found that the combined effects on plant biomass were similar to those of the singular effect of elevated CO_2 (Sherry *et al.* 2008; Walther 2010; Dieleman *et al.* 2012). In addition, the same meta-analysis reported that when applied in combination, the effects of warming and elevated CO_2 were antagonistic and less than additive (Dieleman *et al.* 2012). While the PHACE experiment shows evidence of antagonism with respect to annual productivity and water availability (Morgan *et al.* 2011; Pendall *et al.* 2013), elevated CO_2 and warming were less likely to have antagonistic effects on ecosystem phenology. However, because the singular effects of warming and CO_2 in our study varied from positive to neutral within the growing season, we saw few significant differences from ambient plots when accumulated across the growing season.

Although our method for quantifying greenness correlated well with plant biomass, the relationship between plant biomass and Sample Point estimates of cover derived from the same photographs was variable. Species-specific estimates of plant cover using Sample Point worked well for a subset of the dominant species in our study but did not work for others, despite good agreement between cover and biomass in a similar ecosystem (Booth, Cox & Berryman 2006). However, the lack of a strong relationship between cover and biomass we report may be less satisfactory largely because the Sample Point software and method cannot account for two important aspects of plant biomass – height and differences in biomass density that influence specific leaf area (SLA). Thus, there may be some limitations to using this method for estimates of plant biomass.

EFFECTS OF CLIMATE VARIATION ON ECOSYSTEM PHENOLOGY

In mid- and high-latitude forests, the relationship between greenness and ecosystem phenology is strongly related to temperature (Potter 2003; Zhou 2003). However, in arid land ecosystems, such as the mixed-grass prairie, the timing and amount of precipitation controls virtually all ecological processes, including plant phenology and growth (Loik *et al.* 2004; Knapp *et al.* 2008; Fay *et al.* 2011), as well as greenness (Piao *et al.* 2006; Kurc & Benton 2010). In our study, greenness was highly variable between wet and dry years as well as within a growing season and also related to precipitation prior to the measurements. Climate models for the Great Plains and across the western USA predict increased incidence of drought and larger but less frequent episodic rainfall events (Easterling 2000; Groisman & Knight 2008). Given the sensitivity to precipitation variation we report, predicted changes in precipitation timing and amount will likely have large impacts for ecosystem phenology in the northern Great Plains.

Shifts in the timing of ecosystem activity can also have broader practical and management implications. Plant photosynthetic uptake is generally temperature limited early in the growing season (Lucht 2002; Tanja *et al.* 2003; Fay *et al.* 2011), and spring phenology, including the date of green-up, has already advanced in response to warming (Menzel *et al.* 2006; Cleland *et al.* 2007; Fay *et al.* 2011). Indeed, warming was associated with earlier leaf emergence and flowering, as well as earlier senescence at the PHACE site, and when applied in combination, warming and elevated CO_2 lengthened the growing season (Reyes-Fox *et al.* 2014). Similarly, we observed that in wet springs, warming enhanced greening and in dry springs, elevated CO_2 could enhance greenness alone or in combination with warming. These changes in phenology can inform the timing of livestock grazing that can more precisely coincide with the timing of green-up and maximum production. Ecological restoration efforts can be focused on species and varieties that have the greatest phenological plasticity and therefore greatest ability to adjust to climate variability in the future (Kulpa & Leger 2013).

The contribution of changes in precipitation and atmospheric CO_2 to increased greening across the Northern Hemisphere has been small to date. Nevertheless, when variation in precipitation is considered alone, it appears that grassland ecosystems are especially sensitive to increasing aridity, suggesting that large changes in ecosystem water-use efficiency, such as those associated with climate change, may be associated with especially large effects in these ecosystems (Campos *et al.* 2014). However, elevated CO_2 may dampen these negative effects of drought by increasing water-use efficiency, particularly in low-productivity grasslands that are most vulnerable to changes in water availability.

Conclusions

Vegetation is currently estimated to take up a third of the anthropogenic CO_2 emissions globally (Canadell *et al.* 2007) such that even small changes in vegetation cover and greenness highlight potential consequences for future ecosystem-level carbon uptake potential. Our method of quantifying plot greenness from repeat digital photographs, combined with canopy CO_2 flux measurements, is a useful way to assess how climate change interacts with ecosystem phenology and extends to larger spatial scales (Richardson *et al.* 2010, 2012) to better predict ecosystem-level carbon dynamics. The potential for earlier green-up is also likely to influence vegetation dynamics, potentially favouring species with earlier phenology. In addition, the timing of green-up is important for both domestic and wild herbivores and our results suggest that in addition to maintaining and increasing productivity (Morgan *et al.* 2011), warming (especially when combined with elevated CO_2) may shift the availability of forage for domestic and wild herbivores to earlier in the growing season. Interannual variation in precipitation not only determined the strength and direction of climate change treatments, but also had a much larger effect on vegetation greenness than the influence of elevated CO_2 and warming. Because the relative effects of elevated CO_2 and warming depended on seasonal and interannual precipitation patterns in our study, the potential for grassland ecosystems to adjust to changing climate conditions will depend on the ability of individual species and plant communities to tolerate increasingly variable climate conditions (Weltzin *et al.* 2003; Loarie *et al.* 2009; Craine *et al.* 2012).

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Data accessibility

The data are deposited in the Dryad repository: http://datadryad.org/resource/doi:10.5061/dryad.3mf71 (Zelikova et al. 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. MATLAB code.

Table S1. Dates when elevated atmospheric CO_2 was applied in the spring and turned off in the autumn of each year from 2006 to 2013.

Table S2. Two-way analysis of variance (ANOVA) of the effects of elevated CO_2, warming, and their interaction on seasonally accumulated greenness in the spring, summer, autumn, and annually.

Table S3. A repeated-measured MANOVA analysis of the effects of warming, elevated CO_2, date, and their interaction on percent cover for C_3 graminoids, *Bouteloua gracilis* (a C_4 graminoid), and forbs within year.

Figure S1. Effects of elevated CO_2 and warming on average difference in average plant cover from ambient plots from 2007 to 2012.