LETTER

Impacts of warming and elevated CO_2 on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time

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Abstract

It is unclear how elevated CO_2 (eCO_2) and the corresponding shifts in temperature and precipitation will interact to impact ecosystems over time. During a 7-year experiment in a semi-arid grassland, the response of plant biomass to eCO_2 and warming was largely regulated by interannual precipitation, while the response of plant community composition was more sensitive to experiment duration. The combined effects of eCO_2 and warming on aboveground plant biomass were less positive in 'wet' growing seasons, but total plant biomass was consistently stimulated by ~ 25% due to unique, supra-additive responses of roots. Independent of precipitation, the combined effects of eCO_2 and warming on C_3 graminoids became increasingly positive and supra-additive over time, reversing an initial shift toward C_4 grasses. Soil resources also responded dynamically and non-additively to eCO_2 and warming, shaping the plant responses. Our results suggest grasslands are poised for drastic changes in function and highlight the need for long-term, factorial experiments.

Keywords

Artemisia frigida, *Bouteloua gracilis*, C_3 grass, C_4 grass, climate change, forb, nitrogen, plant productivity, root biomass, soil moisture.

Ecology Letters (2016) **19**: 956–966

INTRODUCTION

Rising air temperatures and atmospheric carbon dioxide concentrations [CO_2] are the most pervasive environmental changes on land. Yet, there is much uncertainty regarding the impact of these global environmental changes on terrestrial ecosystems. Much of the uncertainty is due to the rarity of multi-year, factorial experiments that assess *in situ* effects of elevated [CO_2] (eCO_2) and warming. Studies of eCO_2 alone or warming alone are problematic if warming modifies the response of ecosystems to CO_2 or *vice-versa* (Norby & Luo 2004; Luo *et al.* 2008; Dieleman *et al.* 2012). Short-term studies are problematic because the responses of ecosystems to chronic environmental changes are expected to be dynamic over timescales from days to decades (Shaver *et al.* 2000; Luo *et al.* 2004; Morgan *et al.* 2004, 2007; Nowak *et al.* 2004; Reich *et al.* 2006; Smith *et al.* 2009, 2015; Newton *et al.* 2010; Polley *et al.* 2012; Wu *et al.* 2012; Xu *et al.* 2012; Hovenden *et al.* 2014). Dynamic effects of environmental change are expected because any ecosystem function (e.g. plant production) is a product of numerous ecological processes that operate at different time scales (e.g. photosynthesis, plant demography) and are influenced both directly and indirectly

by environmental change (e.g. via reaction kinetics and biogeochemical feedbacks). Further, although some environmental changes such as eCO_2 proceed in a relatively stable, monotonic fashion, other co-occurring environmental changes are more variable across time (e.g. precipitation).

Impacts of global warming and eCO_2 on ecosystems can vary with two distinct aspects of time: (1) the duration of exposure to altered atmospheric conditions (e.g. experiment duration) and (2) temporal variability in other environmental factors, such as meteorological conditions, that mediate the impacts of warming and eCO_2. For example, during the first months or years of an experiment, the rate and direction of change in plant production due to warming could be mostly determined by the initial physiological responses of plants and microbes; but, over years to decades, the effects on plant production could be determined more indirectly by the cumulative effects of physiological responses on community composition and resource availability in soil (Shaver *et al.* 2000; Luo *et al.* 2004; Luo 2007; Suttle *et al.* 2007; Smith *et al.* 2009; Polley *et al.* 2012; Wu *et al.* 2012). The effects of eCO_2 and warming on ecosystem functions should also vary over time with other environmental factors that regulate physiological processes and ecological interactions, including

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precipitation (Shaver *et al.* 2000; Nowak *et al.* 2004; Newton *et al.* 2010; Hoeppner & Dukes 2012; Shi *et al.* 2015). Warming effects on plant production can be more positive in years with high precipitation due to direct and indirect effects of warming on soil resources (Parton *et al.* 2007; Xu *et al.* 2012). Similarly, effects of eCO_2 on plant production can be more positive in years with low precipitation due to indirect effects of CO_2 on soil moisture (Morgan *et al.* 2004; Hovenden *et al.* 2014). Knowledge of such temporal dynamics is limited, not only because of the rarity of adequate experiments, but also because no prior study has attempted to independently attribute the variable effects of warming and eCO_2 to meteorological conditions *and* experiment duration.

Effects of warming and eCO_2 could be especially dynamic in semi-arid grasslands, which have relatively high rates of turnover in plant community composition (Cleland *et al.* 2013); this could allow relatively rapid shifts in species composition and ecosystem functions due to warming and eCO_2 (Smith *et al.* 2009). Also, biogeochemical processes in semi-arid ecosystems vary widely across years and seasons, largely because precipitation varies substantially over time and many biogeochemical processes are sensitive to water availability (Knapp & Smith 2001; Sala *et al.* 2012; Ahlström *et al.* 2015). The effects of climate change on semi-arid grasslands are of critical importance because this biome covers ~ 30% of Earth's land surface and provides valuable ecosystem services, including secondary production (e.g. beef, wool) and climate regulation (Sala & Paruelo 1997; Poulter *et al.* 2014; Ahlström *et al.* 2015).

Here, we describe the temporal dynamics of eCO_2 and warming effects on a northern mixed-grass prairie in Wyoming, USA. This perennial-dominated grassland type covers more than 350,000 km^2 and accounts for more than a third of the remaining grassland area in the Great Plains of North America (Samson *et al.* 2004). The experiment was a factorial manipulation of CO_2 (ambient and 600 ppm) and canopy temperature (ambient and warmed; + 1.5 °C during the day, + 3 °C at night), achieved using free-air CO_2 enrichment and infrared heaters (LeCain *et al.* 2015). Results from the first few years of the experiment have been reported for plant production and community composition, soil moisture, and nitrogen (N) in plants and soils (Dijkstra *et al.* 2010b, 2012a; Morgan *et al.* 2011; Carrillo *et al.* 2012, 2014; Zelikova *et al.* 2014, 2015). Using data for all these ecosystem properties across the entire 7-year experiment, we present a novel quantification of how the impacts of eCO_2 and warming, and their interactions, were mediated by meteorological conditions and experiment duration.

MATERIALS AND METHODS

Study site

The Prairie Heating and Carbon dioxide Enrichment (PHACE) experiment was located in a pasture with a history of light grazing and negligible slope (41°11′ N, 104°54′ W; 1930 m elevation). Mean annual precipitation at a nearby weather station in Cheyenne, Wyoming was 397 mm and mean annual temperature was 8 °C (1984–2013; GHCND:

USW00024018). Across the 7 years of the experiment, the range of precipitation and temperature observed on-site was similar to that observed in Cheyenne over the prior 30 years (Fig. 1; Appendix S1 in Supporting Information). During the experiment, 55 plant species were identified in biomass harvests. In the year prior to initiation of treatments, six perennial species accounted for more than 90% of the harvested biomass: *Pascopyrum smithii* (C_3 grass; 33%), *Bouteloua gracilis* (C_4 grass; 25%), *Hesperostipa comata* (C_3 grass; 20%), *Carex duriuscula* (C_3 sedge; 6%), *Artemisia frigida* (C_3 semi-woody forb; 5%) and *Sphaeralcea coccinea* (C_3 forb; 3%). Surface soils (0–15 cm) are sandy loams consisting of ≥ 55% sand (mean = 63%) and ≤ 20% clay (mean = 16%), with more clay (≤ 38%) and less sand (≥ 42%) in deeper soil layers. The surface soils are relatively low in carbon (< 3%), N (< 0.3%) and phosphorus (< 0.007%). Nutrient stoichiometry suggests plant growth might be limited by N and phosphorus. Plots were distributed across two blocks distinguished by slightly different soil types (Dijkstra *et al.* 2010b, 2012a).

Experiment and measurements

The elevated CO_2 (eCO_2) treatment increased [CO_2] near the ground to ~ 600 ppm using FACE technology. CO_2 was dispersed and measured 8 cm above the ground. The eCO_2 treatment was implemented during sunlit hours between ~ 1 April and ~ 1 November for each experiment year. The warming treatment increased canopy temperatures by 1.5 °C during the day and 3 °C during the night. Warming was implemented continuously from the beginning to the end of the experiment using infrared lamps. Both treatments were maintained in a factorial design with five plots (3.3 m diameter) per treatment for seven consecutive years (2007–2013; the CO_2 treatment was also implemented in 2006) (LeCain *et al.* 2015). Plant biomass and N concentrations in plant tissues were measured once annually in mid-July, when aboveground biomass was near its maximum (Morgan *et al.* 2011; Dijkstra *et al.* 2012a; Carrillo *et al.* 2014). Each year we sampled aboveground biomass by clipping half the quadrats in a harvest area (1.5 m^2 per plot) and recovered root biomass from three, 15-cm-long, 5-cm-wide, cores taken from an adjacent area. Volumetric soil water content was measured hourly using frequency domain reflectometry sensors. Recovery of nitrate from ion-exchange resins buried in surface soils was used as a time-integrated proxy for the size of the inorganic N pool in soil. Appendix S1 has more details.

Analyses

For each dependent variable, we ran a linear mixed model that included block and plot as random factors (to account for spatial and temporal autocorrelation); the fixed factors were treatment, year (discrete), and pre-treatment values of the dependent variable. Interactions between treatments and with year were also included. We used these models to test the significance of each treatment effect (eCO_2 and warming) and treatment interactions while accounting for pre-existing variation among plots (Fig. 2, Table S1).
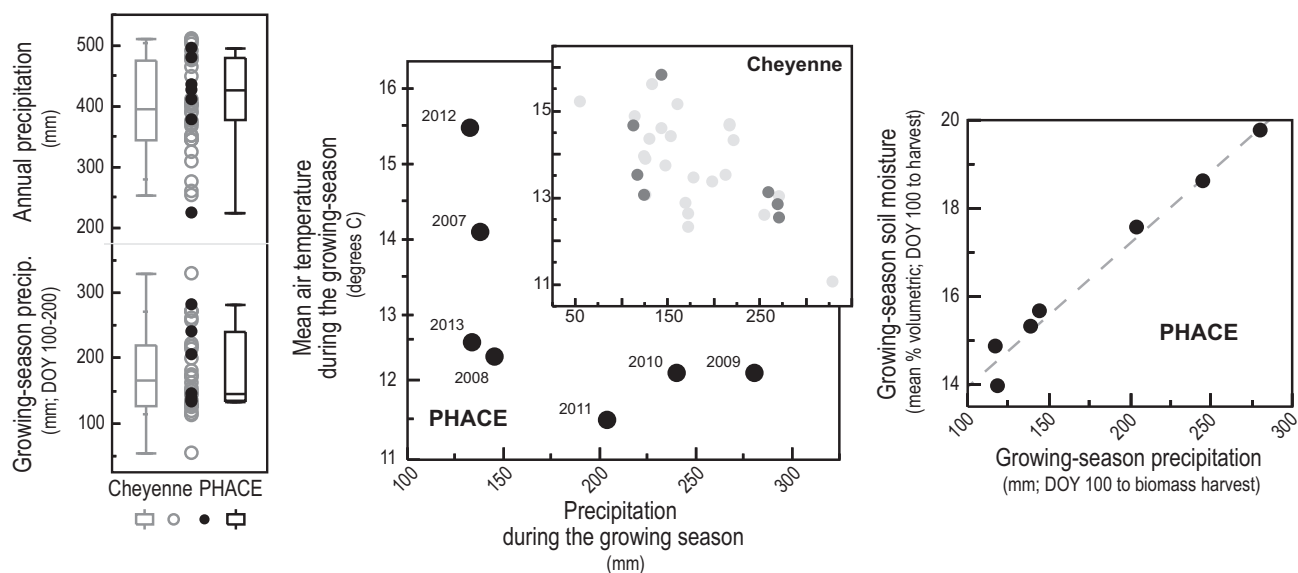
Figure 1 Variability in meteorological conditions across years and growing-seasons for the study site and the nearest long-term weather station (the municipal airport in Cheyenne, WY, which is ~ 8 km from the study site). Data for Cheyenne are for a 30 year record that culminates in the final year of the PHACE experiment, 2013. Data for the study site are shown for the 7 years for which the PHACE treatments were implemented (2007–2013). Each year, harvest of plant biomass began within 4 days of DOY 200.

To evaluate how treatment effects were mediated by specific temporal variables (e.g. experiment duration), we calculated *proportional* treatment effects on ecosystem properties after adjusting the values of each ecosystem property for modelled effects of other temporal variables (e.g. ambient soil moisture and air temperature; the adjustment process is described below). Proportional treatment effects were calculated by subtracting the mean adjusted values of one treatment (e.g. plots with eCO_2 alone) from the mean adjusted values of control plots and then dividing this difference by the mean of control plots. Using linear regression, the sensitivity of adjusted, proportional treatment effects to one temporal variable (e.g. experiment duration) could then be evaluated independently of variation in other temporal variables (e.g. soil moisture and temperature). For example, we determined that experiment duration influenced the combined effects of eCO_2 and warming on soil nitrate if the slope of the relationship between experiment duration and the proportional treatment effects (adjusted for the effects of soil moisture and air temperature) was at least marginally different from zero ($P < 0.1$; Fig. 3b).

The adjusted values of each ecosystem property were derived from the coefficients of a second set of linear mixed models, consistent with the calculation of least-squares means in the ANCOVA framework. The model structure was designed to attribute the generic 'year' and 'treatment × year' effects evident in the first set of analyses (Fig. 2, Table S1) to specific temporal variables. The model structure was the same as that described above, except we removed the discrete 'year' term and added three continuous temporal variables and their interactions with the treatments. The specific temporal variables were: (1) experiment duration, (2) mean ambient air temperature during each growing season, and (3) mean ambient soil moisture during each growing season. To focus on meteorological conditions that most strongly influence

aboveground plant growth in this ecosystem (Derner & Hart 2007; Morgan *et al.* 2011; Zelikova *et al.* 2015), we averaged ambient soil moisture and air temperatures between day of year 100 (early April) and harvest of plant biomass (DOY 196-203). With each of these temporal variables in the same model, the modelled effects of each temporal variable (e.g. experiment duration) are statistically independent of the other temporal variables (soil moisture and air temperature). We included ambient air temperature in these models primarily to better isolate and characterize the effects of experiment duration and soil moisture (reflecting precipitation; Fig. 1). Consistent with calculations of least-squares means, model coefficients and mean values of each temporal variable were used to adjust the plot-level values of each ecosystem property for the modelled effects of any two temporal variables (Snedecor & Cochran 1989; Barrett 2011) (Appendix S1). This allowed us to evaluate independent relationships between each temporal variable and both: (1) adjusted values of each ecosystem property, given mean values of other temporal variables, and (2), proportional treatment effects on the adjusted values of each ecosystem property (Figs 3 and 4).

Although this ANCOVA model structure can determine if treatments have significantly different slopes for the relationship between an ecosystem property and a temporal variable, the interpretation of *F*-tests for the corresponding interaction terms (e.g. CO_2 × experiment duration) is dependent on the transformation used to normalize the distribution of each ecosystem property; this prevents a straightforward interpretation of the *F*-tests as indicators of treatment × time interactions across the different ecosystem properties (Appendix S1). Further, the ANCOVA *F*-tests do not evaluate our primary null hypothesis: that treatment effects do not become *proportionally* larger or smaller with changes in experiment duration or soil moisture.
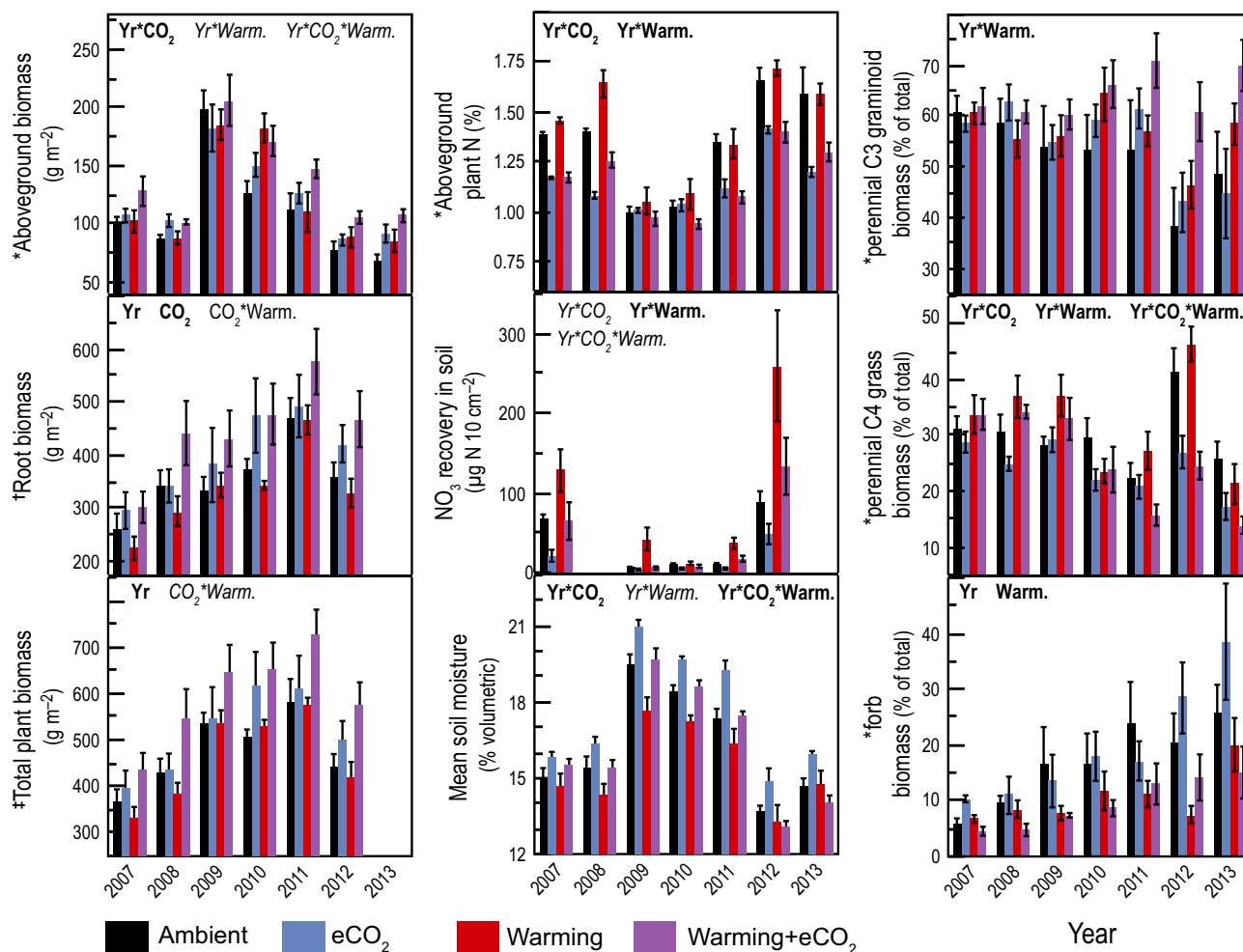
Figure 2 Treatment effects on ecosystem properties across years. Bold print indicates model terms with $P < 0.05$ and *italic print* indicates model terms with $0.05 < P < 0.1$. Treatment interaction terms are also shown in normal print if $0.1 < P < 0.15$ because in factorial designs with low n, risk of Type II errors is likely much greater than that of Type I errors (Smith *et al.* 2002) and interpretation of main effects is conditional upon the interaction (Stehman & Meredith 1995). The significance of main effects (CO_2, Warming, Year) are not shown if a treatment × year term had $P < 0.1$. See Table S1 for full model ouput. *Y-axis labels with an asterisk indicate that the bars for the given ecosystem property show least-squares means (adjusted for the effect of pre-treatment variation among plots). [†]Root biomass was sampled to 15 cm below the soil surface [‡]Total plant biomass is the sum of aboveground and root biomass, excluding crowns.

RESULTS

We observed significant or nearly significant interactions between the eCO_2 and warming treatments for most of the measured ecosystem properties (Fig. 2, Table S1). Thus, below we report and interpret the results of each treatment separately (e.g. 'eCO_2 effects' refers to the difference between controls and eCO_2, ambient temperature plots). During the 7-year experiment, treatment effects were consistent in magnitude and persistent in direction for only a few ecosystem properties. For example, in every year the combination of warming and eCO_2 had positive effects on 'total' plant biomass (shoots + roots, excluding crowns). All measured ecosystem properties varied significantly across years and most showed some evidence of treatment × year interactions (Fig. 2, Table S1). Below we report how meteorological conditions and experiment duration mediated these treatment × year interactions. Each subsequent reference to a

relationship between a temporal variable (e.g. soil moisture) and an ecosystem property, or treatment effects on ecosystem properties, is based on adjusted-values of the ecosystem property that render the relationship independent of the other modelled temporal variables (e.g. experiment duration and ambient air temperature).

Meteorological conditions

Variability in ambient soil moisture across growing seasons was strongly positively correlated with aboveground plant biomass but negatively correlated with indices of N-availability, including nitrate accumulation on buried resins and N concentrations in plant shoots and roots (Fig. 3a, Fig. S1). The magnitude of treatment effects on plant biomass and N-availability also varied with soil moisture. Consistent with an earlier report from this study (Morgan *et al.* 2011), the proportional effects of eCO_2 on aboveground biomass
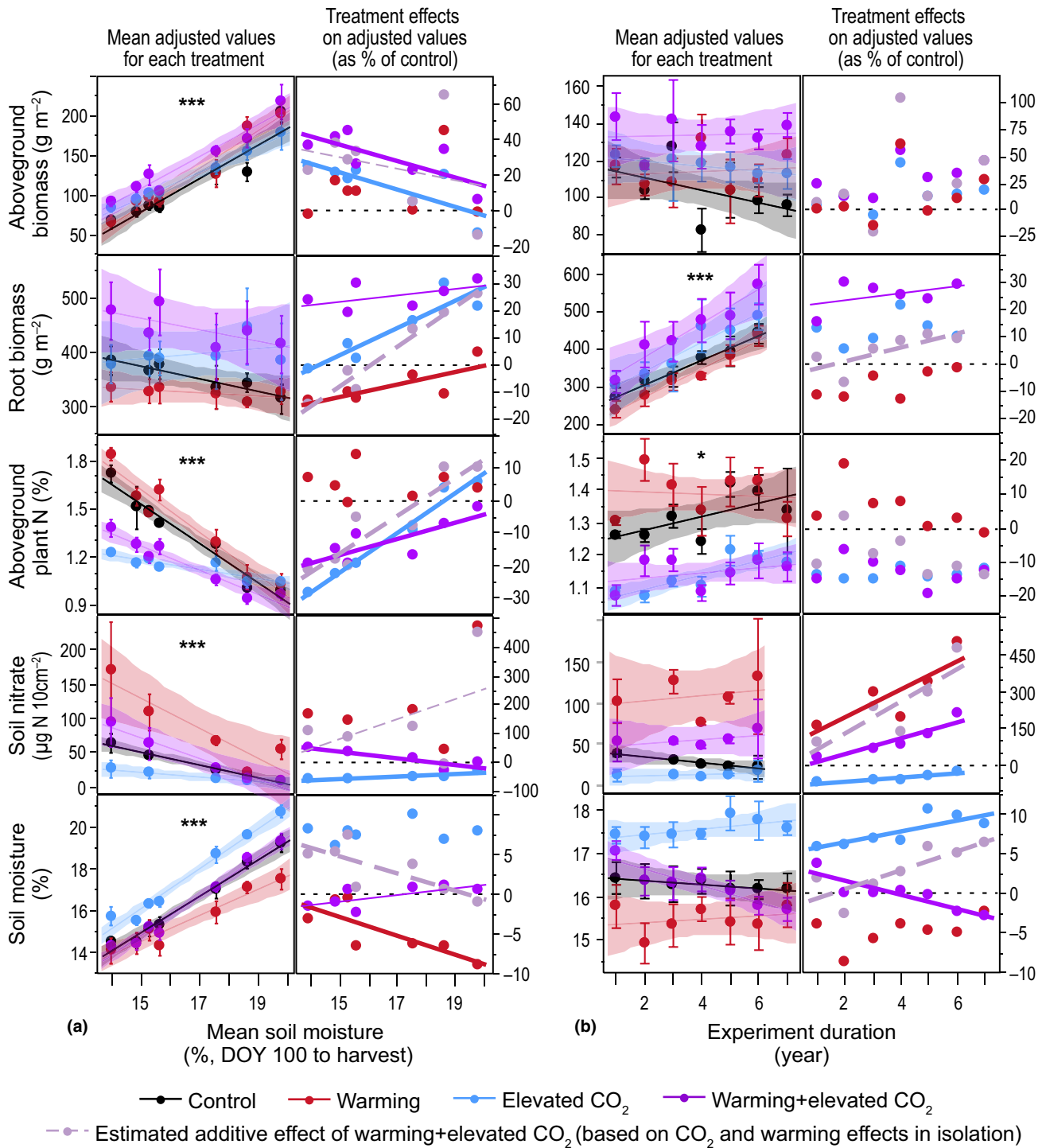
Figure 3 Influence of soil moisture and experiment duration on ecosystem properties and treatment effects on ecosystem properties. For each temporal variable (e.g. soil moisture), the corresponding Y-axes show variability in adjusted, plot-level values for each ecosystem property (given mean values for the other temporal variables, e.g., air temperature and experiment duration). For each treatment, solid lines show the linear trend between the temporal variable and both: (1) adjusted values of each ecosystem property, and (2) proportional treatment effects on each adjusted ecosystem property. Shading shows the 95% confidence intervals of the mean for each treatment and adjusted ecosystem property. Error bars indicate standard error. Asterisks show the statistical significance of the main effects of each temporal variable (*$P < 0.1$, **$P < 0.01$, ***$P < 0.001$). For the panels showing treatment effects, thick lines indicate linear trends with slopes that were at least marginally different from zero ($P < 0.1$); thin lines are shown for comparison when the 'estimated additive' and 'observed' effects of the combined treatment were divergent.

diminished to near zero in 'wet' growing seasons (i.e. with relatively high soil moisture and precipitation; Fig. 1). The combined effects of warming and eCO₂ on aboveground biomass

were also less positive in wet growing seasons, with no apparent warming × CO₂ × soil moisture interaction (i.e. the warming effect on aboveground biomass was not sensitive to
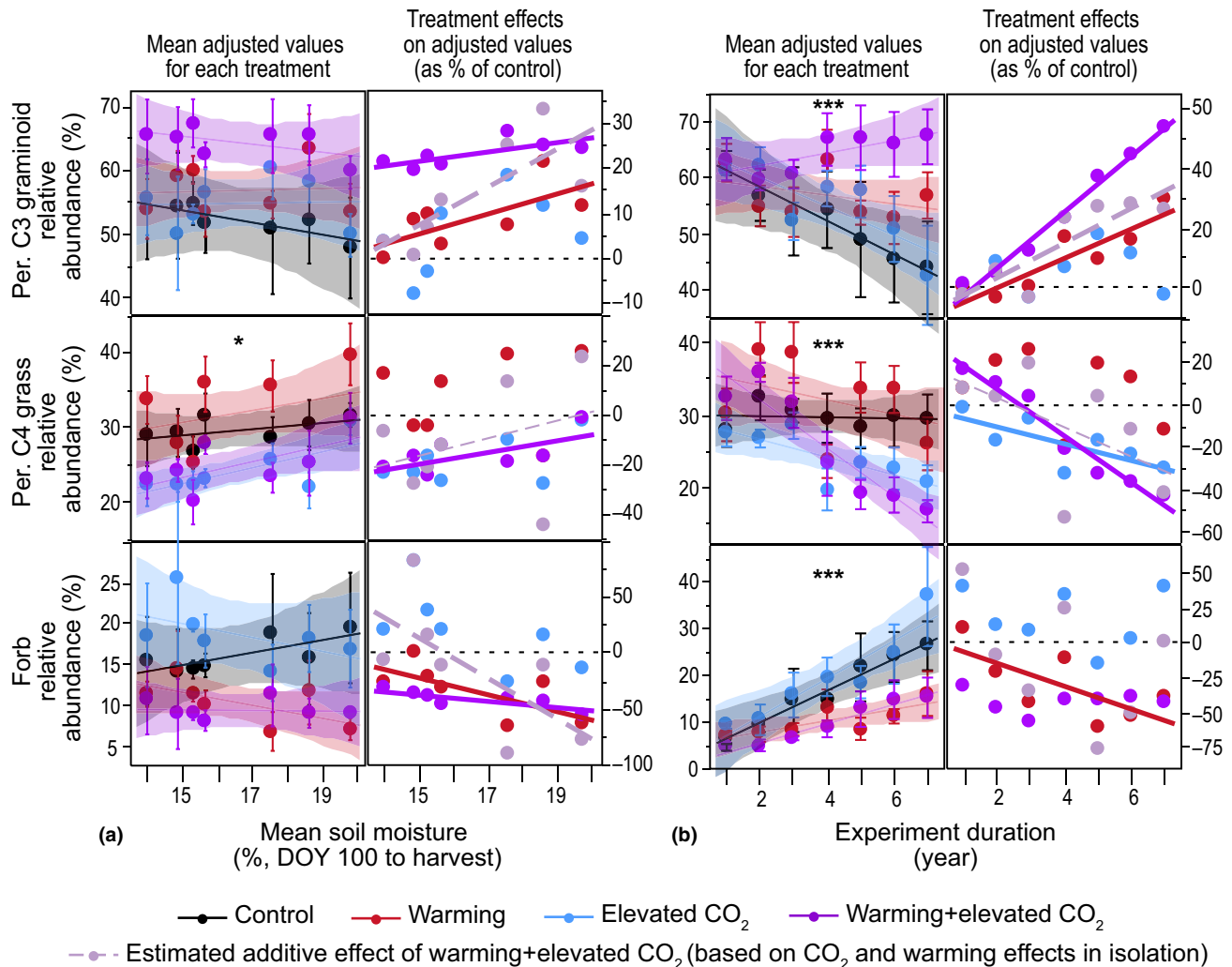
Figure 4 Influence of soil moisture and experiment duration on plant community composition and treatment effects on plant community composition. For each temporal variable (e.g. soil moisture), the *Y*-axes show variability in adjusted, plot-level values of relative abundance (given mean values for the other temporal variables, e.g., air temperature and experiment duration). Linear fits, shading, error bars, and asterisks are as in Fig. 3. For the panels showing treatment effects, thick lines indicate linear trends with slopes that were at least marginally different from zero (*P* < 0.1); thin lines are shown for comparison when the 'estimated additive' and 'observed' effects of the combined treatment were divergent.

soil moisture and warming did not alter the CO_2 × soil moisture interaction). For root biomass, unlike the aboveground effects, the proportional effects of eCO_2 alone and warming alone were more *positive* in wet growing seasons, shifting from approximately neutral to a stimulation of up to 30% compared to controls. A study in a North American tallgrass prairie also showed warming effects on root production were more positive in wet years (Xu *et al.* 2012). In our study, warming and eCO_2 together had supra-additive, *i.e.* synergistic, effects on root biomass in dry growing seasons. Thus, despite no effect of eCO_2 and negative effects of warming in dry growing seasons, eCO_2 and warming together consistently increased root biomass and total biomass by 20–30% (Fig. 3a, Fig. S2). The smaller effects of eCO_2 on aboveground biomass in wet growing seasons were coupled with lower apparent N-availability and diminished effects of eCO_2 on N-availability; the proportional effect of eCO_2 on plant N concentrations and nitrate in soil went from strongly negative

in dry growing seasons to neutral in wet growing seasons (Fig. 3a).

Variability in soil moisture across growing seasons had neutral or relatively small effects on the relative abundance of C_3 graminoids, C_4 grasses, and forbs (including sub-shrubs). However, in wet growing seasons, the effects of warming alone and the combined treatment were more positive for C_3 graminoids and more negative for forbs. Also, the effect of the combined treatment on C_4 grasses was less negative in wet growing seasons (Fig. 4a).

As expected, most of the measured ecosystem properties displayed strong sensitivity to ambient air temperature. Warmer growing seasons were characterized by higher plant biomass aboveground, lower root biomass, lower N concentrations in roots, more nitrate in soil, lower soil moisture, greater relative abundance of C_4 grasses and lower relative abundance of C_3 graminoids (Fig. S3). The magnitude of treatment effects on those ecosystem properties was also sometimes dependent on

growing season temperature. In warmer growing seasons, the effects of eCO_2 were more positive, or less negative, for root biomass, N concentrations in plant shoots, and nitrate in soil. Warmer growing-seasons also had more positive effects of warming alone and the combined treatment on C_3 graminoid abundance and less negative effects of the combined treatment on forb abundance (Fig. S3).

Experiment duration

Independent of growing season precipitation and temperature, some ecosystem properties, and treatment effects on ecosystem properties, changed progressively from the beginning to the end of the experiment (Figs 3b and 4b). This was especially evident for plant community composition. The effect of warming alone on the relative abundance of forbs was initially neutral, but by the end of the experiment, forb abundance in warmed plots was half of that in controls. This increasingly negative effect of warming on forbs was largely attributable to a species that accounted for half of forb biomass in controls during the last 4 years of the experiment: *A. frigida*, a perennial, semi-woody sub-shrub. Negative effects of warming on forbs were also reported in a North American tallgrass prairie (Fay *et al.* 2011). In our combined warming and eCO_2 plots, the relative abundance of C_4 grasses declined continuously over time, causing the proportional effects of warming and eCO_2 together to shift from modestly positive (+ 20%) to strongly negative (−40%). A single species, *B. gracilis*, accounted for > 90% of C_4 grass biomass throughout the experiment. Conversely, the proportional effects of the combined treatment on relative abundance of perennial C_3 graminoids became increasingly positive over time. At the end of the experiment, the relative abundance of C_3 graminoids was > 50% higher in combined treatment plots than in controls (due to increases in the combined treatment plots and decreases in controls). The combined effects of eCO_2 and warming on C_3 graminoid abundance also became increasingly supra-additive over time (Fig. 4b).

Total plant biomass and below ground properties also varied with experiment duration, and differentially so among treatments. The combined effects of eCO_2 and warming on total plant biomass increased from a stimulation of ∼ 20% initially to ∼ 30% by the sixth year of the experiment. The effect of warming on total plant biomass also became more positive over time, shifting from negative initially to neutral later (Fig. S2). By the end of the experiment, nitrate accumulation on buried resins was ∼ 150% higher in the combined treatment (compared to controls). This apparent rise in N-availability also occurred in the warming only treatment, while negative effects of eCO_2 on nitrate in soils diminished over time (Fig. 3b). The proportional effects of the combined treatment on soil moisture shifted from slightly positive to slightly negative over time. This was coincident with a trend towards slightly more positive effects of eCO_2 alone on soil moisture over time. Notably, the combined effects of eCO_2 and warming were non-additive, or increasingly non-additive over time, for total plant biomass, soil nitrate, and soil moisture (Figs 2 and 3b, Fig. S2, Table S1).

DISCUSSION

In this study, the impacts of warming and elevated CO_2 (eCO_2) shifted drastically over time for a suite of ecosystem properties that are, collectively, the foundation of ecosystem services provided by semiarid grasslands. As discussed below, the impacts of simulated climate change on plant biomass were primarily mediated by yearly precipitation, while the impacts on plant community composition were shaped more by experiment duration. However, the impacts of eCO_2 and warming were relatively stable for some ecosystem properties. For example, eCO_2 and warming together consistently increased total plant biomass by ∼ 25% across years. This suggests that in the future, the plant carbon pool in semiarid grasslands could be stimulated by higher [CO_2] and temperature, regardless of precipitation, resulting in a negative feedback to climate change. Notably, the combined effects of eCO_2 and warming on total plant biomass were *larger* than expected based on their effects in isolation. This result, coupled with significant eCO_2 × warming interactions for other ecosystem properties, shows that studies of eCO_2 or warming alone could misrepresent the impacts of climate change on grasslands (see also Dieleman *et al.* 2012). The combination of eCO_2 and warming also had consistent effects on forb abundance over time, resulting in a 40% reduction in forb relative abundance. This result was not apparent in earlier analyses (Morgan *et al.* 2011) and has consequences for future ecosystem functions due to potential differences between graminoids and forbs (e.g. related to leaf morphology, forage quality, or rooting depth) (Dickinson & Dodd 1976; Kindscher & Wells 1995; Craine *et al.* 2002; Tjoelker *et al.* 2005). The subshrub that was mostly responsible for the sensitivity of 'forbs' to future climatic conditions, *A. frigida*, flowers and senesces later than other common species (Dickinson & Dodd 1976; Reyes-Fox *et al.* 2014). Thus, compared to the present state of the mixed-grass prairie, resource availability for wildlife and livestock in the future might be diminished late in the growing season (see also Suttle *et al.* 2007).

Precipitation

The relatively stable effect of eCO_2 and warming together on total plant biomass was a consequence of contrasting impacts of precipitation on treatment effects above- and belowground. Thus, to better understand how precipitation regulates the response of ecosystems to eCO_2 and warming, studies should consider not only shoots (Morgan *et al.* 2004), but also roots (Nowak *et al.* 2004). Unique responses of root biomass to climate change are of importance, not only because roots account for most of the plant carbon pool in grasslands, but because root biomass is pivotal to whole plant function, belowground food webs, and nutrient cycling (Van Noordwijk *et al.* 1998; Mokany *et al.* 2006).

Coincident trends in N-availability indices suggest the treatment effects on plant biomass were sensitive to soil moisture, at least partly, via the influence of precipitation on nutrient cycling. We observed smaller eCO_2 effects on aboveground biomass in wet growing seasons with lower apparent N-availability; this is consistent with other observations that N-

availability can limit the response of plant production to eCO_2 in grasslands (Reich *et al.* 2006; Reich & Hobbie 2013), perhaps especially in years with high spring rainfall (Hovenden *et al.* 2014). For root biomass, the more positive effects of eCO_2 and warming alone in wet growing seasons could indicate greater plant allocation of carbon belowground due to the coupling of enhanced N-limitation with alleviation of other limiting factors (CO_2 and temperature) (Wang & Taub 2010; Poorter *et al.* 2012). The supra-additive effects of warming and eCO_2 together on root biomass in dry growing seasons might reflect shifts in plant carbon allocation due to unique conditions with respect to multiple limiting factors (e.g. perhaps the combination of eCO_2 and warming causes phosphorus-limitation to become more important in dry periods, as compared to N-limitation (Dijkstra *et al.* 2012a)).

Regardless of how treatment effects on plant biomass and N cycling are coupled mechanistically, the reported trends with soil moisture have implications for future forage production and quality, and thus secondary production, in rangelands. For example, if average spring precipitation near the end of the 21st century is 10–30% higher than the present, as is projected for the northern mixed-grass prairie under higher emissions scenarios (Melillo *et al.* 2014), the late century impacts of eCO_2 and warming on aboveground biomass might be better approximated by our observations in a wet year (10–20% stimulation) than in an average year (~ 30% stimulation; Fig. 3a). In this same scenario of future precipitation change, shoot N concentrations might be lower than present norms and insensitive to higher $[CO_2]$ and temperature (Fig. 3a). Thus, if springs throughout the northern mixed-grass prairie are frequently wetter in the future compared to present norms, forage production in the future might typically be high compared to present norms, but of lower quality (due to lower N concentration), regardless of $[CO_2]$ and temperature. During future droughts, which might be more frequent and severe compared to present (Dai 2011; Trenberth *et al.* 2014; Zhao & Dai 2015), the combination of eCO_2 and warming is likely to boost forage production but reduce forage quality via reduced shoot N content, in both cases by more than the average effect observed in this experiment (Fig. 3a).

Experiment duration

Perhaps the most notable shift during the experiment was the complete reversal of the initial advantage to C_4 grasses under the combination of eCO_2 and warming, which coincided with an increasingly positive effect of the combined treatment on C_3 graminoids. Thus, the 'winners' after 3 years of exposure to eCO_2 and warming (Morgan *et al.* 2011) were 'losers' just 4 years later. Due to potential differences in functional traits between C_3 and C_4 graminoids (Dickinson & Dodd 1976; Kindscher & Wells 1995; Epstein *et al.* 1998; Craine *et al.* 2002; Tjoelker *et al.* 2005; Taylor *et al.* 2010), the diminishing abundance of C_4 grasses under both warming and eCO_2 suggests climate change could drastically alter the diversity and functioning of mixed-grass prairies. For example, since the C_3 graminoids in this ecosystem tend to initiate and complete both growth and flowering sooner than the predominant C_4 grass, *B. gracilis* (Dickinson & Dodd 1976; Reyes-Fox *et al.*

2014), a shift towards dominance of C_3 graminoids could alter the seasonal distribution of resource availability for wildlife and livestock. Due to their unique phenology and photosynthetic-pathway, C_4 grasses often have unique responses to droughts, heat waves, and the seasonality of temperature and precipitation (Sage & Kubien 2003; Winslow *et al.* 2003; Derner & Hart 2007; Wang *et al.* 2012; Taylor *et al.* 2014; Irisarri *et al.* 2016); thus, a decline of *B. gracilis* under future climatic conditions would likely alter the response of the mixed-grass prairie to such meteorological conditions.

The changes in plant community composition over time were likely both a cause and consequence of the temporal shifts in treatment effects on soil resources. For three reasons, the increasingly positive effect of the combined treatment on nitrate in soil could have facilitated the divergent effects of that treatment on the abundance of C_3 graminoids and C_4 grasses. First, the positive effect of warming on N-availability might have been largest in the spring, when C_4 grasses are less active (Epstein *et al.* 1998) and ambient soil temperatures, but not soil moisture, probably limit rates of N-mineralization by microbes. Second, C_3 grasses have been shown to benefit disproportionately from N-addition in other grassland experiments (Wedin & Tilman 1996; Reich *et al.* 2001; Sage & Kubien 2003; but see Lauenroth *et al.* 1978; Wilson & Shay 1990), perhaps due to lower N-use-efficiency (Tjoelker *et al.* 2005; Taylor *et al.* 2010). Third, because the direct, positive effects of eCO_2 on photosynthetic rates are greater for C_3 plants, but potentially constrained by N-availability (Ainsworth & Rogers 2007; Leakey *et al.* 2009), the increasingly positive effects of the combined treatment on soil nitrate could have could have allowed for a progressively larger expression of the positive effect of eCO_2 on C_3 photosynthesis. The latter explanation could underlie the increasingly supra-additive response of C_3 graminoid abundance to the combination of warming and eCO_2.

C_3 graminoids also tend to use water less efficiently than C_4 grasses (Tjoelker *et al.* 2005) and are likely to use more water during the primary growing-season (spring to early summer) (Epstein *et al.* 1998). Thus, the increasing dominance of C_3 graminoids in the combined treatment over time, coupled with more positive effects of the combined treatment on total plant biomass, might have caused the impact of this treatment on soil moisture to shift from positive to negative. In turn, because positive effects of eCO_2 on C_4 grasses are mediated by indirect effects on soil moisture (Leakey *et al.* 2009; Dijkstra *et al.* 2010a), the decrease in soil moisture over time in the combined treatment could have contributed to the decrease in C_4 grass abundance.

Such shifts in the response of plant community composition and soil resources to climate change would be expected to interactively influence the response of more emergent ecosystem properties. For example, the increasingly positive effects of the combined treatment on total plant biomass over time may have resulted from progressively positive treatment effects on N-availability and relative abundance of C_3 graminoids (which are typically taller and more productive than the dominant C_4 grass (Derner & Hart 2007)). Further, given the role of plants, soil moisture, and N-availability in driving ecosystem-scale fluxes of energy, water, and greenhouse gases

(Dijkstra *et al.* 2012b, 2013; Pendall *et al.* 2013; Ryan *et al.* 2015), the contribution of the mixed-grass prairie to climate regulation likely evolved in concert with treatment effects on plants and soils.

The strong temporal shifts in root biomass and plant community composition in the control plots (Figs 3b and 4b) suggest the treatments might have interacted with another factor that varied linearly over time (other than duration of exposure to treatments). Within the controls, the progressive decline of C_3 graminoids and rise of forbs, particularly *A. frigida*, could have been caused by a shift in grazing pressure; biennial clipping during the experiment likely increased vegetation removal compared to pre-experiment conditions, when there was light grazing by cattle and wildlife. This speculation is consistent with other observations that increased grazing intensity can increase the abundance of *A. frigida* at the expense of C_3 graminoids, particularly in nutrient-poor soils (Gao *et al.* 2005; Liu *et al.* 2006; Liang *et al.* 2009). Thus, in our study, the negative effect of warming on forb abundance might have been mediated by the positive effect of warming on N-availability. Since grazing is the dominant land use in semiarid grasslands and grazing intensity can substantially impact ecosystem properties (Schuman *et al.* 1999; Porensky *et al.* 2016), future studies should evaluate how grazing mediates the response of grasslands to climate change (Polley *et al.* 2011; Newton *et al.* 2014).

CONCLUSIONS

Our results are consistent with expectations that impacts of eCO_2 and warming on ecosystems will change over time as soil resources vary with precipitation and the duration of exposure to altered atmospheric conditions. Some progressive shifts in treatment effects were only apparent because we independently quantified the role of meteorological conditions *and* experiment duration. For example, after adjusting for growing season precipitation and ambient temperature, the combined effects of eCO_2 and warming on total plant biomass increased over time and the effects of *all* treatments on soil nitrate became progressively positive (or less negative). Future studies could utilize a similar approach to better characterize the temporal evolution of climate change impacts (see also Nowak *et al.* 2004). For the northern mixed-grass prairie, the long-term trajectories of plant productivity and community composition likely depend on whether eCO_2 and warming in combination can persistently increase nitrogen-availability (Parton *et al.* 2007; Newton *et al.* 2010; Dieleman *et al.* 2012; Mueller *et al.* 2013; Reich & Hobbie 2013). Given that $eCO_2 \times$ warming experiments are rare, these results should be valuable for improving models that make long-term and broad-scale predictions regarding the impacts of climate change (Norby & Luo 2004; Luo *etal.* 2008).

ACKNOWLEDGEMENTS

AUTHORSHIP

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