

Elevated CO₂ induces substantial and persistent declines in forage quality irrespective of warming in mixedgrass prairie

DAVID J. AUGUSTINE,^{1,4} DANA M. BLUMENTHAL,¹ TIM L. SPRINGER,² DANIEL R. LECAIN,¹
STACEY A. GUNTER,² AND JUSTIN D. DERNER³

¹Rangeland Resources and Systems Research Unit, USDA Agricultural Research Service, 1701 Centre Avenue,
Fort Collins, Colorado 80525 USA

²Southern Plains Range Research Station, USDA Agricultural Research Service, 2000 18th Street, Woodward, Oklahoma 73801 USA

³Rangeland Resources and Systems Research Unit, USDA Agricultural Research Service, 8408 Hildreth Road,
Cheyenne, Wyoming 82009 USA

Abstract. Increasing atmospheric [CO₂] and temperature are expected to affect the productivity, species composition, biogeochemistry, and therefore the quantity and quality of forage available to herbivores in rangeland ecosystems. Both elevated CO₂ (eCO₂) and warming affect plant tissue chemistry through multiple direct and indirect pathways, such that the cumulative outcomes of these effects are difficult to predict. Here, we report on a 7-yr study examining effects of CO₂ enrichment (to 600 ppm) and infrared warming (+1.5°C day/3°C night) under realistic field conditions on forage quality and quantity in a semiarid, mixedgrass prairie. For the three dominant forage grasses, warming effects on *in vitro* dry matter digestibility (IVDMD) and tissue [N] were detected only in certain years, varied from negative to positive, and were relatively minor. In contrast, eCO₂ substantially reduced IVDMD (two most abundant grasses) and [N] (all three dominant grass species) in most years, except the two wettest years. Furthermore, eCO₂ reduced IVDMD and [N] independent of warming effects. Reduced IVDMD with eCO₂ was related both to reduced [N] and increased acid detergent fiber (ADF) content of grass tissues. For the six most abundant forage species (representing 96% of total forage production), combined warming and eCO₂ increased forage production by 38% and reduced forage [N] by 13% relative to ambient climate. Although the absolute magnitude of the decline in IVDMD and [N] due to combined warming and eCO₂ may seem small (e.g., from 63.3 to 61.1% IVDMD and 1.25 to 1.04% [N] for *Pascopyrum smithii*), such shifts could have substantial consequences for the rate at which ruminants gain weight during the primary growing season in the largest remaining rangeland ecosystem in North America. With forage production increases, declining forage quality could potentially be mitigated by adaptively increasing stocking rates, and through management such as prescribed burning, fertilization at low rates, and legume interseeding to enhance forage quality.

Key words: *Bouteloua gracilis*; forage quality; global warming; Great Plains grassland; *Hesperostipa comata*; livestock production; *Pascopyrum smithii*; rangeland management.

INTRODUCTION

Rangeland ecosystems occupy approximately 30% of the earth's land area, and are used in the production of the majority of the world's livestock (Asner et al. 2004, Ellis and Ramankutty 2008). Increasing atmospheric [CO₂] and mean annual temperature are expected to affect the productivity, species composition, biogeochemistry, and therefore the quantity and quality of forage in rangeland ecosystems. In some semiarid rangelands, warming can reduce temperature limitation and/or exacerbate moisture limitation of primary productivity (Rustad et al. 2001, Dieleman et al. 2012). Elevated atmospheric [CO₂] (eCO₂ hereafter) can offset these

effects by directly increasing photosynthesis (particularly for C₃ grasses) and enhancing water use efficiency of C₃ and C₄ plants (Morgan et al. 2004, 2011, Polley et al. 2013). Due to the combination of direct effects and indirect water-mediated effects on plants, eCO₂ and warming are expected to two important drivers of change in semiarid ecosystems. Warming and eCO₂ may be particularly important in mid-latitude rangelands predicted to experience minor changes in annual precipitation, including a broad swath of central North America (Kunkel et al. 2013, Polley et al. 2013, Reeves et al. 2014). Previous work has shown that eCO₂, alone and in combination with warming, has profound and often positive effects on the productivity of temperate, semiarid rangelands, which translates into increased productivity of the dominant grasses consumed by livestock (Morgan et al. 2004, Polley et al. 2011, Mueller et al. 2016).

Livestock productivity in these rangelands depends not only on the quantity of forage produced, but also on

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⁴E-mail: david.augustine@ars.usda.gov

forage quality. Reduced digestibility can suppress live-stock weight gains and milk production, and reductions in forage N concentrations can impair ruminal metabolizable protein yield (NASEM 2016). Greenhouse studies of forage grasses (Augustine et al. 2011) and a prior field experiment in the shortgrass steppe of North America (Milchunas et al. 2005) showed that eCO₂ reduced forage protein content and digestibility. Elevated CO₂ also often reduces soil N availability and plant N, further suggesting the potential for reductions in forage quality (Luo et al. 2004, Ainsworth and Long 2005, Newton et al. 2010, Reich and Hobbie 2013). However, variation in both the direction and magnitude of eCO₂ effects on forage quality results in an overall neutral effect when averaging across the few existing studies (Dumont et al. 2015).

Relative to the effects of eCO₂, even less is known about the effect of elevated temperature on forage quality (Dumont et al. 2015). Based on a continental analysis of cattle diet quality in North America, Craine et al. (2010) suggest that forage quality is likely to decline with increasing temperature, but this analysis relied on a space-for-time substitution approach that may not hold under future climate change. Increasing temperatures could affect forage quality through multiple pathways. Soil warming can enhance soil nitrogen (N) availability (Rustad et al. 2001, Dieleman et al. 2012), while warming-induced desiccation can impact plant N uptake (Dijkstra et al. 2010). Although few studies have tested how rising temperatures may interact with effects of eCO₂, trends to date suggest that the combination of these changes can also reduce plant N (Dieleman et al. 2012, Mueller et al. 2016). Thus, available evidence suggests declining forage quality could be an important factor for livestock production under future climate change, but measurements of forage quality following experimental CO₂ enrichment or warming remain rare.

Here, we report on a long-term (7-yr) field experiment evaluating the direct and interactive effects of eCO₂ and warming on the digestibility and N content of dominant forage grasses in mixedgrass prairie of Wyoming, USA. This region is characterized by co-dominance of C₃ mid-height grasses (particularly *Pascopyrum smithii* and *Hesperostipa comata*) and the C₄ shortgrass, *Bouteloua gracilis*. These three grasses comprise most of the growing-season diets consumed by livestock in the region (e.g., Milchunas et al. 2008). We measured forage quality of these three dominant grass species in terms of (1) *in vitro* dry matter digestibility (IVDMD), (2) cellulose and lignin content (acid detergent fiber), and (3) N content and evaluated treatment effects on forage quality in the context of the substantial interannual variability in weather conditions over the study period.

METHODS

Study area

We conducted the Prairie Heating And CO₂ Enrichment (PHACE) experiment at the U.S. Department of

Agriculture, Agricultural Research Service's High Plains Grasslands Research Station, Wyoming, USA (latitude 41°11' N, longitude 104°54' W). The ecosystem is a northern mixedgrass prairie dominated by the C₃ grasses *P. smithii* and *H. comata*, and the C₄ grass *B. gracilis*. Other species include the sedge *Carex eleocharis*, the C₃ perennial grass *Koeleria macrantha*, and the perennial forb *Sphaeralcea coccinea*. These six species, all of which are palatable to livestock, comprise between 80% and 99% of total aboveground biomass at the experimental site. Mean annual precipitation is 397 mm, mean precipitation during the key portion of the growing season between day of year (DOY) 100 and 200 is 167 mm, and mean air temperatures are 17.5°C in July and 2.5°C in January. Interannual variability in precipitation in the 30-yr long-term record from nearby Cheyenne, Wyoming, was similar to the variability measured on-site during 2007–2013. During these seven years, on-site precipitation during DOY 100–200 was 137, 144, 280, 237, 205, 132, and 134 mm respectively, which in turn was highly correlated with mean growing-season soil moisture content (Mueller et al. 2016). The soil is a fine loamy, mixed, mesic Aridic Argiustoll with a pH of 7.0, bulk density of 1.2 g/cm³, 62% sand, 23% silt, and 15% clay.

Experiment and measurements

The elevated CO₂ (eCO₂) treatment increased [CO₂] near the ground within the short-statured canopy (~10 cm) to ~600 ppm using Free Air CO₂ Enrichment FACE technology (Miglietta et al. 2001) during sunlit hours in the growing season (~1 April and ~1 November each year). The warming treatment was implemented year-round and increased canopy temperatures (measured with infrared thermometers) by 1.5°C during the day and 3°C during the night using a hexagonal array of 1,000 W ceramic infrared heating elements at each plot (Kimball et al. 2008). Both treatments were maintained in a factorial design with five plots (3.3 m diameter) per treatment for seven consecutive years (2007–2013). Plots were distributed across two blocks (north vs. south) distinguished by slightly different soil types. Aboveground plant biomass was clipped in mid-July each year at peak biomass. A metal grid containing 24 quadrats (25 × 25 cm; total of 1.5 m²) was placed inside each plot, and vegetation in every other quadrat (12 in total) was clipped by species to crown level. Alternate sets of grids were clipped each year, such that any given quadrat was only clipped once every other year. This clipping method approximated the amount of biomass removed by grazers (a long-term average of 50% of aboveground production), as well as the frequency of removal (most plants are grazed once per season under moderate stocking rates in this region; L. M. Porensky, D. J. Augustine, and J. D. Derner, unpublished data). Both season long and rotational grazing management regimes are common in the western Great Plains. Our clipping regime more closely approximates rotational grazing, where plants are

grazed within a short time period, than season-long-grazing, but does not precisely match the temporal pattern or defoliation height of cattle grazing in these rangelands. This compromise was necessary to allow us to measure aboveground production annually. We also note that with stocking rates typical of western Great Plains rangelands, the vast majority of the grazed area (~99% of the landscape after excluding sacrifice areas near water sources or in pasture corners) is not affected annually by dung or urine inputs (Augustine et al. 2013).

Plant sample analyses.—Plant samples were returned to the lab and subjected to additional sorting by species to correct any errors that may have occurred during field sampling. Samples were dried (60°C), weighed, and then ground (by species) through a 1-mm mesh filter. For the three dominant grass species (*B. gracilis*, *P. smithii*, and *H. comata*) we used material subjected to grinding through a 1-mm filter to analyze *in vitro* dry matter digestibility (IVDMD) and acid detergent fiber (ADF; see *Methods* below). For the six most abundant species, a subsample of each coarsely ground sample was ground to a fine powder, and analyzed for N on an elemental analyzer connected to a mass spectrometer (20-20 Stable Isotope Analyzer, Europa Scientific, Cheshire, UK). Crude protein content of plant tissues is equivalent to plant [N] × 6.25; for simplicity, we report plant [N] throughout the paper.

Samples were analyzed for IVDMD following the method of Tilley and Terry (1963) as modified by White et al. (1981). The ruminal inoculum was obtained from four fistulated cross-bred steers that received a daily ration of free-choice grass hay (minimum crude protein, CP, 8%), 1.14 kg 38% cottonseed cube (CP, 40.5%), and 2–3 kg of alfalfa hay (minimum CP, 21%). Ruminal pH values ranged 6.7–7.2. Two blind duplicate samples of each ground sample were analyzed for IVDMD. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by weighing 0.5-g samples of forage into polyester bags (7 × 7 cm, 25-mm pore size; Model F57, Ankom Technology, Macedon, New York, USA) that were heat-sealed at the top using an impulse sealer (Midwest Pacific, Model MP-4; San Jose, California, USA). Acid detergent fiber was determined using Ankom method 12 and method 13, respectively, and the Ankom 2000 Fiber Analyzer (Ankom Technology).

Comparing forage quantity vs. quality.—Our analyses of forage quality focused originally on the three dominant forage grasses, because we anticipated that measurements of these three species would be representative of overall changes in total forage production and quality in response to the treatments, and because these species differ substantially in quality (Milchunas et al. 2005). However, analyses of compositional shifts during the experiment showed that these three species declined in relative abundance over time (Zelikova et al. 2014). Furthermore, multiple unpalatable species that are typically not eaten

by cattle occurred in the experiment. Mueller et al. (2016) previously showed that eCO₂ and warming both strongly influenced aboveground net primary production (ANPP), but did not analyze whether this was true when excluding non-forage plant species. To directly compare the magnitude of treatment effects on forage quality vs. quantity, we performed the following analyses. First, we calculated annual forage production in each plot as the sum of all plant species after excluding 13 species that are typically not consumed by cattle (the most abundant in the experiment being *Artemisia frigida*, *Heterothecha villosa*, and *Vulpia octoflora*; Milchunas et al. 2008). We then calculated the annual proportion of total forage comprised of the three dominant grass species, and found that this proportion declined notably over the course of the experiment (see *Results* and Zelikova et al. 2014). Given that the three dominant species did not adequately represent temporal trends in forage production, we calculated the annual proportion of total forage comprised of the six most abundant forage species, and demonstrated this was both substantial (96%) and stable over time. Due to limited biomass of samples from the fourth, fifth, and sixth most abundant species, we could not analyze IVDMD or ADF, but we did analyze [N] in tissue of all six species throughout the experiment. We therefore analyzed treatment effects on the summed productivity and the biomass-weighted [N] in those six species, which we hereafter refer to as total forage quantity and total forage [N] respectively.

Statistical analyses

For each dependent variable, we ran a linear mixed model that included block and plot as random factors (to account for spatial autocorrelation and repeated measures on plots over time); 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 (eCO₂ and warming) and treatment interactions while accounting for pre-existing variation among plots. We considered any interaction term to be potentially significant at the $P < 0.1$ level (Meredith and Stehman 1991). We further investigated potentially significant interactions by evaluating main effects within years or levels of CO₂ and warming, which we tested for significance at the $P < 0.05$ level. To examine the degree to which IVDMD was related to forage fiber and [N], we fit linear regression models with IVDMD as the response variable, and ADF, [N], and their interaction as predictors.

Predicting consequences for livestock performance

We used the NASEM (2016) beef cattle nutrition model to evaluate potential effects of reduced forage quality on growing-season cattle mass gain in northern mixed-grass prairie. Weight gain of yearling steers

grazing rangeland immediately adjacent to our PHACE experimental site was measured by Derner et al. (2008) for 14 of the years between 1991 and 2006. These years encompassed a similarly wide range of precipitation conditions as during the PHACE experiment. Cattle grazing at a moderate stocking rate in rangeland dominated by *P. smithii* and *B. gracilis* experienced an average daily weight gain (ADG) of 0.95 kg·animal⁻¹·d⁻¹ over the 113-d summer grazing season (Derner et al. 2008). We used values of IVDMD and [N] corresponding to a 50:50 diet of *P. smithii* and *B. gracilis* as measured in the four different PHACE experiment treatments as model inputs. First, we calibrated the model using IVDMD and N values from the ambient climate for an implanted yearling with initial body weight of 285 kg and final body mass of 391 kg, as reported by Derner et al. (2008). We used the empirical model (NASEM 2016), and assumed a ruminal undegraded N value of 61%, derived from Gunter et al. (1995b). We then modeled ADG using forage quality inputs for the other three treatments to evaluate (1) the magnitude of decline in ADG if no supplement is provided, and (2) the amount of supplemental cottonseed meal (commonly used in this region; solvent extracted, 7.3% N and 41% ruminal undegraded N; NRC 1989) that would be needed per animal per day to maintain animal performance at the same level as the ambient climate treatment.

RESULTS

In vitro dry matter digestibility

We did not detect significant eCO₂ × warming or year × eCO₂ × warming interactions for IVDMD for any grass species. Thus, we focus first on the effects of eCO₂ for each species, followed by warming effects. For the rhizomatous C₃ grass, *P. smithii*, eCO₂ effects varied by year (year × eCO₂ interaction, $P < 0.001$). We detected the largest reductions in IVDMD (absolute decline of 2.5–3.8%; $P < 0.001$) in 2007, 2008, 2012, and 2013, a modest reduction (1.5%; $P = 0.05$) in 2011, and no effect in the wet years of 2009 and 2010 ($P \geq 0.17$; Fig. 1a). Averaged across all years, eCO₂ reduced *P. smithii* IVDMD from 63.3 to 61.1%. We did not detect any effect of warming on *P. smithii* IVDMD (year × warming, $P = 0.53$; warming main effect, $P = 0.10$).

For the C₃ bunchgrass, *H. comata*, eCO₂ effects varied substantially across years (year × eCO₂ interaction, $P = 0.002$). Consistent with *P. smithii*, *H. comata* exhibited large negative eCO₂ effects on IVDMD in two of the driest years, 2007 and 2013 (–2.8% and –2.7% respectively; $P \leq 0.02$). In contrast, *H. comata* exhibited a positive eCO₂ effect (+2.7%) in the wettest year, 2009, and no significant effects in remaining years ($P \geq 0.10$). Warming effects also varied with year for *H. comata* (year × warming interaction, $P = 0.03$) due to a positive effect in 2008 (+3.8%, $P = 0.001$), a marginal positive effect in 2009 (+2.0%; $P = 0.07$), and no effect during the other five

years ($P \geq 0.33$; Fig. 1b). Averaged across all years, *H. comata* IVDMD under the ambient climate (55.2%) was similar to IVDMD with warming and eCO₂ (55.4%).

For the C₄ shortgrass, *B. gracilis*, eCO₂ reduced IVDMD (eCO₂ main effect, $P < 0.001$), but exhibited potential variation in the effect among years (year × eCO₂ interaction, $P = 0.084$) (Fig. 1c). Analysis of eCO₂ effects on *B. gracilis* by year revealed large negative effects of eCO₂ on IVDMD in four of seven years (absolute reduction in IVDMD of –1.9% to –3.8%; $P < 0.02$), a marginal negative effect in 2013 (–1.6%, $P = 0.051$), and no effect in both the wettest (2009; $P = 0.11$) and the driest years (2012; $P = 0.47$; Fig. 1c). Unlike the C₃ grasses, warming consistently reduced *B. gracilis* IVDMD across all years (year × warming interaction, $P = 0.26$; warming main effect, $P = 0.046$), but the warming effect (–0.8%) was small relative to the eCO₂ effect (mean IVDMD reduced by –2.1%). Averaged across all years, the combination of warming and eCO₂ reduced IVDMD of *B. gracilis* from 63.8% to 60.9%.

Nitrogen

Consistent with results for IVDMD, we found no interactions between warming and eCO₂ for [N] in *P. smithii* and *B. gracilis*, but we detected a significant year × eCO₂ × warming interaction for *H. comata* (Fig. 2; see below). For the C₃ grass *P. smithii*, eCO₂ effects varied by year (year × CO₂, $P = 0.007$), with [N] reduced in 2007, 2008, 2011, 2012, and 2013 (–0.27% to –0.41%; $P < 0.05$) but unaffected during the two wettest years, 2009 and 2010 (Fig. 2a). Warming enhanced [N] in 2008, but had no significant effect in any other year (Fig. 2a). Overall, mean [N] for *P. smithii* declined from 1.25% under ambient climate to 1.04% with eCO₂ and warming. *H. comata* was the only species for which we detected a potential year × warming × eCO₂ interaction ($P = 0.023$). However, analysis of the warming × eCO₂ interaction slices for individual years revealed no significant interactions in six of seven years ($P > 0.10$), and only a marginal warming × eCO₂ interaction in 2009 ($P = 0.097$). This reflects an increase in [N] with warming alone but not in combination with eCO₂ (Fig. 2b). Other than in the wettest year (2009), eCO₂ significantly reduced [N] in *H. comata*, which is counter to the findings for IVDMD. Warming enhanced [N] in *H. comata* in 2008 (as also occurred for the other two grass species in 2008), and reduced it in 2013, with no effect in the remaining years (Fig. 2b).

Consistent with IVDMD results, we found eCO₂ and warming effects for *B. gracilis* varied by year ($P < 0.001$ for both interactions with year). Elevated CO₂ reduced [N] (absolute decline of –0.22% to –0.35%) in five of seven years ($P < 0.001$), and had no effect in the two wettest years of 2009 and 2010 ($P \geq 0.50$; Fig. 2c). Warming enhanced [N] in 2008 (+0.25%; $P < 0.001$) and 2012 (+0.14%), but had no effect in the other five years ($P \geq 0.09$). Overall, mean [N] for *B. gracilis* declined

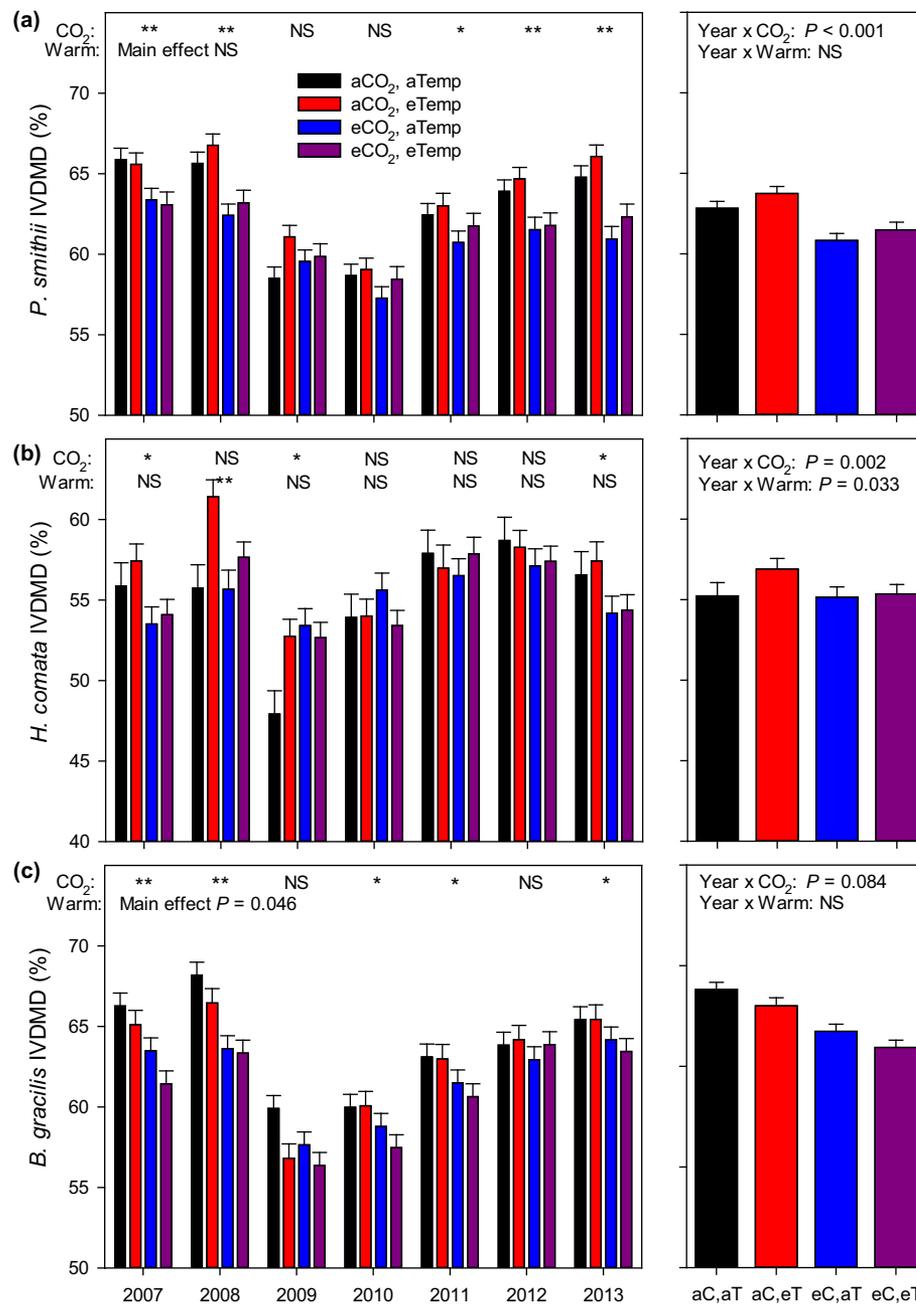


FIG. 1. Effects of elevated atmospheric [CO₂] (eCO₂) and warming (eTemp) treatments on *in vitro* dry matter digestibility (IVDMD) of the three dominant grass species from mixed-grass prairie of southeastern Wyoming, USA: (a) *Pascopyrum smithii*, (b) *Hesperostipa comata*, and (c) *Bouteloua gracilis*. Panels on the right show treatment means averaged across years; error bars show SE. Symbols above the yearly means indicate whether CO₂ and warming treatment significantly affected IVDMD at the *P* < 0.01 level (**), the *P* < 0.05 level (*), or were not significant (NS). Text above the experiment-wide means indicates significance of tests for year × CO₂ and year × warming interactions.

from 1.48% under ambient climate to 1.34% with eCO₂ and warming.

Acid detergent fiber

The least digestible portion of forage is represented by ADF, which includes lignin and cellulose but not

hemicellulose. Consistent with findings for IVDMD and [N], we did not detect eCO₂ × warming or eCO₂ × warming × year interactions (*P* ≥ 0.14). For ADF in *P. smithii*, eCO₂ effects potentially varied by year (*P* = 0.06), so we examined yearly eCO₂ contrasts. ADF increased with eCO₂ in 2008 and 2013 (*P* ≤ 0.05; Fig. 3a). Warming did not affect ADF in *P. smithii*

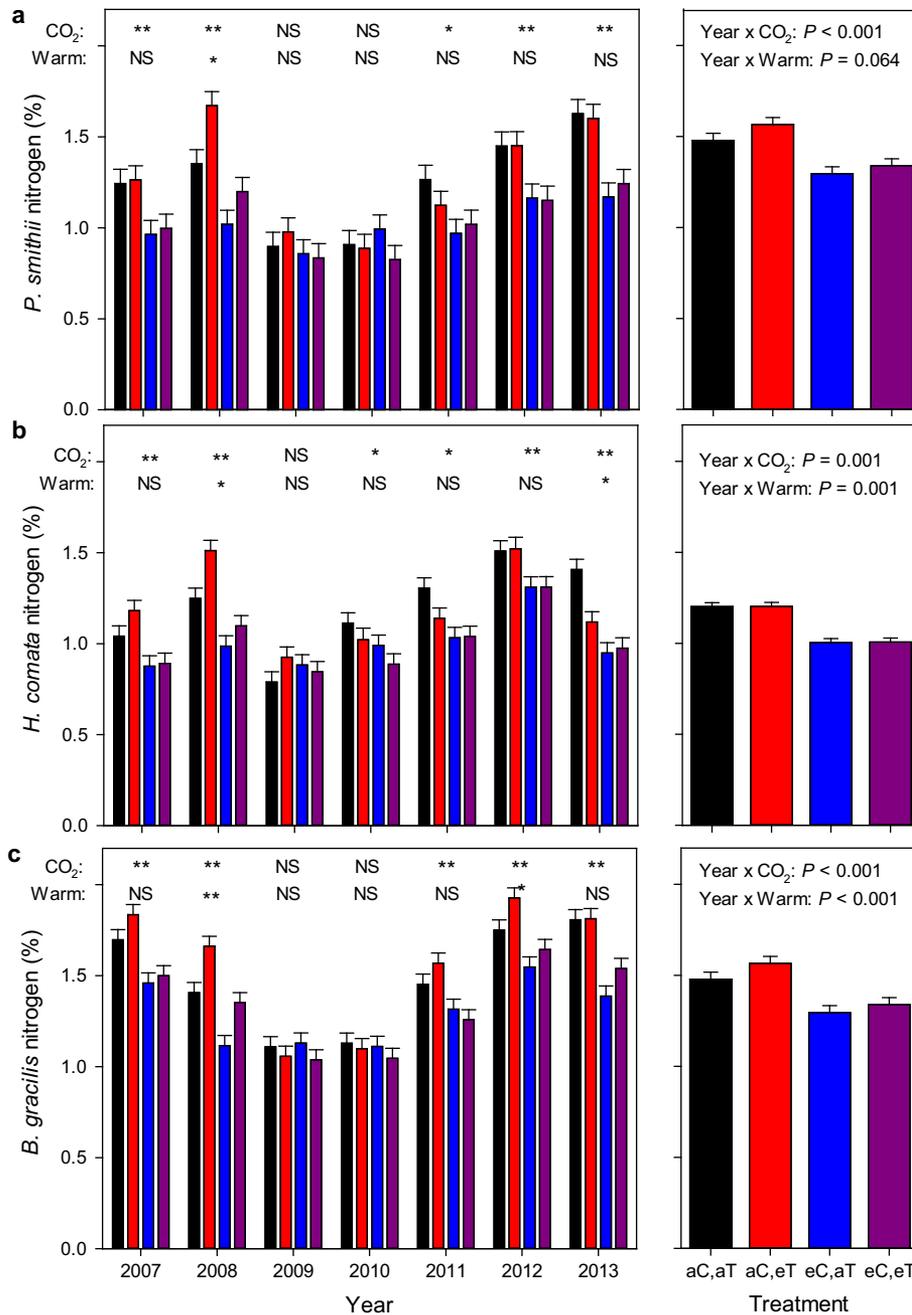


FIG. 2. Effects of climate treatments on the concentration of nitrogen ([N]) in the three dominant grass species from mixed-grass prairie of southeastern Wyoming. Bar colors and text indicating significance of statistical tests follow Fig. 1.

($P = 0.43$). ADF in *H. comata* was not influenced by eCO₂ ($P = 0.79$), but warming effects on ADF in *H. comata* potentially varied by year ($P = 0.09$). Analysis of yearly contrasts showed warming reduced ADF in the wettest year of 2009 ($P = 0.04$) but not any other years (Fig. 3b). ADF in *B. gracilis* varied substantially among years ($P < 0.001$), increased with eCO₂ ($P = 0.01$), and declined with warming ($P = 0.02$; Fig. 3c).

Relationships among IVDMD, [N], and ADF

Finally, we examined the degree to which variation in IVDMD for each species was related to variation in [N] and ADF, as a way to examine how warming and eCO₂ may be influencing IVDMD through their effects on [N] and ADF. The total digestible nutrient content of forages (of which IVDMD is a direct laboratory measure)

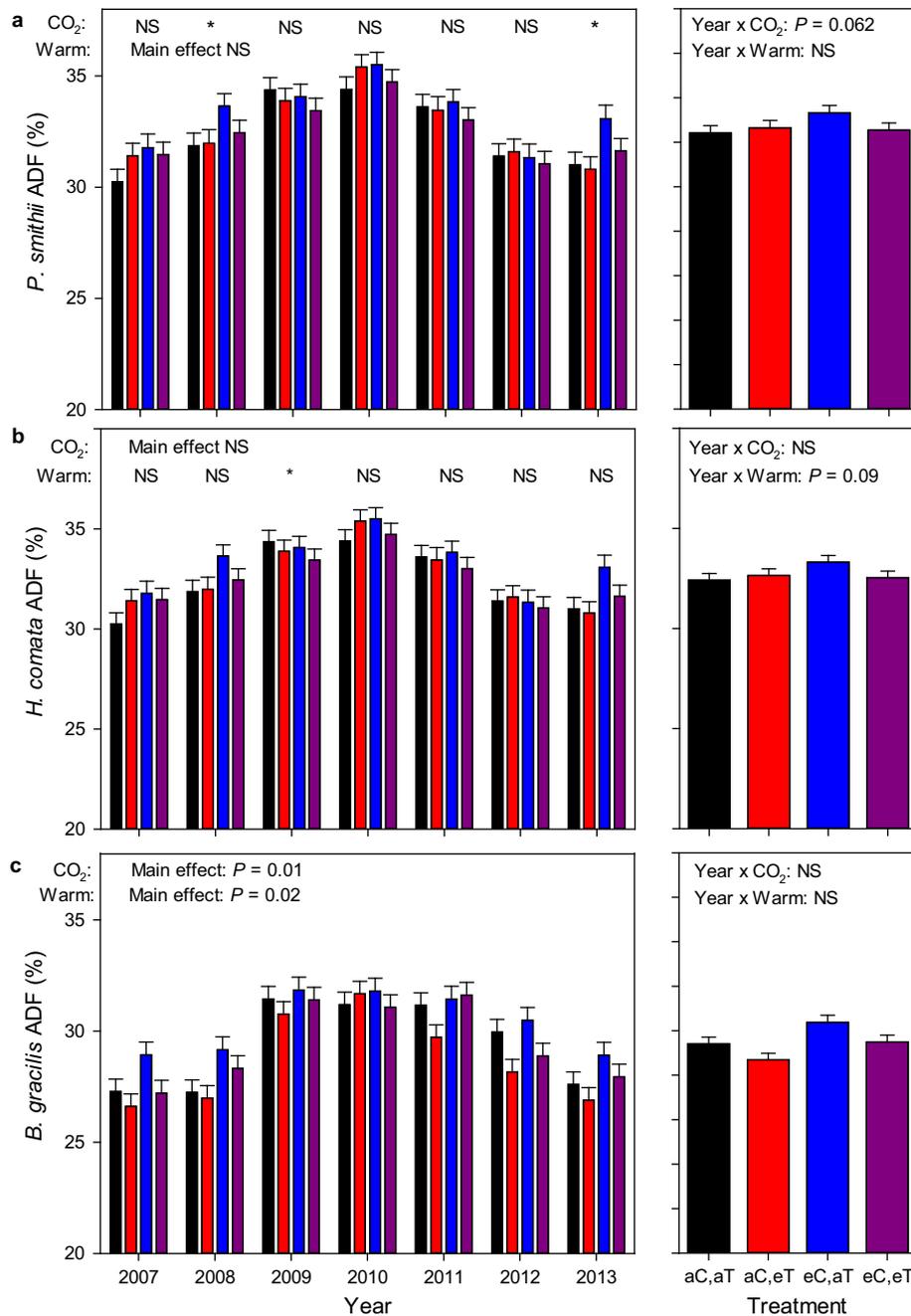


FIG. 3. Effects of climate treatments on acid-detergent fiber (ADF) concentrations in the three dominant grass species from mixed-grass prairie of southeastern Wyoming. Bar colors and text indicating significance of statistical tests follow Fig. 1.

is often predicted from ADF content of forages, or from more complex assessments including sub-fractions of the ADF and crude protein content (NASEM 2016). Here, we simply examined the amount of variation in IVDMD that could be predicted from ADF and [N], in order to compare their relative importance in affecting IVDMD in mixedgrass prairie, and we additionally tested for an interaction term between ADF and [N], to

assess whether these two chemical components of the forage influence one another's digestibility. Across all years and treatments, IVDMD for all three species was positively correlated with [N], negatively correlated with ADF, and all three models included an ADF × N interaction term (Table 1). The similarity in standardized betas and parameter estimates for all three species (Table 1) indicates that warming and CO₂-induced

TABLE 1. Linear regression models for in vitro dry matter digestibility (IVDMD) as a function of acid-detergent fiber and nitrogen concentration for three dominant grass species in the mixed-grass prairie of southeastern Wyoming, USA.

Species	Prediction equation	Model r^2	Standardized betas		P		
			ADF	N	ADF	N	ADF × N
<i>Pascopyrum smithii</i>	$= 84.25 - 0.81 (\%ADF) + 4.10 (\%N) + 0.65 (\%ADF \times \%N) - 32.75 (\%N) - 1.15$	0.57	-0.504	0.383	<0.001	<0.001	0.092
<i>Hesperostipa comata</i>	$= 64.67 - 0.44 (\%ADF) + 5.77 (\%N) + 0.67 (\%ADF \times \%N) - 34.34 (\%N) - 1.07$	0.53	-0.408	0.417	<0.001	<0.001	0.045
<i>Bouteloua gracilis</i>	$= 75.94 - 0.67 (\%ADF) + 4.52 (\%N) + 0.68 (\%ADF \times \%N) - 29.49 (\%N) - 1.42$	0.55	-0.667	0.391	<0.001	<0.001	0.065

changes in ADF and [N] both contribute to a similar degree to variation in the digestibility of these grasses. Correlation coefficients for these models (r^2 of 0.55, 0.57, and 0.53 for *B. gracilis*, *P. smithii*, and *H. comata* respectively) indicate that changes in tissue [N] and ADF can explain just over half of the observed variation in IVDMD for each grass species. The nature of these relationships, including the interaction between ADF and [N] on IVDMD, can be visualized in Fig. 4, where reducing ADF from 36% to 30% has a much stronger influence on IVDMD for tissues with low [N] (e.g., 0.8%) than for tissues with high [N] (e.g., 1.8%).

Total forage biomass and [N]

To what extent do these three dominant grass species represent changes in total forage production by all species, including forbs and subdominant grasses, over the course of this experiment? After excluding non-forage plant species, the three dominant grasses comprised an average of 76.0% of forage production during the seven years of the experiment. However, relative abundance of these three species as a percentage of total forage production declined over time, from 83.3% in 2007 to 67.5% in 2013. This shift occurred because three other subdominant forage species, *Carex duriuscula* (perennial C₃ sedge), *Koeleria macrantha* (perennial C₃ grass), and *Sphaeralcea coccinea* (perennial forb), increased in relative abundance over time, from 15.2% of forage production in 2007 to 24.8% in 2013. To better understand how eCO₂ and warming influence the total amount and

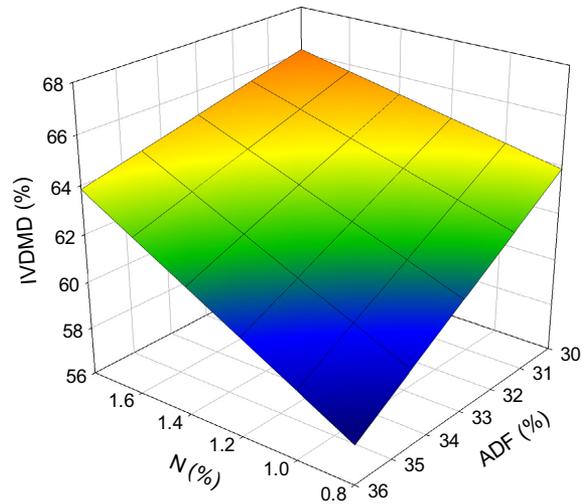


FIG. 4. Predicted relationships among acid-detergent fiber (ADF), tissue nitrogen (N) concentration, and in vitro dry matter digestibility (IVDMD) for the dominant forage grass in mixed-grass prairie, *P. smithii*, based on samples collected from different climate treatments during 2007–2013. We also observed a similarly shaped IVDMD response surface for *H. comata* and *B. gracilis* (see Results and Table 1).

quality of forage available to livestock, we analyzed treatment effects on the sum of the 6 most abundant forage species, which collectively represented a large (average of 96.0%) and temporally consistent percentage of forage production (98.5–92.3% over the seven years).

Effects of eCO₂ and warming on forage production varied by year (CO₂ × warming × year interaction, *P* = 0.055). We then tested for eCO₂ × warming interactions within each year. In the absence of such an interaction, we evaluated contrasts for main effects of CO₂ and warming within years; in the presence of a significant interaction within a year, we report pairwise differences among treatment means (Fig. 5). Treatment effects increased in complexity and magnitude over time, with no effects in 2007, a marginal eCO₂ effect in 2008 (*P* = 0.093), and CO₂ × warming interactions in 2009, 2010, 2011, and 2013. The interaction in 2011 was subadditive (i.e., the effect of CO₂ and warming combined was less than expected based on their additive effects). In contrast, in 2009, 2011, and 2013, the positive effect of CO₂ and warming combined was greater than expected based on their additive effects (Fig. 5). Finally, during the drought of 2012, warming alone enhanced forage production, while CO₂ had no effect. Averaged across all years of the study, we found that while neither warming nor eCO₂ alone substantially enhanced total forage production, in combination they increased forage production from 93 g/m² under ambient climate to 128 g/m² under the simulated future climate (38% increase; Fig. 5). Despite the complex treatment interactions in some years, eCO₂ and warming together consistently increased forage production, by 18–53 g·m⁻²·yr⁻¹, with no trend over time (regression of year vs. net forage production increase, *P* = 0.20).

Effects of eCO₂ and warming on forage [N] (Fig. 6) were similar to the patterns observed for the three dominant grass species discussed previously (Fig. 2). We did not detect a significant CO₂ × warming × year interaction (*P* = 0.37), and hence focus on yearly variation in CO₂ and warming effects (Fig. 6). The strongest effect on forage [N] was a significant reduction with eCO₂ in five of seven years (i.e., in all years except the two wettest years of 2009 and 2010). Warming did not affect forage [N] in most years, but did enhance forage [N] in 2008. Averaged across years, the influence of warming alone was negligible, whereas the eCO₂ effect was substantial (Fig. 6). Forage [N] declined from 1.33% under ambient climate to 1.15% under the simulated future climate (13% decrease).

Predicted effects on livestock performance

The NAESM (2016) model predicts that a yearling consuming a 50:50 diet of *P. smithii* and *B. gracilis* grown under ambient climate would gain 0.96 kg/d, and body mass gain would be limited by ruminal metabolizable N (i.e., protein) yield (Table 2). Warming alone is not predicted to reduce animal performance or necessitate increased provision of supplement. However, with combined warming and eCO₂, the measured decline in forage quality is predicted to reduce cattle weight gain by 12.5%, from 0.96 to 0.84 kg·animal⁻¹·d⁻¹ (Table 2), which over a 113 d growing season results in a cumulative loss of 13.6 kg/animal. These predictions assume that forage quantity is not limiting dry matter intake by the cattle, which is warranted based on the 38% increase in forage production we measured in the combined warming + eCO₂ treatment.

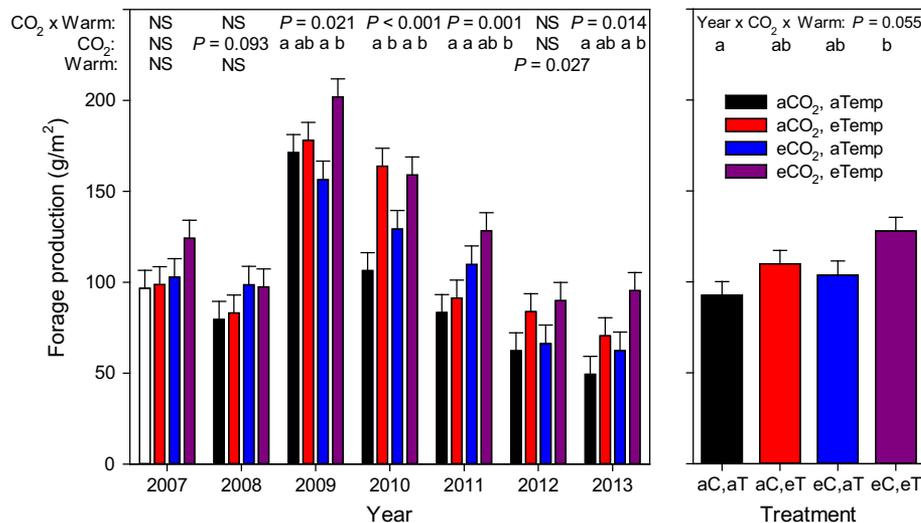


FIG. 5. Effects of climate treatments on total forage production in mixed-grass prairie of southeastern Wyoming. The panel on the left shows yearly least square means and SE and the panel on the right shows treatment means across all years. For forage production, we detected a potentially significant year × CO₂ × warming interaction (*P* = 0.055), so in the left-hand panel, we report tests for the significance of the CO₂ × warming interaction each year. If this interaction was not significant, we report tests for the main effects of CO₂ and warming within each year. Otherwise, different letters above each treatment means indicate significant differences from one another (*P* < 0.05).

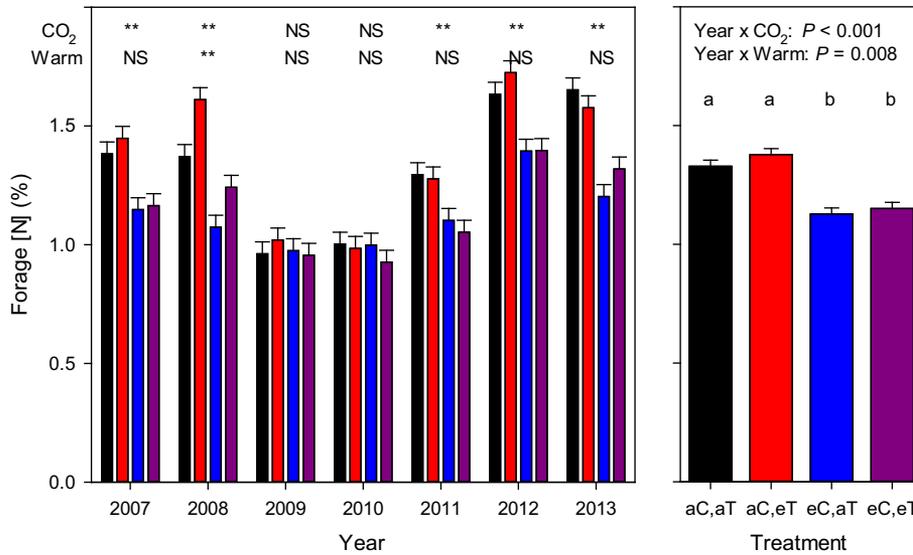


FIG. 6. Effects of climate treatments on nitrogen concentrations in forage produced in mixed-grass prairie of southeastern Wyoming. The panel on the left shows yearly least square means and SE and the panel on the right shows treatment means across all years. We did not detect year \times CO₂ \times warming or CO₂ \times warming interactions, so we report significance of the tests for year \times CO₂ and year \times warming interactions. Treatment notations and colors follow Fig. 5.

DISCUSSION

Understanding how climate change will influence forage and livestock in semiarid rangelands is critical to the long-term sustainability of these agricultural systems, where economic margins are low (Dunn et al. 2010, Rolfe et al. 2016). Livestock managers contend with substantial year-to-year variability in forage production and quality, and often vary stocking rates and animal distribution in flexible ways to mitigate this variation (e.g., Derner et al. 2008, O'Reagain et al. 2014). However, these strategies are often based on past experience (potentially gained over multiple generations), and do not incorporate consideration of directional, long-term shifts in forage production and quality that could arise from climate change.

Our experimental manipulation of atmospheric [CO₂] and temperature over seven years, encompassing both drought and wet conditions, revealed that the combination of eCO₂ and warming substantially increased forage production, by an average of 38%. This suggests that future fluctuations in forage production will occur around an increasing mean level of productivity. Species compositional shifts in this experiment include a significant increase in C₄ grass (but not C₃ grass) production with eCO₂ + warming during the first three years (2007–2009; Morgan et al. 2011), and increased production with eCO₂ + warming primarily due to C₃ graminoids during the last four years (Mueller et al. 2016). Such compositional shifts may be related to variation in the degree to which warming vs. eCO₂ affected forage production in years with varying weather conditions (Fig. 4). However, the

TABLE 2. Predicted effects of changes in forage quality (IVDMD and [N]) associated with climate treatments on average daily mass gain (ADG, kg·animal⁻¹·d⁻¹) of yearling cattle based on the NASEM (2016) beef cattle nutrition model calibrated using forage quality measurements from the ambient climate treatment and measurements of ADG for yearlings grazing northern mixed-grass prairie at our study site (Derner et al. 2008).

	Treatments			
	aCO ₂ , aTemp	aCO ₂ , eTemp	eCO ₂ , aTemp	eCO ₂ , eTemp
IVDMD (% of diet)	63.3	63.4	61.3	61.2
[N] (% of diet)	1.48	1.57	1.30	1.34
Predicted voluntary dry matter intake (kg/d)	7.89	7.89	7.91	7.91
Allowable ADG (kg/d) based on ME intake	1.48	1.01	1.33	1.32
Allowable ADG (kg/d) based on ruminal metabolizable N yield	0.96	1.01	0.82	0.84
Supplement required to maintain ADG at the same level as ambient climate diet (kg/d)	–	–	0.22	0.19

Note: Predicted realized ADG in each treatment is the minimum of the allowable ADG based on metabolizable energy (ME) intake or ruminal metabolizable N yield. Dashes indicate treatments where no supplement is required to maintain ADG at the same level as ambient climate diet.

relatively consistent increase in forage production under warming + eCO₂ throughout the experiment suggests enhanced forage production is not dependent upon the relative dominance of C₃ vs. C₄ grasses. It also shows that the observed decline in the three dominant forage grasses (Zelikova et al. 2014), has been compensated for by increases in other palatable species.

Although forage production was enhanced by projected future climatic conditions, this came with a substantial reduction in forage quality, which would negatively affect ruminant weight gains. Most of the annual weight gain by cattle in rangelands of the western Great Plains occurs during the growing season, when rapidly growing forages typically have high [N] and cell solubles, and cell walls are not as lignified. Using IVDMD as our measure of the total digestible nutrient value of the grasses in our experiment, we found that the combination of warming and eCO₂ reduced digestibility of the dominant C₃ grass from 62.8% to 61.5% and reduced digestibility of the dominant C₄ grass from 63.3% to 61.2%. Although these absolute decreases may seem small, such a reduction reflects both increased fiber content and reduced [N] (and hence crude protein) in forage at time that is critical for rapid animal mass gain. The impact of reduced digestibility on livestock mass gains will depend on the species, size, and reproductive state. The NASEM (2016) beef cattle nutrition model calibrated for yearling cattle grazing under ambient climate conditions at our study site (Derner et al. 2008) predicts that the decline in IVDMD and [N] due to eCO₂ + warming for a 50:50 mixed diet of *P. smithii* and *B. gracilis* would reduce weight gains by 12.5% (0.12 kg·animal⁻¹·d⁻¹), or 13.6 kg/animal over a 113 d growing season. Mitigating this decline would require provision of 0.2 kg of cottonseed meal supplement·animal⁻¹·d⁻¹, with incurred economic costs of the supplement plus labor and equipment costs to provide the supplement. Either reduced weight gain or the need to provide supplement would clearly affect the profitability of livestock operations, although predictions in monetary terms would be strongly influenced by market conditions. Reduced mass gain could also affect future performance of cows through increased time to mating age and/or reduced conception rates. Shifts in relative productivity in C₃ vs. C₄ species would only minimally affect our predictions, but given our IVDMD results, increased C₃ production would reduce the predicted loss while increased C₄ production would increase the predicted loss in weight gain.

Our findings regarding forage quality are consistent with a previous field experiment in the shortgrass steppe of Colorado that manipulated atmospheric [CO₂] but not warming (Milchunas et al. 2005). We extend their results by demonstrating a CO₂-induced decline in digestibility and [N], and increase in ADF, of similar magnitude for the mixedgrass prairie of Wyoming, and by showing that warming did not notably influence forage quality or alter the effects of eCO₂. Cellulose and hemicellulose in plant cell walls are potentially digestible, but the rate depends on both the degree of cell wall

lignification and the availability of N in the rumen (van Soest 1994). Both increased ADF (an index of lignification) and reduced [N] in forage from the eCO₂ + warming treatment contributed equally to a model explaining variation in IVDMD. Our results for eCO₂ and warming effects on ADF and N were consistent with both the expected (predicted from Table 1) and measured changes in IVDMD in response to the treatments for *P. smithii* and *H. comata*, suggesting that effects of eCO₂ and warming on IVDMD operate primarily through changes in fiber and protein content.

For *B. gracilis*, eCO₂ effects on ADF and [N] were consistent with findings for IVDMD. In contrast, the warming treatment reduced ADF and enhanced [N] in *B. gracilis* in some years, but did not enhance IVDMD as we might expect based on the modeled relationship. Variation in forage ADF and [N] only explains a moderate amount of the variation in *B. gracilis* IVDMD (e.g., r^2 of 0.55 in Table 1), suggesting that additional changes in plant tissue chemistry contribute to declines in its digestibility. ADF is a coarse measure of low-quality fiber in the diet because it represents a combination of indigestible lignin and digestible cellulose. Changes in the ratios of these fractions, in the digestibility of different components of the proteins in the diet, and in other aspects of plant chemistry could also be contributing to changes in digestibility.

While warming and eCO₂ both consistently enhanced forage quantity across wet and dry years, the decline in forage quality was due largely to eCO₂ alone, and was less consistent. For all three dominant grasses, IVDMD did not decline in the wettest year, whereas declines that were more consistent across species occurred in average and dry years. For forage [N], we found no effect of warming and eCO₂ during the two wettest years, but consistent effects across years ranging from slightly above-average precipitation to drought. Given that forage quality (both IVDMD and [N]) is lowest in wet years, our results show that eCO₂ and warming reduce year-to-year variation in forage quality by reducing forage quality in drier years to levels similar to those occurring under ambient climate in wet years. Such reductions in forage quality may amplify the negative effects of drought on livestock production.

An additional consideration in attempting to project how forage quality may affect ruminant livestock performance is whether the forage contains sufficient protein to sustain effective functioning of rumen microbes. Forages with low [N] have long been known to impair rumen function, and cattle consuming such forages can benefit from the provision of high-protein supplements to stimulate ruminal microbial protein yield (NASEM 2016). A meta-analysis by Gunter et al. (1995a) showed that optimization of apparent ruminal N digestion would occur at a dietary N to IVDMD ratio of 4.57. In our PHACE experiment, the eCO₂ + warming treatment had the highest ratio of dietary N to IVDMD at 2.34, which suggests a deficiency of ruminally degraded N for

cattle grazing herbage from any of the treatments (Gunter et al. 1995a). Late in the growing season, effects on ruminant livestock performance may be more strongly related to declines in forage [N], which for our samples collected in July was already approaching critically low levels in the eCO₂ + warming treatment. If treatment differences persist as vegetation senescens over the course of the growing season (as demonstrated by Milchunas et al. [2005] for eCO₂ effects), it is likely that eCO₂ will increase the need for protein supplementation throughout the year, and especially during the dormant season. Determining when to provide high-protein supplements to grazing livestock is often challenging, and providing supplement is costly. These challenges are likely to increase with rising atmospheric CO₂ (see also Craine et al. 2010). Additional research assessing the effects of eCO₂ on the phenological patterns of forage quality, and potential interactions with the timing and intensity of defoliation, is clearly needed.

The mechanisms by which eCO₂ suppressed [N] of both C₃ and C₄ grasses in our study, at both ambient and elevated temperature, are not fully known. While eCO₂ often reduces soil N availability, these effects can be offset by elevated temperature (Loiseau and Soussana 2000, Rustad et al. 2001, Dieleman et al. 2012). Over the course of our study, eCO₂ consistently reduced soil inorganic N (Mueller et al. 2016). However, warming enhanced soil inorganic N, such that the combination of eCO₂ and warming led to similar, slightly smaller (2008) or slightly larger (2011, 2012) soil inorganic N pools compared to the ambient treatment (Dijkstra et al. 2010, Mueller et al. 2016). We found that eCO₂ suppressed forage [N] at both ambient and elevated temperature, calling into question whether soil N supply is the sole factor limiting plant [N]. At the same time, the amount of above-ground plant biomass produced was greatest in the combined treatment. One interpretation is that N dilution drives plant [N], such that low N supply combined with a small increase in plant growth (eCO₂ treatment) leads to similar plant [N] as enhanced N supply combined with a large increase in plant biomass (eCO₂ + warming treatment). Another possibility is that reduced plant leaf [N] under eCO₂ may be related to physiological changes in leaves of grasses growing at eCO₂ that inhibit leaf NO₃ assimilation, either independent of or in combination with mechanisms related to soil N supply rates. Particularly with long-term exposure to CO₂, C₃ plants can exhibit physiological changes associated with stomatal closure that include decreased transpiration, decline in RuBisCO activity and amount, enhanced leaf carbohydrate content, and reduced leaf [N] (Stitt and Krapp 1999, Lecain et al. 2003, Pérez et al. 2005, Vicente et al. 2016). We also note that in rangelands grazed by ruminants, some of the N will be cycled through dung and urine, which could alter rates of soil N supply to plants relative to the conditions in our experiment, and potentially increase N loss from the system via N volatilization (Allard et al. 2003). Finally, one may postulate that a

warming-induced phenological shift toward growth earlier in the season would reduce the quality of plant tissues sampled in July. However, previous work showed that the combined warming and eCO₂ treatment altered phenology by extending both ends of the growing season (earlier greenup and later senescence) because of the water savings associated with eCO₂ (Reyes-Fox et al. 2014). Furthermore, if a warming-induced shift in peak maturity was driving our results, we would expect large negative effects of the warming treatment alone on forage quality, which we did not observe.

Management implications

Atmospheric [CO₂] has already increased from ~280 ppm in the preindustrial era to 320 ppm in 1965 and 405 ppm in 2017. Future increases to the level of 600 ppm simulated in our experiment are currently projected with greater certainty than any other component of climate change, although the predicted time frame over which this will occur varies from 30 to 70 yr (data available online).⁵ Given our findings and the well-documented mechanisms relating eCO₂, stomatal closure, and water savings to reduced forage quality of plants in water-limited rangelands, how can livestock managers implement adaptive management strategies to reduce or offset weight gain losses associated with declining forage quality? Grazed grasses regrow leaves with increased protein and reduced cellulose and lignin concentrations compared to ungrazed grasses in rangelands worldwide (Hobbs 1996), and specifically in the western Great Plains (Milchunas et al. 1995, 2005). Thus, given our projections of increased forage production, reduced forage quality, and declining weight gain per animal in the coming decades, a clear solution is for producers in the North American Great Plains, where plants are well adapted to ungulate grazing (Milchunas et al. 2008), is to adaptively increase stocking rates to match forage availability (Smart et al. 2010). Increased stocking with increased forage production would maintain the grazing pressure index (and grazing efficiency) at current levels, thereby either maintaining or increasing total mass gain per unit area (Smart et al. 2010). Grazing-induced changes to vegetation communities in northern mixed-grass prairie are slow, continuous and reversible (Porensky et al. 2016) due to a long evolutionary history of ungulate grazing (Milchunas et al. 2008). In rangelands without such an evolutionary history, increases in stocking rates may have greater potential to detrimentally alter plant communities or ecosystem states (e.g., Bestelmeyer et al. 2013, O'Regain et al. 2014). Given the uncertainties inherent in predicting climate change and plant responses, we urge caution in how livestock producers adjust stocking rates. Stocking rates should emphasize flexibility within and across grazing seasons for adaptation to weather fluctuations (Ritten et al.

⁵ www.ipcc-data.org

2010, Hamilton et al. 2016), with the recognition that such fluctuations could increase in magnitude (Swain and Hayhoe 2015). Depending on how grazing pressure is managed in space and time, increased grazing pressure could also help offset declines in forage quality, and offset declines in per-animal mass gains. We also note that while plant community shifts did not negatively affect forage production in our study, strong eCO₂ and warming responses of invasive weeds in adjacent PHACE subplots suggest the potential for community changes that would reduce forage quantity and quality in rangelands experiencing soil surface disturbance (Blumenthal et al. 2013, 2016).

Other management strategies to mitigate declining forage quality could include (1) increased provision of protein supplements, (2) N fertilization of rangeland (e.g., Samuel et al. 1980, Springer and Taliaferro 2001) at rates low enough to avoid invasion of undesired plants (Seabloom et al. 2015), (3) interseeding of legumes, which can be favored under eCO₂ but inhibited by selective grazing (Mortenson et al. 2005, Newton et al. 2014, Jaurena et al. 2016), (4) adjusting seasonality of grazing to account for changing phenology (Reyes-Fox et al. 2014) and to facilitate livestock consumption and control of invasive plants (Rinella and Bellows 2016), and (5) strategic use of patch burning to manipulate forage quality and livestock grazing distribution (Allred et al. 2011, Augustine and Derner 2014). Finally, we suggest that tactical monitoring of forage quality and quantity should play an increasingly important role in livestock production systems, and be used to guide adjustments in stocking rates and the application of other adaptive management strategies. Monitoring of forage quality could occur both via plant tissue samples collected using protocols that control for phenology, and via livestock fecal samples, which already are collected and analyzed in a consistent manner across much of the western US (Craine et al. 2010, Tolleson and Schafer 2014). Detecting directional long-term trends in forage quality will be challenging in the variable climates that characterize most rangelands worldwide, emphasizing the need to establish consistent and cost-effective monitoring protocols as soon as possible.

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DATA AVAILABILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.b32q9>