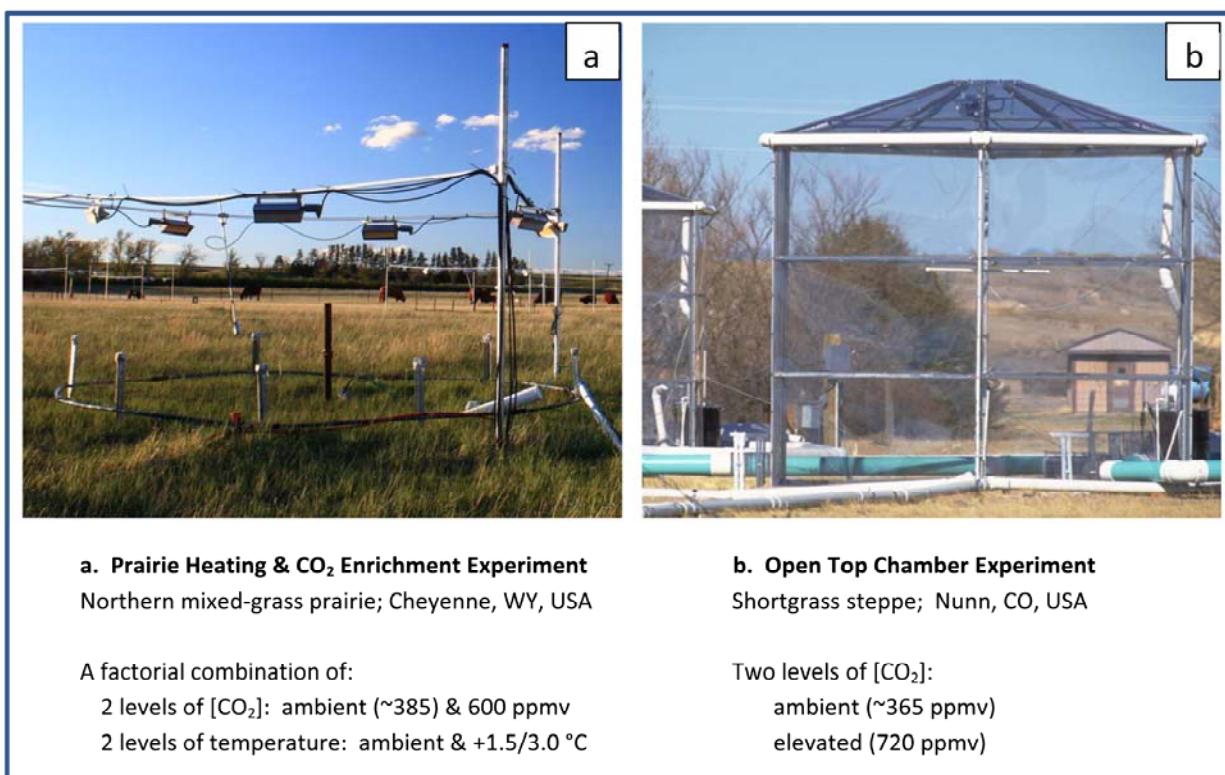


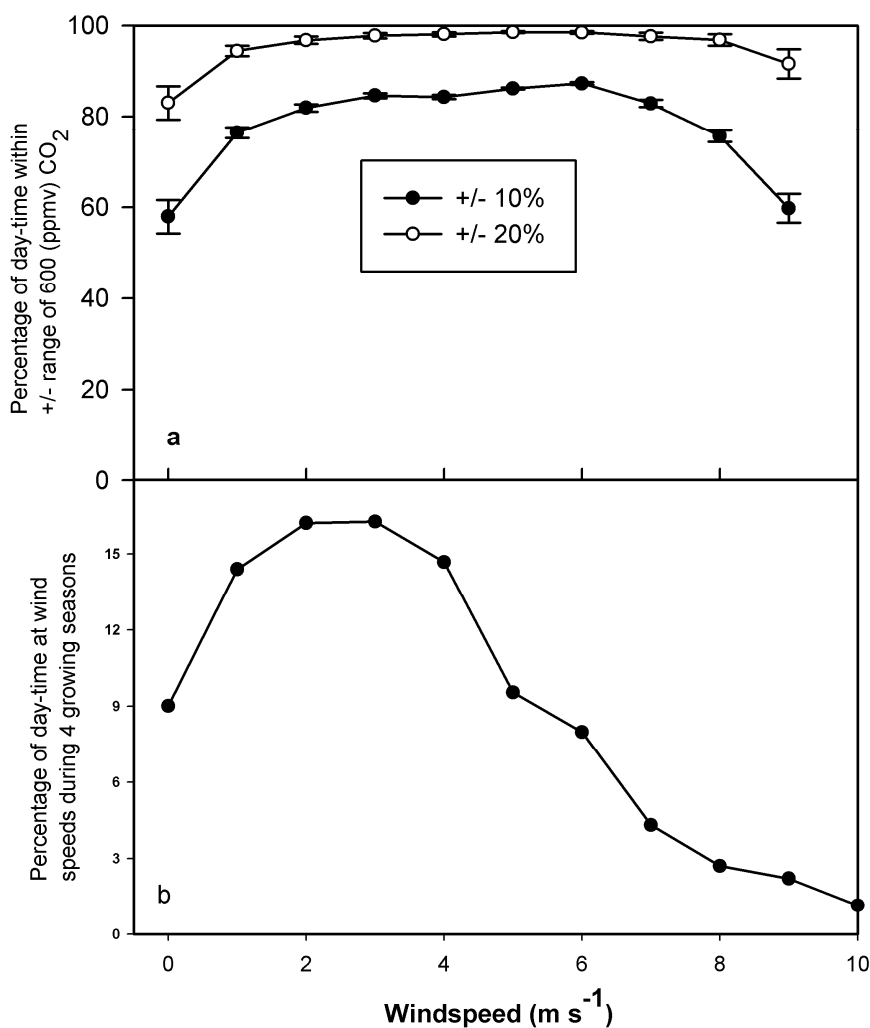
SUPPLEMENTARY APPENDIX I. Experimental Methods and System Performance

Experiments. Photographs and basic experimental information are given (Supplementary Fig. 1) for two field experiments that have examined the responses of northern mixed-grass prairie and shortgrass steppe ecosystems of the western Great Plains to CO₂ enrichment (panels a and b), and warming (panel a). Details of the experimental layout of the **Prairie Heating and CO₂ Enrichment (PHACE)** Experiment can be found in the Methods section of the article, while information on the performance of the CO₂ enrichment and warming systems is presented in two subsequent sections of this appendix. A final section, Soil Water Conversions, discusses transformation of soil water content data into soil matric potential.



Supplementary Fig. 1. The **Prairie Heating and CO₂ Enrichment (PHACE)** Experiment (panel a) and the **Open Top Chamber Experiment** (panel b).

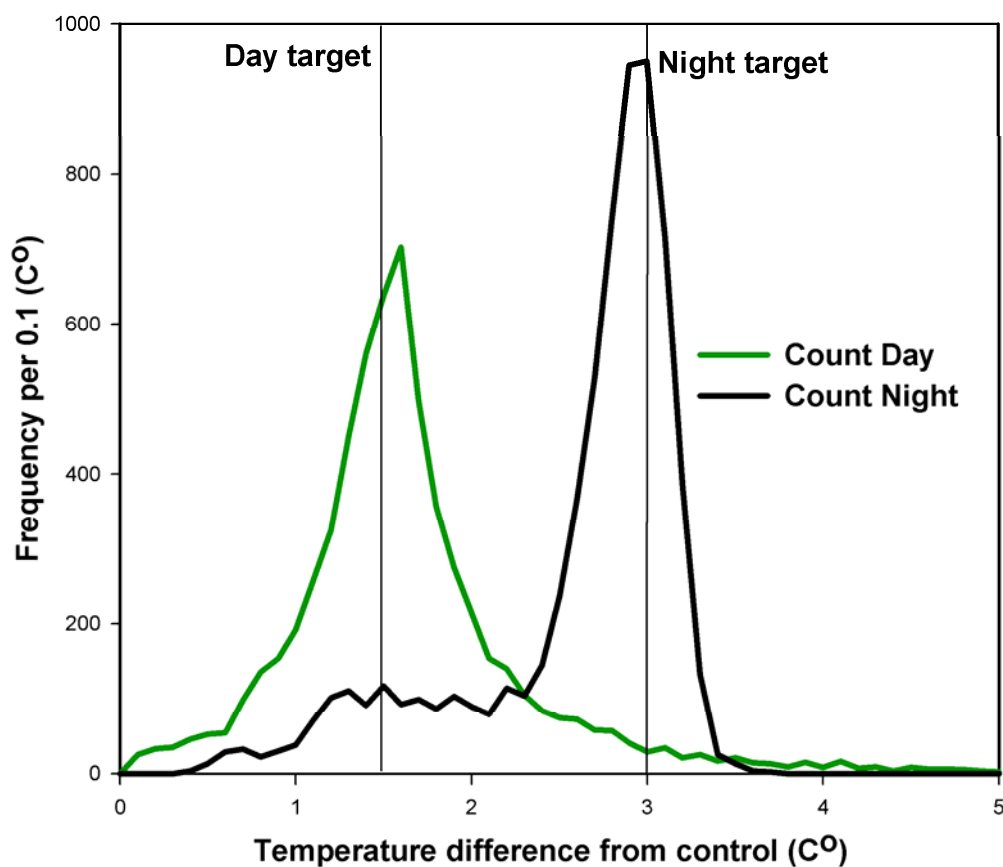
Performance of Free Air CO₂ Enrichment (FACE) System. The Free Air CO₂ Enrichment (FACE) technology used in this experiment is essentially the same as that used by Hovenden et al.²⁹. The main differences between the sites were that: 1) our FACE rings are 3.3 m in diameter, approximately twice the diameter of the 1.5 m rings installed in Tasmania, and 2) the average growing season wind speed measured at our site, 4.1 m s⁻¹, is higher than the 2.7 m s⁻¹ wind speed at TasFACE²⁹. An analysis of PHACE CO₂ control over varying wind-speed shows that the elevated CO₂ control (measured at ring center) is within 10% of the target concentration (600 ppmv) about 85% of the time at the most common wind-speeds (1 to 4 m s⁻¹) and well within 20% of the target nearly all of the time (Supplementary Fig. 2). CO₂ control is less accurate under calm wind and at wind speeds higher than 8 m s⁻¹. Such control is similar to Hovenden et al.²⁹ and exceeds the established expectation for FACE performance of maintaining CO₂ within 20% of the set point 80% of the time. Hovenden et al.²⁹ present more details on the horizontal patterns in CO₂ across the rings and the dynamics and patterns of CO₂ control of this FACE system.



Supplementary Fig. 2. Impact of wind speed on FACE performance.

Performance of the Temperature Free-Air Controlled Enhancement (T-FACE) System.

Performance of the warming system was excellent. Daytime temperature differentials were within 0.5 °C of the daytime 1.5 °C target temperature 69% of the time and the nighttime 3.0 °C target temperature 72% of the time. However, there was some bias in both measurements with average daytime temperature differentials of 1.6 °C and average nighttime differentials of 2.6 °C (Supplementary Fig. 3). This bias was due in large part to including transition periods between daytime and nighttime warming in the analysis.



Supplementary Fig. 3. Frequency distribution of day/night temperature differentials between eight T-FACE warmed and eight control plots as measured with infrared radiometers for the entire year 2008.

Warming significantly increased air and soil temperatures (Supplementary Table 1). Air temperature at canopy height was 0.7 °C warmer throughout the growing season in heated compared to control plots. Soil temperatures at 3 and 10 cm depths were 2.5 and 1.9 °C warmer in heated compared to control plots.

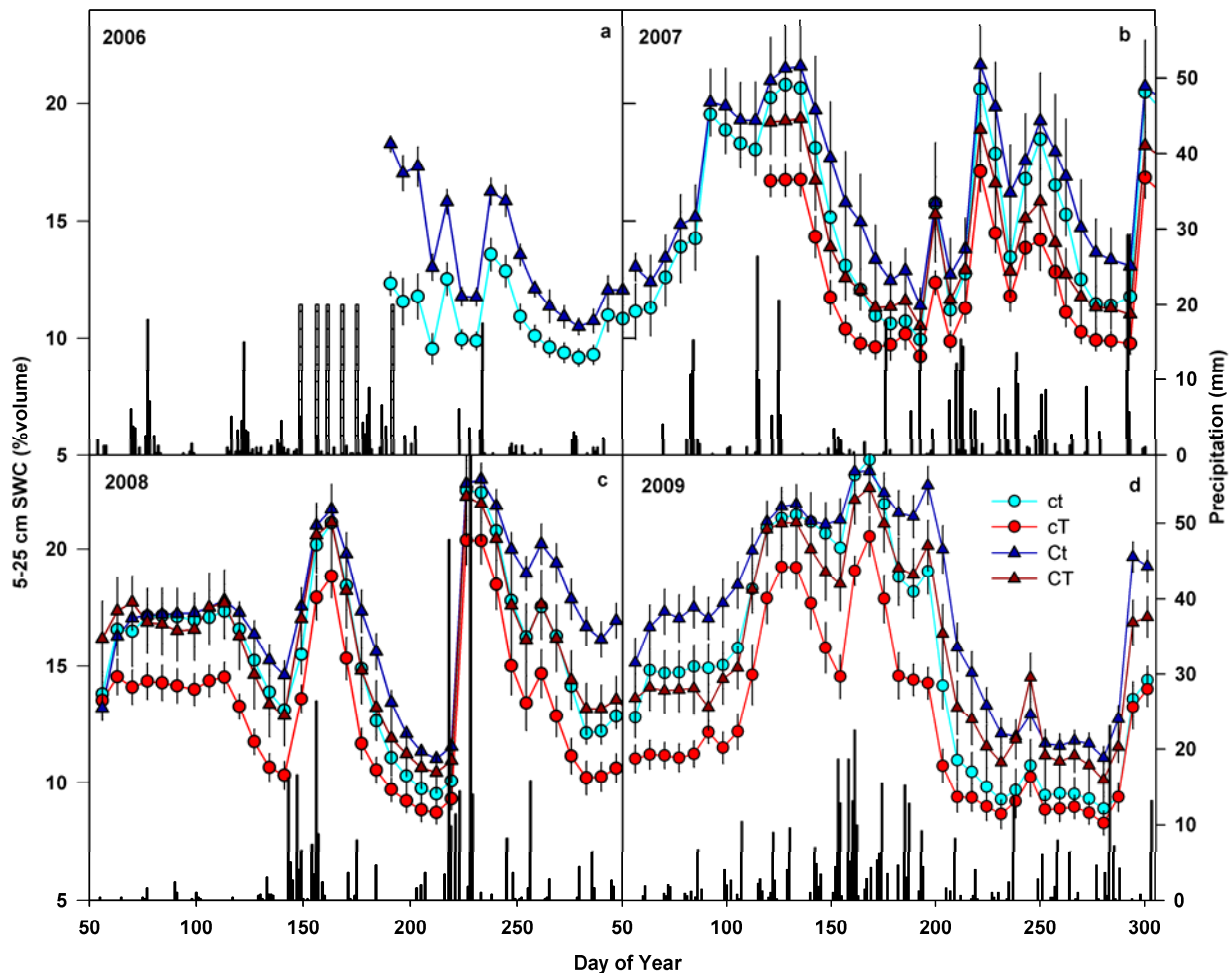
Supplementary Table 1. Average air and soil temperatures

Treatment	Average Annual Temperatures (°C)		
	Air	Soil 3 cm	Soil 10 cm
ct	10.8 (0.10) a	13.2 (0.17) a	12.7 (0.08) a
Ct	10.7 (0.09) a	12.8 (0.17) a	12.5 (0.08) a
cT	11.4 (0.09) b	15.7 (0.17) b	14.6 (0.08) b
CT	11.6 (0.09) b	15.3 (0.17) b	14.4 (0.08) b

Near-annual (day of year 50-308) daily temperatures averaged across years (2007-2009) and determined with illuminated, thin-wire thermocouples placed at canopy height, plus thermocouples placed in the soil at 3 and 10 cm depths below the soil surface. Data analysis was conducted using SAS.STAT software, Version 9.2, Proc GLIMMIX, copyright © 2002-2008, SAS Institute Inc., Cary, NC, USA. Air and soil temperatures were compared among treatments using estimates provided from a repeated measures general linear model. A fixed effect spline was used to fit the general trend of temperatures across day of year for each year. A random effects radial smoother was used to fit an individual spline for each replication of treatment. Letters following mean temperatures (s.e.m. in parentheses) grouped according to the Bonferroni Grouping (Version 9.2 of the SAS System for Windows), such that means with different letters are significantly different ($\alpha=0.05$).

Soil Water Conversions. The relationship between early-season (DOY100-200) control plot (**ct**) SWC and biomass enhancement ratio (BER) developed from the present PHACE experiment differed significantly from the curve developed from the previous open top chamber (OTC) experiment (data not shown); we speculated that contrasting soils between the two experiments might be involved in these differences. Soils at the OTC site had higher sand content, indicating that soil water would be held under less tension at low SWC compared to the PHACE site soil which had higher clay content³⁰. Expressing soil water availability in terms of matric potential (ψ_m), a measure of the tension with which water is held in soils that accounts for differences in soil texture and structure, should theoretically produce a more consistent relationship between the two experiments. Directly measured SWC values from control (**ct**), non-CO₂ enriched plots in the PHACE and OTC experiments were converted to ψ_m using Rosetta (version 1.2), a computer program for estimating soil hydraulic parameters using pedotransfer functions (<http://www.ars.usda.gov/Services/docs.htm?docid=8953>). Physical properties used in the construction of the soil water release curve at the PHACE experiment included: 1) average site soil texture (59.6 % sand, 18.7 % silt, 21.7 % clay) and bulk density (1.31 g cm⁻³) determined at all PHACE rings between 0-30 cm depth, and averaged across rings; and 2) soil moisture retention of nine representative surface soils at the PHACE experiment subjected to 1/3 bar tension (field capacity=37.5%). The soil at the OTC site was a Remmit fine sandy loam (Ustollic camborthids). Physical soil properties used from the OTC upper soil layers were texture (73.4% sand, 17% silt, 9.6 % clay), bulk density (1.35 g cm⁻³), field capacity (18%), and wilting point (4%), all obtained from Mosier et al.³¹. Since we were interested in evaluating the effects of SWC on the biomass responses of plants to CO₂, we limited our analysis to the upper soil layers where most roots reside³². We used different instrumentation and measured different depths in these two experiments. Volumetric SWC was determined approximately weekly at the OTC site using a Troxler model 4301 neutron probe (Troxler Electronics Lab., Research Triangle Park, NC, USA) at 30 cm depth and deeper. Time domain reflectometry was used to determine soil moisture in the upper 15 cm of the soil horizon where neutron probes are less accurate. We averaged the TDR and 30 cm depth neutron probe readings to obtain an average SWC for the top 45 cm. In the PHACE experiment, SWC was obtained from an average of two Sentek probes positioned at 10 and 20 cm depth. Although the approximate sample measurement depths differ in the two experiments (0-45 cm for OTC, 5-25 cm for PHACE), the measurement depths are in the zone of maximum rooting for these grasslands. Further, the slightly lower average sample depth for the OTC experiment (22 cm for OTC vs. 15 cm for PHACE) is a physiologically more relevant comparison since rooting depth in these dry grasslands tends to be deeper in more sandy soils. In 2006, the first year of the PHACE experiment, Sentek probes were not installed and functioning until mid-summer (DOY 188). Alternate soil water data, representative of the control plots (**ct**) were obtained from an on-site weather station for DOY 100-150, taking the average measurements from two TDR probes situated 10 and 30 cm below the soil surface. These on-site soil water measurements were of no use from DOY 151-187 when water was added to all plots (Supplementary Fig. 4), so for this 36-day period, soil moisture was estimated with the Daycent model³³ (average for 10 and 20 cm depths). Sentek soil moisture data were used after DOY 187. Since soil water is relatively unavailable to plants when ψ_m falls below -15 bars³⁰, we assumed plant growth during such periods to be essentially nil, and therefore disregard such periods in computing an average ψ_m for developing the relationships between early-season ψ_m and BER.

SUPPLEMENTARY APPENDIX II. Soil Water Content. Throughout this experiment, and consistent with our predictions, SWC tended to be highest at 600 ppmv CO₂ (Ct) and lowest in the warming treatment (cT), with the control (ct) and CO₂-enriched and warming treatments (CT) often having similar, intermediate SWCs compared to the other two treatments (Supplementary Fig. 4).



Supplementary Fig. 4. Responses of soil water content (SWC) to CO₂ and warming treatments. Average and s.e.m. (error bars) weekly soil water content (SWC) (5-25 cm depth) for plots exposed to present-day ambient CO₂ and temperature (ct), 1.5/3 °C day/night warming (cT), 600 ppmv CO₂ (Ct), and 600 ppmv CO₂ and 1.5/3 °C day/night warming (CT). Results are given for 2006 and the first few months of 2007 when only the CO₂ treatment was operating (and therefore, each mean and s.e.m. are computed from ten replicate samples per treatment per date), and for 2007-2009 when all four combinations of CO₂ and warming were imposed (5 replicate samples per treatment per date). Total annual precipitation amounts for the four years were 397, 353, 357 and 453 mm for 2006-2009, respectively. Six supplemental hand watering events of 20 mm applications each (part of another experiment not reported here) are included in the annual precipitation amount in 2006, and are indicated by open histograms with bars.

When averaged across the three years (DOY 50-308 each year) in which both CO₂ and warming treatments were imposed (2007-2009), SWC was significantly ($\alpha=0.05$) highest in the CO₂-enriched treatment and lowest in the warming treatment (Supplementary Table 2), demonstrating the opposing effects of these two global change treatments on SWC. Intermediate in SWC were the control (**ct**) and combined CO₂-enriched plus warming treatments (**CT**) which had similar SWCs (15.5% and 15.6% respectively). Similar results were observed within individual years.

Supplementary Table 2. Effects of global change treatments on annual soil water content.

Treatment	Average Volumetric Soil Water Content (%)			
	07-09	2007	2008	2009
ct	15.5 (0.28) b	15.2 (0.48) b	15.9 (0.46) b	15.3 (0.68) a
Ct	17.3 (0.28) a	16.9 (0.48) a	17.7 (0.46) a	17.5 (0.68) a
cT	13.1 (0.28) c	13.4 (0.48) c	13.5 (0.46) c	12.1 (0.68) b
CT	15.6 (0.28) b	15.3 (0.48) a,b	15.6 (0.46) b	15.9 (0.68) a

Volumetric soil water content (SWC) measured with Sentek EnviroSMART soil water sensors positioned at 10 and 20 cm depth below the soil surface and averaged over those two depths. Resultant mean values (s.e.m. in parentheses) of SWC are assumed to represent SWC from approximately 5-25 cm depth, and are calculated from approximate weekly mean values averaged across/within the three years (2007-2009) from days of year 50-308 (for each of 4 treatments, with 5 replications each). Data analysis was conducted using SAS/STAT software, Version 9.2, Proc GLIMMIX, copyright © 2002-2008, SAS Institute Inc., Cary, NC, USA. Soil water content was compared among treatments using estimates provided from a repeated measures general linear model. A fixed effect spline was used to fit the general trend of mean SWC across days of year for each year. A random effects radial smoother was used to fit an individual spline for each replication of treatment. Letters following mean SWCs group treatment means according to the Bonferroni Grouping (Version 9.2 of the SAS System for Windows), such that means with different letters are significantly different ($\alpha=0.05$).

SUPPLEMENTARY APPENDIX III. Global Change Treatments and Plant Responses.

As with all experiments, understanding the limitations of experimental protocol is critical. Here we discuss three such issues: the warming and CO₂ methodologies, the ET modeling exercise, and the observed superior performance of C₄ vs. C₃ grasses to CO₂ and warming.

Warming & CO₂ methodologies: features and limitations. Among warming technologies, infrared heaters seem to have the fewest drawbacks in warming field plots to evaluate the effects of climate change on intact ecosystems³⁴. Harte et al.³⁵ were the first to use such technology, and applied it in a constant power mode. This apparently resulted in fairly high canopy warming during stable night-time conditions, and little warming under more turbulent daytime atmospheric conditions³⁶. The T-FACE system, which uses a proportional integral derivative feed-back system with infrared thermometers positioned above the canopy to sense and respond to canopy temperatures, is able to achieve fairly good control of canopy temperature set-points (see Supplementary Appendix I). However, the warming treatment does not include the additional moisture which is expected in a warmer atmosphere. Most projections of climate change suggest vapor pressure will rise with warming, with relative humidity (RH) remaining fairly constant³⁷. This likely does not apply as strictly to native grasslands, many of which are located in dry continental interior regions and/or on the lee side of mountain ranges where orographic precipitation removes considerable moisture. Nevertheless, had we been able to increase humidity in the field as will occur to some extent as temperatures continue to climb, the desiccating effects would have been smaller than what we observed in our study.

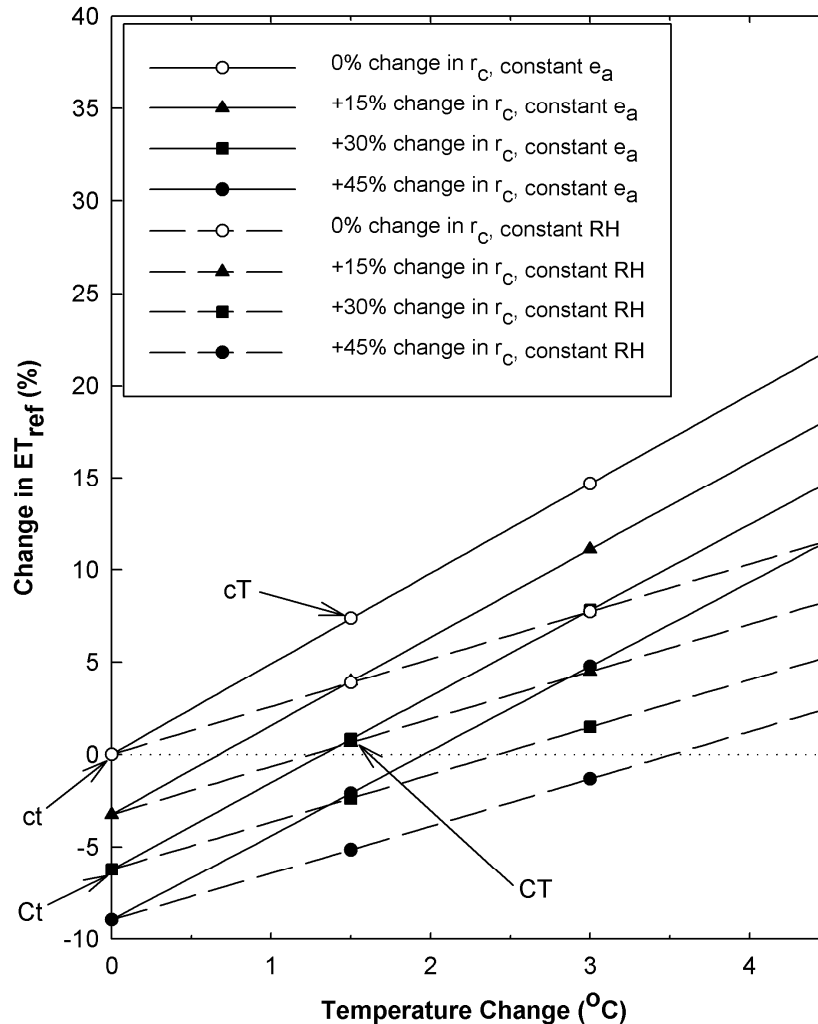
To evaluate the effect of this on ET, we repeated analyses depicted in Figure 4, and added an additional scenario of ambient RH remaining constant (e_a increasing) as temperature increases (broken lines in Supplementary Fig. 5). The results suggest that the 1.5 °C daytime warming in our experiment, in a field environment with fairly constant e_a (solid lines), resulted in an increase in ET_{ref} similar to that simulated by 3.0 °C daytime warming under conditions of constant ambient RH. Thus, in terms of its evaporative effects, our warming treatment had the effect of an approximate 3.0 °C in temperature, assuming ambient RH remains constant. This means our conclusions about the effect of CO₂ off-setting the desiccating effect of warming in our experiment is quite conservative.

However, it should be noted that the small plot size may have minimized canopy warming due to CO₂. A small amount of warming is expected to occur in CO₂-enriched atmospheres because CO₂ reduces transpiration; therefore, more energy is exchanged through surface warming and re-radiation. We were unable to detect any such warming with the infrared thermometers used in the T-FACE control system in comparisons of canopy temperature between elevated CO₂ (Ct) and control plots (ct) under non-limiting conditions of soil water. We suspect the narrow leaves of the dominant grasses plus turbulence due to frequent windy conditions minimized changes in leaf temperature due to stomatal closure⁴¹. Fetch of our plots may also have been insufficient for the canopy energy balance to fully equilibrate to CO₂-induced stomatal closure, meaning canopy temperature in a future CO₂-enriched world could be slightly higher, and therefore ET slightly greater than experienced in our plots. This potential under-representation of ET was likely minor considering the narrow leaves and often windy conditions at the site, and was less than the over-representation of desiccation in our warming treatment.

ET Modeling: Stomatal resistance vs. leaf area responses. A limitation of our modeling exercise (Supplementary Fig. 5) is that it does not account for treatment differences in leaf area. Elevated CO₂ increased peak AGB by an average 33% in the first three years of the experiment, so leaf area was likely 25-30% greater in CO₂ enriched plots by mid-July in those years (leaf area differences would have been less than AGB since AGB includes a small fraction of non-transpiring stem tissues). However, for the remainder of the year, AGB is lower. In spring and early summer, the plant community is growing and expanding its leaf area up to the time of peak AGB. Afterwards, leaves begin to senesce and transpirational surface declines. So while increases in leaf area would tend to counter the water savings effects of increased stomatal resistance, these leaf area differences, integrated over the entire growing season, were considerably less than the reported 30-40% increases in stomatal resistance that occur when native C₃ and C₄ grasses are exposed to CO₂-enriched atmospheres^{25, 26}. Even with moderate increases in peak AGB, our SWC results (Fig. 1 and Supplementary Fig. 4) suggest that CO₂ treatment effects on stomatal resistance dominated seasonal plant community transpirational responses. This was especially the case for our contrast of greatest interest, between present day (ct) and future CO₂-enriched and warmer conditions (CT), in which average differences (2007-2009) in peak AGB were only 12%.

Supplementary Fig. 5 is useful for considering the interactions of CO₂-induced increases in canopy resistance with increases in temperature. It suggests that an increase in CO₂ to 600 ppmv reduces ET_{ref} roughly equivalent to predicted increases in ET_r due to a 2-3 °C increase in daytime temperature. Thus, increases in ambient CO₂ to 600 ppmv should compensate for moderate warming effects on ET. However, more severe warming will eventually overcome this anti-transpirant response due to limits on stomatal-based water conservation¹². Understanding this apparent tipping point and properly scaling it spatially to regional and higher levels is a challenging but essential problem that must be solved to accurately predict the hydraulic responses of semi-arid ecosystems to climate change.

C₄ vs. C₃ responses. The discovery that productivity of only the C₄ grasses (primarily *Bouteloua gracilis*) was enhanced under future warmed and CO₂-enriched conditions suggests a competitive advantage for C₄ vs. C₃ grasses. This finding, although novel, is not too surprising since we know C₄ grasses are especially adapted to warm environments^{8, 13}, and can respond to CO₂, especially under water-limiting conditions⁵. However, past research has shown mixed responses of *B. gracilis* to CO₂, with one recent experiment showing no sensitivity of aboveground biomass to CO₂²¹ but previous work showing higher photosynthesis⁴² and growth^{42, 43} in *B. gracilis* exposed to CO₂-enriched atmospheres. And while many C₄ grasses are known to be well adapted to warm periods and droughts, the extensive drought which visited the Great Plains during the Dust Bowl of the 1930's almost entirely eliminated some C₄ grasses, while some C₃ grasses that were able to take advantage of early-season soil moisture due to their cool-season metabolism expanded their range⁴⁴. Interestingly, the range of *B. grama* increased dramatically during the Dust Bowl. Collectively, these findings suggest an expansion of *B. gracilis* in future CO₂-enriched and warmer environments, but caution should be used in extrapolating these results to other mixed C₃/C₄ grasslands without additional realistic field experimentation. Indeed, we will need to corroborate these findings for our site with further years of data since long-term ecological responses to perturbations can change over time.



Supplementary Fig. 5. Percent changes in reference evapotranspiration (ET_{ref}) for a grass surface as affected by temperature and changes in canopy resistance (r_c). Percent changes in ET_{ref} calculated using the ASCE standardized evapotranspiration equation³⁸ versus a range of temperature increases following Kimball and Bernacchi³⁹ and Kimball⁴⁰. The calculations were done using observed site weather station data from the PHACE Project for the 1 April – 16 October 2007, 2008, and 2009 growing seasons. The total calculated ET_{ref} for the three seasons was 2490 mm (average of 5.1 mm/day). The calculations were done for zero, +15, +30, and +45% changes in r_c , as might be expected from CO_2 -induced increases in stomatal closure. Two humidity scenarios are considered: 1) changes in r_c under constant vapor pressure (e_a), the conditions of our experiment (solid lines in figure); and 2) changes in r_c under conditions of constant relative humidity (RH), the predicted future environment for regions in which air vapor pressure is closely linked to air temperature (broken lines). The procedure assumes a value of 50 s/m for daytime surface or grass canopy resistance and 200 s/m at night. Nighttime ET_{ref} is accounted, but because of nighttime stomatal closure and because of higher temperatures and wind speeds in daytime, daytime ET_{ref} considerably exceeds that at nighttime.

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