

C₄ grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland

Jack A. Morgan¹, Daniel R. LeCain¹, Elise Pendall², Dana M. Blumenthal¹, Bruce A. Kimball³, Yolima Carrillo², David G. Williams⁴, Jana Heisler-White⁴, Feike A. Dijkstra^{1,5} & Mark West¹

Global warming is predicted to induce desiccation in many world regions through increases in evaporative demand^{1–3}. Rising CO₂ may counter that trend by improving plant water-use efficiency^{4,5}. However, it is not clear how important this CO₂-enhanced water use efficiency might be in offsetting warming-induced desiccation because higher CO₂ also leads to higher plant biomass, and therefore greater transpirational surface^{2,6,7}. Furthermore, although warming is predicted to favour warm-season, C₄ grasses, rising CO₂ should favour C₃, or cool-season plants⁸. Here we show in a semi-arid grassland that elevated CO₂ can completely reverse the desiccating effects of moderate warming. Although enrichment of air to 600 p.p.m.v. CO₂ increased soil water content (SWC), 1.5/3.0 °C day/night warming resulted in desiccation, such that combined CO₂ enrichment and warming had no effect on SWC relative to control plots. As predicted, elevated CO₂ favoured C₃ grasses and enhanced stand productivity, whereas warming favoured C₄ grasses. Combined warming and CO₂ enrichment stimulated above-ground growth of C₄ grasses in 2 of 3 years when soil moisture most limited plant productivity. The results indicate that in a warmer, CO₂-enriched world, both SWC and productivity in semi-arid grasslands may be higher than previously expected.

Grass-dominated, dry rangelands account for over 30% of Earth's terrestrial surface^{9,10} and provide most of the forage for the world's domestic livestock. Among the most important of these include the vast Central Asian steppes, North American prairies, Australian rangelands, plus extensive grazing lands of Africa, South America and the Mediterranean. Productivity of these lands, which is under increasing pressure with human population growth, is primarily limited by water^{9,11}. Soil water availability is driven by complex interactions between precipitation, temperature^{1,11}, ambient CO₂ (ref. 4) and soil properties, rendering rangelands and the livestock and cultures they support particularly susceptible to climate change and rising CO₂. Higher CO₂ concentrations induce stomatal closure, which reduces leaf transpiration and increases plant water-use efficiency and SWC⁴. Despite agreement among global climate models that climate change will induce drought-like conditions in several rangeland-dominated world regions^{1,2}, considerable uncertainty remains as to how stomatal closure from CO₂ might interact with increased temperature to influence stand-level evapotranspiration, soil water dynamics and plant productivity. Many believe that CO₂-induced reductions in transpiration at the leaf level will be largely offset at the canopy level by increases in leaf area^{2,6,7,12}.

Most rangelands are dominated by graminoids, and contain a mixture of the two major photosynthetic pathway classes of plants, C₃ and C₄. Today, C₄ grasses are most common in grasslands and savannas within 40° of the Equator⁸, and are predicted to become more abundant with increasing temperature¹³. However, C₃ plants, owing to their CO₂-limited photosynthetic metabolism, may become more competitive in future CO₂-enriched rangelands^{8,14}. The relative abundances

of these two photosynthetic types govern spatial and temporal aspects of water and nutrient cycling, net primary production and plant-animal interactions^{13,15,16}, yet there is considerable uncertainty about which group will be favoured under future, warmer, CO₂-enriched conditions.

In the Prairie Heating and CO₂ Enrichment (PHACE) experiment, we evaluated the responses of native mixed-grass prairie to 1 year of CO₂ enrichment (2006) (from present ambient (385 p.p.m.v.) to elevated (600 p.p.m.v.) CO₂, treatments c and C respectively), followed by 3 more years (2007–2009) of combined CO₂ enrichment and warming (present ambient, and elevated, temperatures (1.5/3.0 °C warmer canopy day/night), treatments t and T respectively) using free-air CO₂ enrichment (FACE) technology for CO₂ (ref. 17) and T-FACE technology for warming¹⁸ (Supplementary Appendix I). CO₂ enrichment and warming had opposing effects on soil water balance. Elevated CO₂ (Ct) increased annual SWC (17.3% averaged over 2007–2009), whereas warming reduced it (13.1%), such that there was no difference between control (ct; 15.5%) and CO₂-enriched and warmed plots (CT; 15.6%) (Supplementary Appendix II). The same pattern of compensating treatment effects on SWC was observed in the early growing season (Fig. 1), the time of year when SWC most controls annual productivity in this grassland¹⁹. These results are similar to but more consistent than those reported in a semi-arid Australian temperate grassland²⁰, and illustrate that the water conservation effects

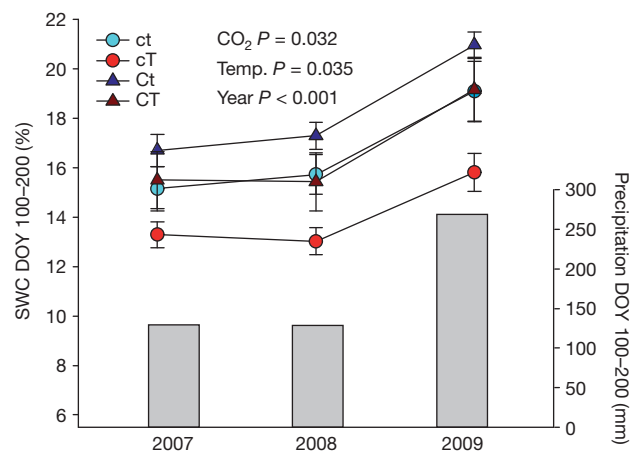


Figure 1 | Responses of SWC to CO₂ and warming. Average and s.e.m. (error bars) of volumetric SWC (5–25 cm depth) for plots exposed to present-day ambient CO₂ and temperature (ct), 1.5/3 °C day/night warming (cT), 600 p.p.m.v. CO₂ (Ct), and 600 p.p.m.v. CO₂ and 1.5/3 °C day/night warming (CT) (five replications per treatment). Data are averaged over days of year (DOY) 100–200, the early- to mid-growing season when soil water most limits productivity. Precipitation amounts for this same period are also presented. Significance ($P \leq 0.05$) for main effects and year are given in the figure.

¹USDA-ARS, Rangeland Resources Research Unit and Northern Plains Area, Fort Collins, Colorado 80526, USA. ²Department of Botany and Program in Ecology, University of Wyoming, Laramie, Wyoming 82071, USA. ³US Arid-Land Agricultural Research Center, USDA, Agricultural Research Service, Maricopa, Arizona 85238, USA. ⁴Departments of Botany, Renewable Resources, and Program in Ecology, University of Wyoming, Laramie, Wyoming 82071, USA. ⁵Faculty of Agriculture, Food and Natural Resources, The University of Sydney, Sydney, New South Wales 2006, Australia.

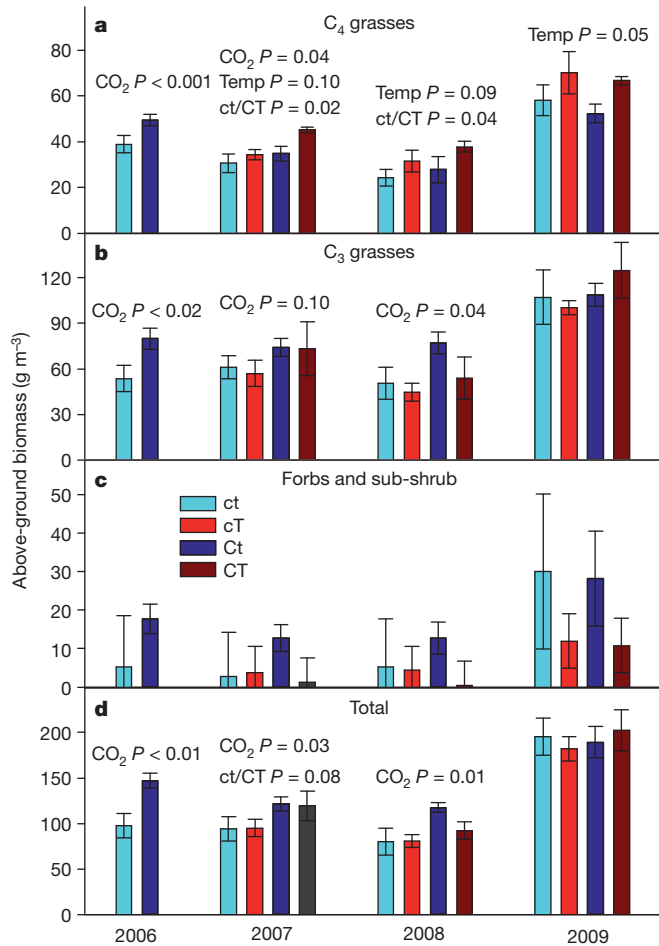
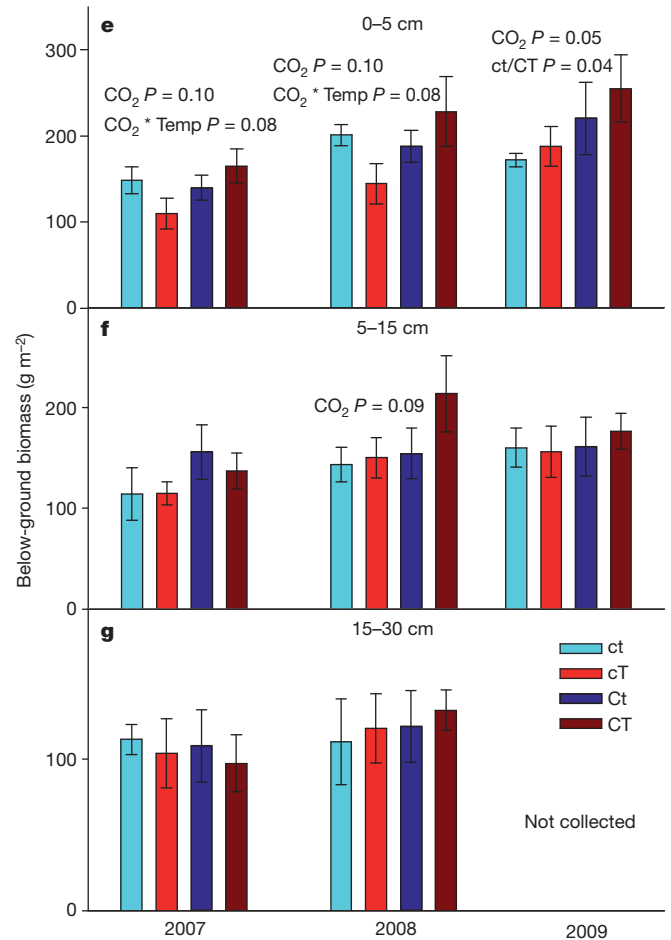


Figure 2 | Plant biomass responses to CO₂ and warming. Treatment effects (details in Fig. 1) on biomass in mid-July. AGB and s.e.m. (error bars) are given for C₄ (a) and C₃ grasses (b), forbs and sub-shrub (c) and total plant community (d). Below-ground biomass and s.e.m. (error bars) is given for soil depths of 0–5



(e), 5–15 (f) and 15–30 (g) cm. Results from 2006 include only the CO₂ treatment. Significant contrasts between present-day (ct) and future (CT) conditions are represented above histograms as ct/CT. *P* values are given for analyses of main effects, interactions and the ct/CT contrast when *P* ≤ 0.10.

of elevated CO₂ can completely cancel the desiccating effects of moderately warmer temperatures.

Exposure of the prairie to 600 p.p.m.v. CO₂ increased peak total above-ground biomass (AGB, an estimate of above-ground net primary productivity) by an average 33% (Fig. 2d) in the first 3 years of the experiment when annual precipitation amounts were within 7% of the site 132-year average of 388 mm. We attribute this strong, positive response of AGB to CO₂ in this semi-arid grassland to the higher SWC in CO₂-enriched plots⁴. CO₂ enrichment had no effect on AGB in 2009, a relatively wet year for the site when annual precipitation was 17% higher than the long-term mean (Supplementary Appendix II). We suspect that higher SWC in 2009 (Fig. 1) minimized the potential water-relations benefit of CO₂ enrichment on plant productivity.

To evaluate further the effect of CO₂ on SWC and plant productivity, we examined how responses of AGB to increasing CO₂ varied with early-season soil matric potential (ψ_m) averaged during periods of active growth ($\psi_m \geq -1.5$ MPa). The ratio of AGB of plants exposed to 600 p.p.m.v. CO₂ to that of plants exposed to present-day CO₂ (biomass enhancement ratio) was used for this evaluation. We incorporated results from another CO₂ enrichment experiment in the Colorado shortgrass steppe to include the other semi-arid grassland of the western Great Plains²¹ (Supplementary Appendix I, Supplementary Fig. 1). A strong negative relationship was observed between ψ_m and biomass enhancement ratio (Fig. 3), which we attribute to (1) the enhancement of water use efficiency under elevated CO₂ and increasing water stress^{4,5}, and (2) the use of a physiologically relevant environmental driver (ψ_m rather than SWC; Supplementary Appendix I, soil water conversions)

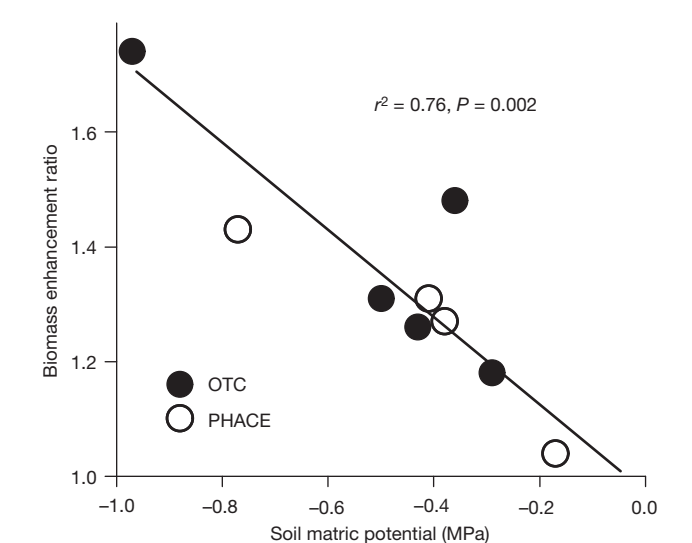


Figure 3 | Response of biomass enhancement ratio to soil matric potential. Effects of early-season (DOY 100–200) rooting zone soil matric potential (ψ_m) on biomass enhancement ratio, the ratio of mid-July harvested above-ground plant biomass in CO₂-enriched plots divided by plant biomass from ambient CO₂ plots. Data are from the PHACE experiment (4 years' data, *n* = 4), and from a previous open top chamber CO₂ enrichment experiment (5 years' data, *n* = 5) conducted on Colorado shortgrass steppe²¹. For further details, see Supplementary Appendix I, soil water conversions.

averaged over periods of physiological activity. This robust relationship from two different ecosystems suggests that CO₂ will increase plant productivity most when plants are water-limited yet still active.

The positive effect of CO₂ on production was also apparent in below-ground plant biomass. However, because root turnover in semi-arid perennial grasslands occurs every 5–7 years (ref. 22), we did not detect a significant ($P \leq 0.05$) effect on roots until the fourth year of our experiment (2009), when below-ground plant biomass in the 0–5 cm depth layer was 32% higher in CO₂-enriched plots (Fig. 2e).

Warming has the potential to increase production by extending the duration of the growing season, and by increasing nitrogen uptake, efficiency of nitrogen use²³ and biological activity. In our experiment, however, warming by itself did not significantly affect total AGB (Fig. 2d) or below-ground plant biomass (Fig. 2e–g), despite increased plant nitrogen content²⁴. Warming-induced desiccation in this semi-arid mixed-grass prairie (Fig. 1) and the preponderance of cool-season C₃ grasses (55% of AGB) probably limited its ability to respond positively to warming.

Although water was a dominant driver in the responses of both C₃ and C₄ grasses to CO₂ and temperature, there was also evidence of unique functional group responses. C₃ grass AGB was 34% greater in CO₂-enriched compared with ambient plots from 2006 to 2008 ($P < 0.05$ in 2006 and 2008; $P = 0.1$ in 2007) (Fig. 2b), but did not respond to warming. In contrast, AGB of C₄ grasses was 28% greater in CO₂-enriched plots in the first 2 years, but was also greater under warming ($P \leq 0.10$), most notably the wet year (2009) when C₄ AGB was 23% greater in heated plots ($P = 0.05$) (Fig. 2a). Comparing present (ct) to future (CT) conditions, C₃ grasses did not respond, but C₄ AGB significantly increased with the combination of warming and CO₂ enrichment in 2007 and 2008 (Fig. 2). These results are consistent with our physiological understanding of C₃ and C₄ plant functional types^{8,13}, but they demonstrate for the first time in a field experiment a potential advantage for C₄ grasses in a CO₂-enriched and warmer future. Further research will be needed to determine the extent to which these results can be applied to other C₃ and C₄ grasses and grassland ecosystems (Supplementary Appendix III, C₄ versus C₃ responses). Small population sizes and high year-to-year variability limited our capacity to detect treatment effects on forbs and subshrubs (Fig. 2c).

Our results suggest that even with modest increases in plant growth (2007), rising atmospheric CO₂ may counter the pervasive desiccating effects of warming in semi-arid grassland ecosystems. The most likely explanation is a CO₂-induced suppression of transpiration⁴. A modelling analysis using our site's weather data shows the effects of different hypothetical increases in canopy resistance to water loss (r_c) such as might occur from stomatal closure, and different temperature increases on grass reference evapotranspiration rate (ET_{ref}, Fig. 4), the evapotranspiration rate of non-stressed grassland. Differences in ET_{ref} reflect not only the rate of evapotranspiration when soil water is readily available to plants, but also how long grass can continue to transpire and grow after major rain events before exhausting its soil water supply. At ambient CO₂, a daytime warming of 1.5 °C (our cT treatment daytime temperature; roughly 90% of evapotranspiration occurs during daytime) is predicted to increase ET_{ref} by 7%. At ambient temperature, an increase in r_c of 30% would be required to decrease ET_{ref} by a similar amount, 6%. This condition is similar to our Ct treatment (Fig. 4); leaf gas exchange measurements on the dominant C₃ and C₄ grasses of this²⁵ and other²⁶ natural ecosystems suggest a 30–40% increase in stomatal resistance occurs in native grasses under CO₂-enriched and non-stressed conditions. Thus, when daytime warming of 1.5 °C is combined with an increase of 30% in r_c (our CT treatment), the temperature effect and the CO₂ effect through increased stomatal resistance almost exactly offset one another, so that an increase of about only 1% in ET_{ref} is predicted. This modelling result mirrors the observed offsetting effects of CO₂ and temperature on SWC (Figs 1 and Supplementary Figure 4). The analysis does not account for treatment

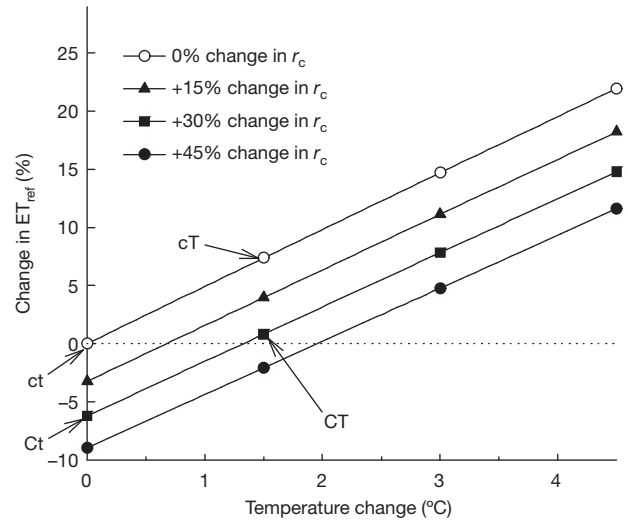


Figure 4 | Percentage changes in ET_{ref} for a grass surface as affected by temperature and changes in r_c . Percentage changes in ET_{ref} calculated using the American Society of Civil Engineers standardized evapotranspiration equation versus a range of temperature increases following Kimball²⁸. Calculations used observed weather 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 2,490 mm (average of 5.1 mm d⁻¹). Calculations were done for zero, +15, +30 and +45% changes in canopy resistance to water vapour (r_c), as might be expected from CO₂-induced stomatal closure. For further details, see Supplementary Appendix III, Supplementary Fig. 5.

effects on leaf area, although such differences were probably small compared with 30–40% increases in stomatal resistance we would expect under CO₂ enrichment (Supplementary Appendix III, evapotranspiration modelling). A protracted drought that depletes soil water stores will of course eventually eliminate these CO₂-induced water savings; we have yet to experience such conditions in this experiment. Thus, although rising CO₂ may ameliorate the effects of warming-induced drought through plant stomatal closure, it is unlikely to eliminate severe and protracted droughts predicted for rangelands in regions like southwestern North America or the Mediterranean, where both substantive temperature increase and reduced precipitation are predicted^{1,2}.

The particular responses reported here are to a single set of simulated global change conditions that are within the prediction possibilities of the Intergovernmental Panel on Climate Change for the latter half of this century²⁷. The manipulations are not without their artefactual issues, which must be considered in scaling such information temporally and beyond the experimental site (Supplementary Appendix III, warming & CO₂ methodologies). Nevertheless, our results clearly illustrate the importance of compensating CO₂ and warming effects in semi-arid ecosystems. These contrasting, water-mediated effects must be accounted for in accurately predicting the susceptibility of such systems to climate change.

METHODS SUMMARY

Experiment. The experiment was conducted in northern mixed-grass prairie west of Cheyenne, Wyoming, USA. It had a factorial combination of two levels of CO₂ (385 p.p.m.v. and elevated 600 p.p.m.v.), and two temperature (present ambient, and elevated (1.5/3.0 °C warmer day/night)) regimes, with five replications each. Treatments were randomly assigned to 20 3.3-m diameter circular plots in two soil types (blocks). Details of the experimental site and set up are available elsewhere²⁴.

AGB was measured by species in 2005 (the year before CO₂ treatments), in 2006 (the first year of CO₂ treatments) and from 2007 to 2009 (the years of combined elevated CO₂ and warming treatments) during mid-July. A metal wire grid divided into 24–25 cm × 25 cm quadrats (1.5 m² total) was placed over each plot and vegetation in every other quadrat (12 in total) was clipped to the crown, dried at 60 °C and weighed. Plant species were subsequently grouped into three functional groups for analyses: C₄ grasses, C₃ grasses, and Forbs and sub-shrub.

Beginning in 2007, three soil cores (30 cm deep, 5 cm diameter) were collected at about the same time as the AGB samples from each plot, divided into 0–5, 5–15 and 15–30 cm depths, and the three core samples composited into one sample per depth. Fine roots (less than 1 mm) were handpicked from the composited samples, and root fragments were washed, weighed and ash-corrected.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions J.A.M., E.P., D.M.B., B.A.K., D.G.W. and M.W. conceived the study. J.A.M., D.R.L., E.P., D.M.B., Y.C., D.G.W., J.H.-W. and F.A.D. performed the experiment. B.A.K. designed the warming system and conducted the evapotranspiration analysis. J.A.M. wrote the paper and the remaining authors contributed to the writing. Statistical analyses using SAS/STAT software, version 9.2, Proc GLIMMIX were performed by M.W. and J.A.M. The regression analyses using JMP software were performed by D.M.B. and J.A.M. Figures were developed by D.R.L.

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METHODS

Site description. The experiment was conducted at the US Department of Agriculture-Agricultural Research Service High Plains Grasslands Research Station, west of Cheyenne, Wyoming, USA (41° 11' N, 104° 54' W), elevation 1930 m. The ecosystem is a northern mixed-grass prairie, with a plant community of about 55% cool-season C₃ grasses (mostly *Pascopyrum smithii* and *Hesperostipa comata*, both perennials), 25% warm-season C₄ grasses (almost exclusively the perennial *Bouteloua gracilis*), and 20% sedges, forbs and small shrubs. Annual precipitation is 384 mm, mean air temperatures are 17.5 °C in summer and -2.5 °C in winter. The average annual wind speed is 6 m s⁻¹, with a growing season wind speed of 4.1 m s⁻¹.

A 2.4-ha site, which had been grazed by cattle at least since 1974, was fenced to prevent cattle entering in 2005. Soils are an Ascalon variant loam (fine-loamy, mixed-mesic) at the north end of the study site, and an Altvan loam (fine-loamy over sandy, mixed-mesic) at the south end.

Experimental layout. The experiment has a factorial combination of two levels of CO₂ (present ambient (385 p.p.m.v.), and elevated (600 p.p.m.v.), c and C respectively), and two temperature (present ambient, and elevated (1.5/3.0 °C warmer day/night), t and T respectively) regimes, with five replications making 20 experimental plots. Assuming an annual increase of CO₂ concentration of 2.3 p.p.m.v., our treatment combinations represent CO₂ and temperatures expected near the end of this century. We chose differential day/night temperatures based on the Fourth Intergovernmental Panel on Climate Change assessment, which suggests that daily minimal temperatures will increase faster than maximal temperatures²⁷. Furthermore, the equilibrium global mean surface air temperature for a doubling of atmospheric CO₂ is likely to lie between 2 and 4.5 °C. For our target CO₂ concentration of 600 p.p.m.v., our average day/night temperature of 2.25 °C lies at the upper third of a range we calculate to be between approximately 1.1–2.5 °C. The four treatment combinations were designated as follows: ct, ambient CO₂, non-heated; Ct, elevated CO₂, non-heated; cT, ambient CO₂, heated; CT, elevated CO₂, heated. Treatments were randomly assigned to 20 plots in northern and southern soil-type field blocks. Details are available elsewhere²⁴, including the FACE¹⁷ and infrared warming¹⁸ apparatus used to alter ambient CO₂ and temperature (see also Supplementary Appendix I).

Experimental rings and microclimate. To accommodate the FACE system, experimental plots were circular with a diameter of 3.3 m (area of 8.6 m²). Before instrumentation was installed, hexagonal trenches 60 cm deep were dug around the circumference of each plot and a plastic barrier installed to isolate treated plots hydraulically from outside non-treated soils. This hydraulic separation helped maintain any water-relations effects of the treatments.

Most of the plot area was maintained as native northern mixed prairie and was partitioned into soil and plant sampling areas, plus sections for other measurements, including SWC and air and soil temperature. Volumetric SWC was

measured daily using frequency domain reflectometry sensors at 10 and 20 cm soil depths (Sentek EnviroSMART sensors, Sentek Sensor Technologies) placed approximately 75 cm from the ring centres. Daily total precipitation was recorded with a rain gauge (Onset corp. S-RGA-M002).

Plant biomass. AGB was measured by species in 2005 (the year before CO₂ treatments), in 2006 (the first year of CO₂ treatments) and from 2007–2009 (the years of combined elevated-CO₂ and warming treatments) during mid-July. Mid-July is the approximate time of peak seasonal AGB, and provides a good estimate of above-ground net primary production. A metal wire grid divided into 24–25 cm × 25 cm quadrats (1.5 m² total) was placed over each plot (all sampled vegetation being within a metre of the ring centre), and vegetation in every other quadrat (12 in total) was clipped to the crown, dried at 60 °C and weighed. This defoliation protocol removed 50% of the green vegetation and represented the grazing removal for these grasslands. To correct for initial plot differences in species abundance, the 2005 pre-treatment harvest data were used to calculate overall means of each plant species' biomass across all 20 plots, plus the deviation for each species from that mean in every plot. These deviations were either added to or subtracted from individual species' biomass data collected in all plots for the subsequent treatment years, 2006–2009, thereby correcting for initial plot differences in plant species abundance. Plant species were subsequently grouped into three functional groups for analyses: C₄ grasses, C₃ grasses and sedges, and forbs and sub-shrub.

Beginning in 2007, near the time of the peak standing AGB harvest, three soil cores (30 cm deep, 5 cm diameter) were collected from each plot, divided into 0–5, 5–15 and 15–30 cm depths, and composited into one sample per depth. Fine roots (less than 1 mm) were handpicked from the composited samples, and root fragments were washed, weighed and ash corrected.

Statistical analyses. Data analysis used SAS/STAT software, version 9.2, Proc GLIMMIX, © 2002–2008 SAS Institute. Mean AGB and below-ground plant biomass were compared among all four treatments (five replications each) and years by fitting a repeated-measures general linear model to biomass. Plots within treatments represented subjects on which repeated measures were taken. Treatments and year were fitted as fixed-effects. Contrasts were constructed to estimate and test the effects of CO₂, warming and their interaction on biomass within years, owing to climatic differences among the four years. We hypothesized that increasing CO₂ would increase plant biomass (one-tailed test), but that warming would have no net effect on biomass (two-tailed test). Analyses of mean SWC (averaged annually for days of year 100–200) were conducted across years 2007–2009 when both CO₂ and warming treatments were operational. Regression curves of SWC and ψ_m with biomass enhancement ratio using data from the PHACE and open top chamber experiments were computed using JMP software, version 8.0.1, 2009 SAS Institute. Throughout, a *P* value of ≤ 0.05 is the standard for indicating significance, although marginal significance (*P* ≤ 0.10) is reported in figures, and sometimes commented on in the text as deemed appropriate.