# LETTER

# C$_4$ grasses prosper as carbon dioxide eliminates desiccation in warmed semi–arid grassland

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**Global warming is predicted to induce desiccation in many world regions through increases in evaporative demand[1–3]. Rising CO$_2$ may counter that trend by improving plant water-use efficiency[4,5]. However, it is not clear how important this CO$_2$-enhanced water use efficiency might be in offsetting warming-induced desiccation because higher CO$_2$ also leads to higher plant biomass, and therefore greater transpirational surface[2,6,7]. Furthermore, although warming is predicted to favour warm-season, C$_4$ grasses, rising CO$_2$ should favour C$_3$, or cool-season plants[8]. Here we show in a semi-arid grassland that elevated CO$_2$ can completely reverse the desiccating effects of moderate warming. Although enrichment of air to 600 p.p.m.v. CO$_2$ increased soil water content (SWC), 1.5/3.0 °C day/night warming resulted in desiccation, such that combined CO$_2$ enrichment and warming had no effect on SWC relative to control plots. As predicted, elevated CO$_2$ favoured C$_3$ grasses and enhanced stand productivity, whereas warming favoured C$_4$ grasses. Combined warming and CO$_2$ enrichment stimulated above-ground growth of C$_4$ grasses in 2 of 3 years when soil moisture most limited plant productivity. The results indicate that in a warmer, CO$_2$-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 CO$_2$ (ref. 4) and soil properties, rendering rangelands and the livestock and cultures they support particularly susceptible to climate change and rising CO$_2$. Higher CO$_2$ concentrations induce stomatal closure, which reduces leaf transpiration and increases plant water-use efficiency and SWC[4]. 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 CO$_2$ might interact with increased temperature to influence stand-level evapotranspiration, soil water dynamics and plant productivity. Many believe that CO$_2$-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, C$_3$ and C$_4$. Today, C$_4$ grasses are most common in grasslands and savannas within 40° of the Equator[8], and are predicted to become more abundant with increasing temperature[13]. However, C$_3$ plants, owing to their CO$_2$-limited photosynthetic metabolism, may become more competitive in future CO$_2$-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, CO$_2$-enriched conditions.

In the Prairie Heating and CO$_2$ Enrichment (PHACE) experiment, we evaluated the responses of native mixed-grass prairie to 1 year of CO$_2$ enrichment (2006) (from present ambient (385 p.p.m.v.) to elevated (600 p.p.m.v.) CO$_2$, treatments c and C respectively), followed by 3 more years (2007–2009) of combined CO$_2$ 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 CO$_2$ enrichment (FACE) technology for CO$_2$ (ref. 17) and T-FACE technology for warming[18] (Supplementary Appendix I). CO$_2$ enrichment and warming had opposing effects on soil water balance. Elevated CO$_2$ (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 CO$_2$-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 grassland[19]. These results are similar to but more consistent than those reported in a semi-arid Australian temperate grassland[20], and illustrate that the water conservation effects
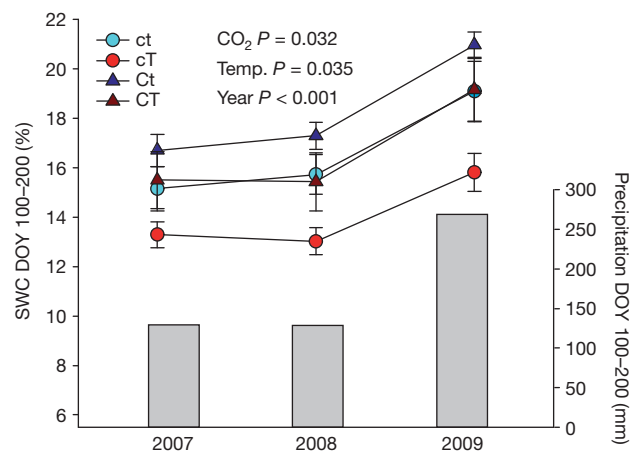


**Figure 1 | Responses of SWC to CO$_2$ and warming.** Average and s.e.m. (error bars) of volumetric SWC (5–25 cm depth) for plots exposed to present-day ambient CO$_2$ and temperature (ct), 1.5/3 °C day/night warming (cT), 600 p.p.m.v. CO$_2$ (Ct), and 600 p.p.m.v. CO$_2$ and 1.5/3 °C day/night warming (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.

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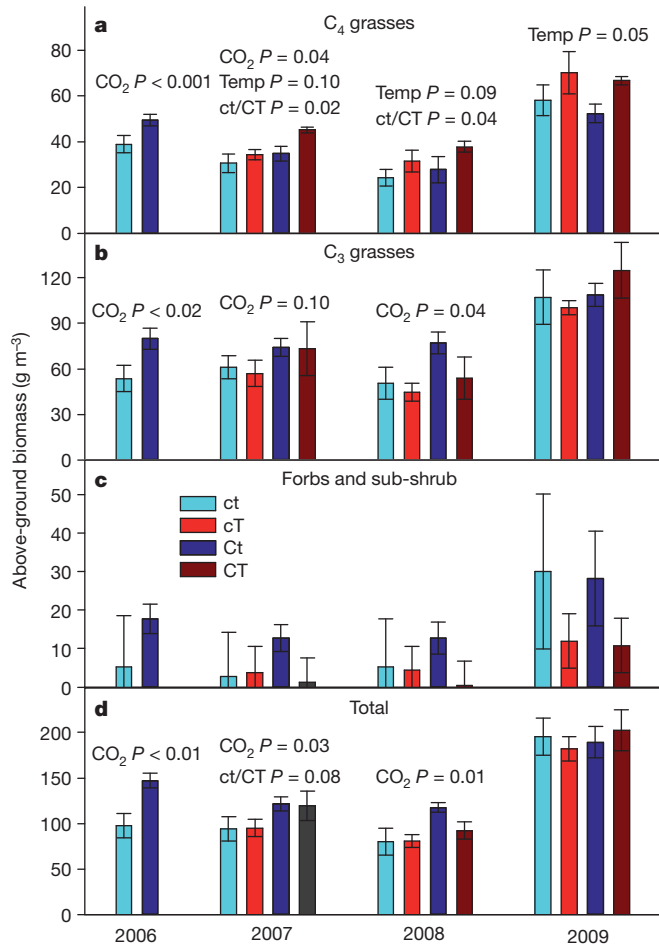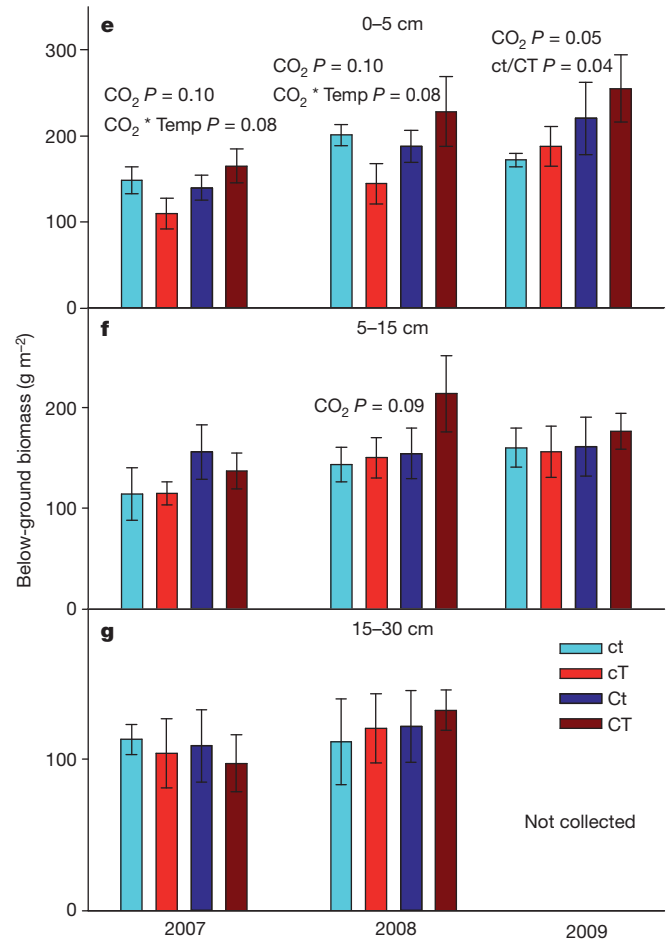
**Figure 2 | Plant biomass responses to $CO_2$ and warming.** Treatment effects (details in Fig. 1) on biomass in mid-July. AGB and s.e.m. (error bars) are given for $C_4$ (**a**) and $C_3$ grasses (**b**), forbs and sub-shrub (**c**) and total plant community (**d**). Below-ground biomass and s.e.m. (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 $CO_2$ 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 $P \le 0.10$.

of elevated $CO_2$ can completely cancel the desiccating effects of moderately warmer temperatures.

Exposure of the prairie to 600 p.p.m.v. $CO_2$ 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 $CO_2$ in this semi-arid grassland to the higher SWC in $CO_2$-enriched plots[4]. $CO_2$ 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 $CO_2$ enrichment on plant productivity.

To evaluate further the effect of $CO_2$ on SWC and plant productivity, we examined how responses of AGB to increasing $CO_2$ varied with early-season soil matric potential ($\psi_m$) averaged during periods of active growth ($\psi_m \ge -1.5$ MPa). The ratio of AGB of plants exposed to 600 p.p.m.v. $CO_2$ to that of plants exposed to present-day $CO_2$ (biomass enhancement ratio) was used for this evaluation. We incorporated results from another $CO_2$ enrichment experiment in the Colorado shortgrass steppe to include the other semi-arid grassland of the western Great Plains[21] (Supplementary Appendix I, Supplementary Fig. 1). A strong negative relationship was observed between $\psi_m$ and biomass enhancement ratio (Fig. 3), which we attribute to (1) the enhancement of water use efficiency under elevated $CO_2$ and increasing water stress[4,5], and (2) the use of a physiologically relevant environmental driver ($\psi_m$ rather than SWC; Supplementary Appendix I, soil water conversions)
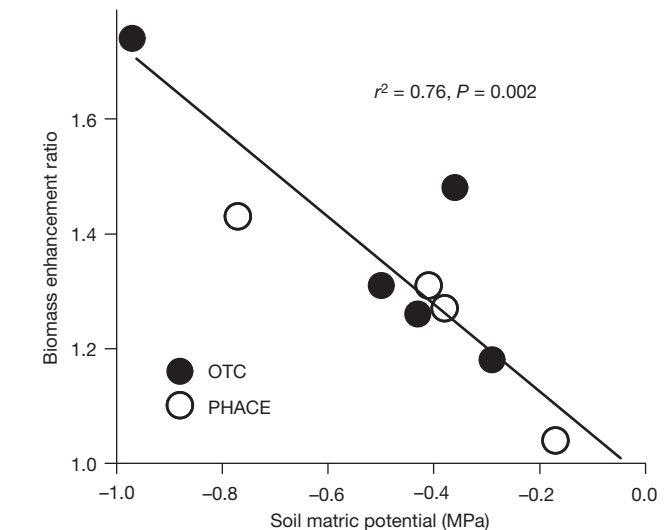


**Figure 3 | Response of biomass enhancement ratio to soil matric potential.** Effects of early-season (DOY 100–200) rooting zone soil matric potential ($\psi_m$) on biomass enhancement ratio, the ratio of mid-July harvested above-ground plant biomass in $CO_2$-enriched plots divided by plant biomass from ambient $CO_2$ plots. Data are from the PHACE experiment (4 years' data, $n = 4$), and from a previous open top chamber $CO_2$ enrichment experiment (5 years' data, $n = 5$) conducted on Colorado shortgrass steppe[21]. 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 $CO_2$ will increase plant productivity most when plants are water-limited yet still active.

The positive effect of $CO_2$ 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 $CO_2$-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 use[23] 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 content[24]. Warming-induced desiccation in this semi-arid mixed-grass prairie (Fig. 1) and the preponderance of cool-season $C_3$ grasses (55% of AGB) probably limited its ability to respond positively to warming.

Although water was a dominant driver in the responses of both $C_3$ and $C_4$ grasses to $CO_2$ and temperature, there was also evidence of unique functional group responses. $C_3$ grass AGB was 34% greater in $CO_2$-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 $C_4$ grasses was 28% greater in $CO_2$-enriched plots in the first 2 years, but was also greater under warming ($P \leq 0.10$), most notably the wet year (2009) when $C_4$ AGB was 23% greater in heated plots ($P = 0.05$) (Fig. 2a). Comparing present (ct) to future (CT) conditions, $C_3$ grasses did not respond, but $C_4$ AGB significantly increased with the combination of warming and $CO_2$ enrichment in 2007 and 2008 (Fig. 2). These results are consistent with our physiological understanding of $C_3$ and $C_4$ plant functional types[8,13], but they demonstrate for the first time in a field experiment a potential advantage for $C_4$ grasses in a $CO_2$-enriched and warmer future. Further research will be needed to determine the extent to which these results can be applied to other $C_3$ and $C_4$ grasses and grassland ecosystems (Supplementary Appendix III, $C_4$ versus $C_3$ responses). Small population sizes and high year-to-year variability limited our capacity to detect treatment effects on forbs and sub-shrubs (Fig. 2c).

Our results suggest that even with modest increases in plant growth (2007), rising atmospheric $CO_2$ may counter the pervasive desiccating effects of warming in semi-arid grassland ecosystems. The most likely explanation is a $CO_2$-induced suppression of transpiration[4]. 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 $CO_2$, 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 $C_3$ and $C_4$ grasses of this[25] and other[26] natural ecosystems suggest a 30–40% increase in stomatal resistance occurs in native grasses under $CO_2$-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 $CO_2$ 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 $CO_2$ and temperature on SWC (Figs 1 and Supplementary Figure 4). The analysis does not account for treatment
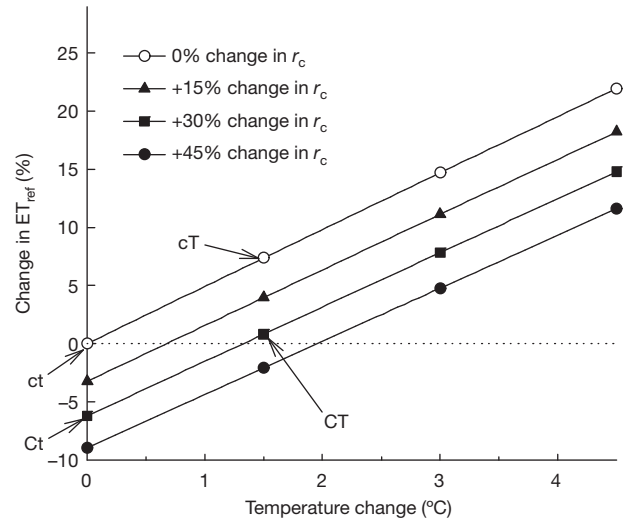


**Figure 4 | Percentage changes in $ET_{ref}$ for a grass surface as affected by temperature and changes in $r_c$.** Percentage changes in $ET_{ref}$ calculated using the American Society of Civil Engineers standardized evapotranspiration equation versus a range of temperature increases following Kimball[28]. 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 $CO_2$-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 $CO_2$ enrichment (Supplementary Appendix III, evapotranspiration modelling). A protracted drought that depletes soil water stores will of course eventually eliminate these $CO_2$-induced water savings; we have yet to experience such conditions in this experiment. Thus, although rising $CO_2$ 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 century[27]. 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 & $CO_2$ methodologies). Nevertheless, our results clearly illustrate the importance of compensating $CO_2$ 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 $CO_2$ (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 elsewhere[24].

AGB was measured by species in 2005 (the year before $CO_2$ treatments), in 2006 (the first year of $CO_2$ treatments) and from 2007 to 2009 (the years of combined elevated $CO_2$ 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: $C_4$ grasses, $C_3$ 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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## 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 $C_3$ grasses (mostly *Pascopyrum smthii* and *Hesperostipa comata*, both perennials), 25% warm-season $C_4$ 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 $CO_2$ (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 $CO_2$ concentration of 2.3 p.p.m.v., our treatment combinations represent $CO_2$ 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 temperatures[27]. Furthermore, the equilibrium global mean surface air temperature for a doubling of atmospheric $CO_2$ is likely to lie between 2 and 4.5 °C. For our target $CO_2$ 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 $CO_2$, non-heated; Ct, elevated $CO_2$, non-heated; cT, ambient $CO_2$, heated; CT, elevated $CO_2$, heated. Treatments were randomly assigned to 20 plots in northern and southern soil-type field blocks. Details are available elsewhere[24], including the FACE[17] and infrared warming[18] apparatus used to alter ambient $CO_2$ 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 m$^2$). 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 $CO_2$ treatments), in 2006 (the first year of $CO_2$ treatments) and from 2007–2009 (the years of combined elevated-$CO_2$ 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 m$^2$ 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: $C_4$ grasses, $C_3$ 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 $CO_2$, warming and their interaction on biomass within years, owing to climatic differences among the four years. We hypothesized that increasing $CO_2$ 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 $CO_2$ and warming treatments were operational. Regression curves of SWC and $\psi_m$ 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 ($P \leq 0.10$) is reported in figures, and sometimes commented on in the text as deemed appropriate.