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CO_2-caused change in plant species composition rivals the shift in vegetation between mid-grass and tallgrass prairies

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Abstract

Atmospheric CO_2 enrichment usually changes the relative contributions of plant species to biomass production of grasslands, but the types of species favored and mechanisms by which change is mediated differ among ecosystems. We measured changes in the contributions of C_3 perennial forbs and C_4 grasses to aboveground biomass production of tallgrass prairie assemblages grown along a field CO_2 gradient (250–500 μmol mol^{-1}) in central Texas USA. Vegetation was grown on three soil types and irrigated each season with water equivalent to the growing season mean of precipitation for the area. We predicted that CO_2 enrichment would increase the forb contribution to community production, and favor tall-grasses over mid-grasses by increasing soil water content and reducing the frequency with which soil water fell below a limitation threshold. CO_2 enrichment favored forbs over grasses on only one of three soil types, a Mollisol. The grass fraction of production increased dramatically across the CO_2 gradient on all soils. Contribution of the tall-grass *Sorghastrum nutans* to production increased at elevated CO_2 on the two most coarse-textured of the soils studied, a clay Mollisol and sandy Alfisol. The CO_2-caused increase in *Sorghastrum* was accompanied by an offsetting decline in production of the mid-grass *Bouteloua curtipendula*. Increased CO_2 favored the tall-grass over mid-grass by increasing soil water content and apparently intensifying competition for light or other resources (Mollisol) or reducing the frequency with which soil water dipped below threshold levels (Alfisol). An increase in CO_2 of 250 μmol mol^{-1} above the pre-industrial level thus led to a shift in the relative production of established species that is similar in magnitude to differences observed between mid-grass and tallgrass prairies along a precipitation gradient in the central USA. By reducing water limitation to plants, atmospheric CO_2 enrichment may alter the composition and even structure of grassland vegetation.

Keywords: aboveground biomass production, forbs, grassland, plant functional group, precipitation, soil texture, soil water content, subambient CO_2, threshold, water availability

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Introduction

Atmospheric carbon dioxide (CO_2) concentration has increased by about 40% since the 19th century, from the pre-industrial level of 270 ppmv to the current level of 385 ppmv (Petit *et al*., 1999; Keeling *et al*., 2009), and is expected to reach double the pre-industrial concentration during this century (IPCC, 2007). CO_2 enrichment usually alters the contributions of plant species to biomass production of grasslands (Leadley *et al*., 1999; Edwards *et al*., 2001; Niklaus *et al*., 2001; Polley *et al*., 2003; Morgan *et al*., 2004), but the types of species favored by CO_2 enrichment vary among grassland ecosystems. Several studies have shown that CO_2 enrichment favors forbs over grasses (Potvin & Vasseur, 1997; Leadley *et al*., 1999; Niklaus *et al*., 2001; Teyssonneyre *et al*., 2002), for example, but this trend is not universal (Owensby *et al*., 1999; Morgan *et al*., 2001; Zavaleta *et al*., 2003; Kammann *et al*., 2005). Our ability to predict vegetation change, defined here as a shift in plant species abundances or composition, thus is limited, despite its relevance for management and potential consequences for ecosystem processes (Polley *et al*., 2011).

Vegetation change at elevated CO_2 often is linked to an increase in soil water content (Chiariello & Field, 1996; Grünzweig & Körner, 2001; Morgan *et al*., 2004; but see, Edwards *et al*., 2001; Zavaleta *et al*., 2003), but the mechanism by which change is mediated varies among ecosystems. An increase in soil water content could contribute to species change by differentially increasing seed production and seedling recruitment among species (Niklaus *et al*., 2001; Morgan *et al*., 2004)

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or altering competitive interactions among established plants (Polley *et al*., 2003; Dijkstra *et al*., 2010).

The response of dominant members of the plant community to CO_2 also varies among experiments. CO_2 enrichment usually increases production of one or more species without affecting biomass of other dominant and co-dominant species, rather than by causing opposing changes in dominant species. CO_2 enrichment increased the contribution of *Stipa comata* to aboveground production of shortgrass steppe but did not affect growth of the other two dominant grasses (Morgan *et al*., 2004). Similarly, elevated CO_2 altered proportional contributions of dominants to community biomass of calcareous grassland by differentially stimulating production among species, rather than by also causing negative biomass responses in some species (Leadley *et al*., 1999). In only one grassland study of which we are aware did CO_2 enrichment cause strong opposing responses among species or species groups. CO_2 enrichment favored perennial forbs at the expense of the dominant grass species in C_3/C_4 grassland, apparently by increasing soil water content (Polley *et al*., 2003).

Despite findings that higher CO_2 increases soil water content and changes species abundances in grasslands, there is surprisingly little evidence that CO_2 enrichment can increase soil water content sufficiently to cause opposing changes in the production of species or functional groups that co-exist as dominant members of the plant community at the current CO_2 level. That is, there is little evidence that CO_2 enrichment elicits the type and magnitude of directional change in composition that may be observed in plant communities along regional gradients of precipitation.

Several factors affect soil water content and its availability to plants, and thus potentially determine how plant communities respond to the water-savings effect of CO_2 enrichment. Soil texture is among the most important of these factors at both local and regional scales. Water-holding capacity is greater and water loss to drainage below the rooting zone is smaller in fine- than coarse-textured soils. As a result, we anticipate that the water-saving effect of CO_2 on vegetation generally will be smaller in fine- than coarse-textured soils.

We measured soil water content and the contributions of perennial forb and grass species to aboveground biomass production of tallgrass prairie assemblages during the first four growing seasons of exposure to a pre-industrial to elevated gradient in CO_2 concentration. Mixtures of perennial species were grown on three soils of different texture along the field CO_2 gradient (250–500 μmol mol^{-1}). Our objective was to determine whether the effects of CO_2 enrichment on species contributions to aboveground production of prairie communities could be explained by accompanying changes in soil water content. Specifically, we hypothesized that (1) CO_2 enrichment would increase the relative contribution of C_3 perennial forbs at the expense of C_4 perennial grasses and of tall-grasses at the expense of mid-grass species by increasing soil water content, and (2) opposing changes in the contributions of different growth forms or species to production along the CO_2 gradient would be greater in coarse- than fine-textured soils. We predicted that CO_2 enrichment from subambient to elevated concentrations would elicit opposing changes in growth forms or species, as observed previously in C_3/C_4 grassland (Polley *et al*., 2003), by reducing the frequency with which soil water content fell below threshold levels of water stress, rather than by simply increasing mean values of water content. We anticipated that stomatal conductance and transpiration would be particularly responsive to CO_2 at subambient concentrations (Anderson *et al*., 2001). Water availability most directly mediates vegetation dynamics when soil water falls below threshold levels that separate non-limiting from water-limiting states for one or more species. Opposing changes in species production could result from water limitation if thresholds between limiting and non-limiting states differ among species.

Material and methods

CO_2 chambers

We used unique, elongated field chambers to expose assemblages of tallgrass prairie species to a continuous gradient in CO_2 spanning pre-industrial to elevated concentrations (Polley *et al*., 2008; Fay *et al*., 2009). The Lysimeter CO_2 gradient facility is located in central Texas, USA (31°05′N, 97°20′W) and consists of two transparent, tunnel-shaped chambers each 1.2 m wide and tall and 60 m long, aligned parallel along a north-south axis. Each chamber is divided into 10 consecutive compartments each 5 m long. Aerial growth of chambered vegetation is enclosed in a transparent polyethylene film which transmits >90% of incident irradiance with minimal effects on spectral quality. Chambers depend upon photosynthesis during daylight and upon respiration at night to create CO_2 gradients. Air introduced into chambers during daylight is progressively depleted of CO_2 by photosynthesizing plants as it is moved by blowers toward the air outlet of the system. The direction of air flow is reversed at night, and respiration by the enclosed ecosystem progressively increases the CO_2 concentration of chamber air. Desired CO_2 concentration gradients are maintained by automatically varying the rate of air flow through chambers in response to changes to photosynthesis (daylight) or respiration rates (night). During daylight, pure CO_2 is injected into air introduced into the south end of one chamber (elevated chamber) to elevate the CO_2 concentration to 500 μmol mol^{-1}. The CO_2 level is depleted to

370 μmol mol^{-1} at the north end of the chamber. Ambient air is introduced into the south end of the second chamber (subambient chamber) to initiate an ambient to subambient gradient in CO_2 (370–250 μmol mol^{-1}). Night-time CO_2 concentrations are regulated at about 130 μmol mol^{-1} above daytime values along each chamber. Air temperature and vapor pressure deficit are regulated near ambient values by cooling and dehumidifying air at 5-m intervals along chambers. CO_2 treatments have been maintained each growing season from early April through mid-November since CO_2 control was initiated in 2006.

Soil monoliths

We installed four steel boxes containing intact soil monoliths (each 1 m wide and long × 1.5 m deep) beneath each of the twenty 5-m long compartments. Three soil types of contrasting physical and hydrological properties are represented. These include a Mollisol from the Austin series (Udorthentic Haplustolls), a Vertisol from the Houston Black series (Udic Haplusterts), and a sandy loam Alfisol from the Bastsil (or Bastrop) series (Udic Paleustalfs). Two monoliths of each of two soil types were randomly placed beneath each 5-m long compartment. Twelve compartments contain two monoliths each of the Mollisol and Vertisol (40–45% and 49–55% clay content, respectively; Fay *et al.*, 2009). Remaining compartments contain two monoliths of the Alfisol (60–73% sand content, 0–50 cm depth) and two of monoliths of either the Mollisol (four compartments) or Vertisol (four compartments).

Perennial species characteristic of tallgrass prairie in central Texas were transplanted into 60 monoliths in June 2003, 3 years before CO_2 treatment was initiated. Eight plants of each of four C_4 grass species [*Bouteloua curtipendula*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Tridens albescens* (Vasey) Wooton & Standl. (white tridens)] and three forb species [*Salvia azurea* Michx. Ex Lam. (pitcher sage), *Solidago canadensis* L. (Canada goldenrod), and the legume *Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald (Illinois bundleflower)] were transplanted into each monolith (total density = 56 plants m^{-2}; hereafter referenced by genus). Monoliths received ambient precipitation prior to CO_2 treatment, with two exceptions. We irrigated monoliths to maintain soil water content near field capacity during the summer following planting (2003) and added 150 mm of water to each monolith during the dry summer of 2005. Precipitation during 2005 was 71% of the 97-year mean for the site (878 mm). We excluded precipitation after initiating CO_2 control in 2006 and irrigated each monolith to simulate the average of growing season precipitation in central Texas (560 mm). The seasonal timing of water input was varied among years, however. Water input was scheduled to mimic the typical seasonal pattern of precipitation in central Texas during 2007 and 2009, with a maximum during spring and minimum during late summer (August, September). This seasonal pattern of water input was reversed in 2008 by adding larger amounts of water in summer than spring. The seasonal total of irrigation was applied in equal monthly increments during 2006.

We measured volumetric soil water content weekly (biweekly in 2006) in the center of each monolith with a neutron probe. Neutron attenuation was measured at 0.2- to 0.3-m increments to 0.9 m depth. We measured xylem pressure potentials (Ψ) on leaf blades of one of the dominant grasses (*Sorghastrum*) with a pressure chamber (Model 3005; Soil Moisture Equipment, Golita, CA, USA). Leaves were accessed through zippered openings in the polyethylene coverings of 5 m compartments, excised, and immediately placed in the pressure chamber. Xylem potentials were measured every 2 weeks at mid-day (1100–1400 Central Standard Time) during the 2006, 2007, and 2008 seasons. For each soil type, we measured the Ψ of each of two leaves at eight CO_2 concentrations along the gradient.

Aboveground biomass production was determined by clipping vegetation in each monolith to 5 cm height at the end of each growing season (November). At harvest, plants from each monolith were sorted by species. Harvested tissues were weighed after oven drying for 72 h at 60 °C. In order to minimize effects of harvesting on element cycling, plant material that was removed in November was returned to the soil surface of the monolith from which it was harvested in January. Plant material was shredded with a wood chipper to simulate effects of late-season mowing before it was returned.

We used between-year change in the proportional contribution of dominant species to aboveground production as an index of vegetation change. Change was calculated for each monolith by subtracting the dominant's contribution to production in 1 year from its contribution to production in the following year. In order to assess the possible role of 'threshold' levels of soil water content in vegetation change, we calculated a water limitation threshold (Θ_{WLT}) for each soil. The Θ_{WLT} is defined as the soil-specific content of water in the 50–90 cm depth increment at which the value of the mid-day Ψ of *Sorghastrum*, averaged across CO_2 treatments and years, was ≤ -1.85 MPa. At this Ψ, leaf net assimilation rates of *Sorghastrum*, the dominant grass in our experiment, are about 20% of those measured at leaf water potentials >-0.50 MPa (Polley *et al.*, 1992). The Ψ of *Sorghastrum* was more highly correlated with water content over the 50–90 cm depth increment of each soil type than with water content over the 0–50 cm increment. The Θ_{WLT} equaled 9.5, 11.5, and 14.5 cm^3 H_2O per cm^3 soil for the Alfisol, Mollisol, and Vertisol soils, respectively. For each year and soil, we then calculated the fraction of weekly measurements of water content in the 50–90 cm depth that were smaller than Θ_{WLT} ($f\Theta_{WLT}$).

We used the linear relationship between CO_2 concentration during daylight and distance along chambers (Fay *et al.*, 2009) to calculate the mean CO_2 concentration to which each monolith was exposed. These values of CO_2 were used as the independent variable in regression analyses in which soil water content, Ψ, or between-year changes in either biomass production of dominant species or the contribution of dominants to community production were dependent variables. CO_2 concentration was correlated with other potential predictor variables. We distinguished the unique contribution of CO_2 to variation in dependent variables by using the residuals from a regression of CO_2 on the other predictor variable as the independent variable in regression analysis.

Results

Aboveground production

Aboveground biomass production of prairie assemblages differed among soils and years. Aboveground production was 31% lower on the Mollisol (316 g m^{-2}) than Alfisol and Vertisol soils (461 and 459 g m^{-2}, respectively) when averaged across all monoliths and for the pre-treatment year and 4 years of CO_2 treatment (Fig. S1). Aboveground production was greater on all soils in 2008 and 2009 than during the pre-treatment year of 2005 and first 2 years of CO_2 exposure. Aboveground production, averaged across all monoliths of a given soil type, increased by 46%, 32%, and 36% for the Mollisol, Alfisol, and Vertisol soils, respectively, from 2007 to 2008. The sharp increase in production in 2008 resulted from an increase in the production of C_4 grasses. Production of forbs declined from 2006 through 2009 on all soils. Forb production was greatest on the Vertisol.

The grass contribution to aboveground production increased with time on all soils (Fig. 1). The fractional contribution of C_4 grasses to end-of-season biomass increased, on average, from 0.71, 0.80, and 0.66 on the Mollisol, Alfisol, and Vertisol soils in 2005 prior to CO_2 treatment to 0.87, 0.93, and 0.81, respectively, following the fourth year of CO_2 exposure.

The increase in grass dominance was not expressed equally at subambient and elevated CO_2 concentrations on all soils, however. The contribution of grasses to total production was a negative linear function of CO_2 concentration by the end of 2009 for the Mollisol (Fig. 2). The grass contribution declined from 0.96 to 0.81 and, consequently, the forb contribution rose from 0.04 to 0.19 as CO_2 increased from 265 to 500 μmol mol^{-1} on the Mollisol. There was no correlation between the grass fraction of production and CO_2 in 2008 or 2009 for the Alfisol ($P = 0.18$ and 0.45, respectively) or Vertisol ($P = 0.49$ and 0.48, respectively).

Species relative abundances

Mollisol. The identity of the dominant grass and temporal trends in grass production differed among soils and along the CO_2 gradient. CO_2 effects on species relative abundances were most strongly expressed on the Mollisol. Aboveground production was similar among the three dominant grasses, *Bouteloua*, *Schizachyrium*, and *Sorghastrum*, following the pre-treatment year of 2005 (not shown). Production of *Bouteloua* almost doubled during the first year of CO_2 treatment, increasing from an average of 60.8–114.9 g m^{-2} (Fig. S2), but did not respond to CO_2 (Table S1). Across CO_2 treatments, *Bouteloua* remained the dominant grass on the Mollisol during subsequent years. Beginning in 2007, the contribution of *Bouteloua* to production of assemblages declined dramatically at elevated CO_2 mostly because *Sorghastrum* production increased by an average of 269 g m^{-2} per 100 μmol mol^{-1} increase in CO_2 (Table S1, $n = 24$). This trend for CO_2 enrichment to favor *Sorghastrum* over *Bouteloua* was reinforced in 2008 when *Sorghastrum* production rose sharply at elevated CO_2 even as *Bouteloua* production declined at elevated CO_2 and increased at subambient CO_2 (Fig. 3a, Table S1). These changes reinforced a strong tradeoff in species relative abundances along the CO_2 gradient on the Mollisol. This tradeoff in species abundances was even stronger in 2009. As estimated by regressions developed using data averaged for 2008 and 2009, the *Bouteloua* fraction decreased from 0.75 to 0.15 as CO_2 increased from 280 to 480 μmol mol^{-1},

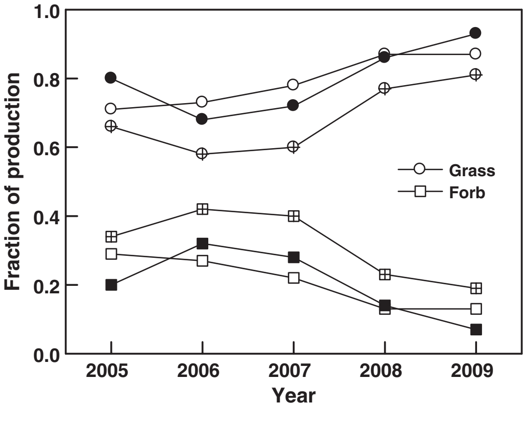

Fig. 1 Contributions of perennial C_4 grasses (circles) and C_3 forbs (squares) to aboveground production of tallgrass prairie vegetation on each of three soil types (Mollisol, open symbols; Alfisol, closed symbols; Vertisol, hatched symbols) during the pre-treatment year (2005) and 4 years of CO_2 exposure.

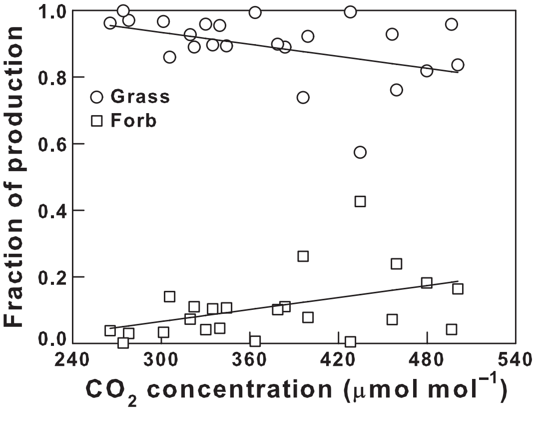

Fig. 2 Contributions of perennial C_4 grasses (circles) and C_3 forbs (squares) to aboveground production of tallgrass prairie vegetation as a function of CO_2 treatment for the Mollisol soil following the fourth year of CO_2 exposure (2009). Lines were derived using linear regression ($r^2 = 0.15$, $P = 0.04$, $n = 23$).

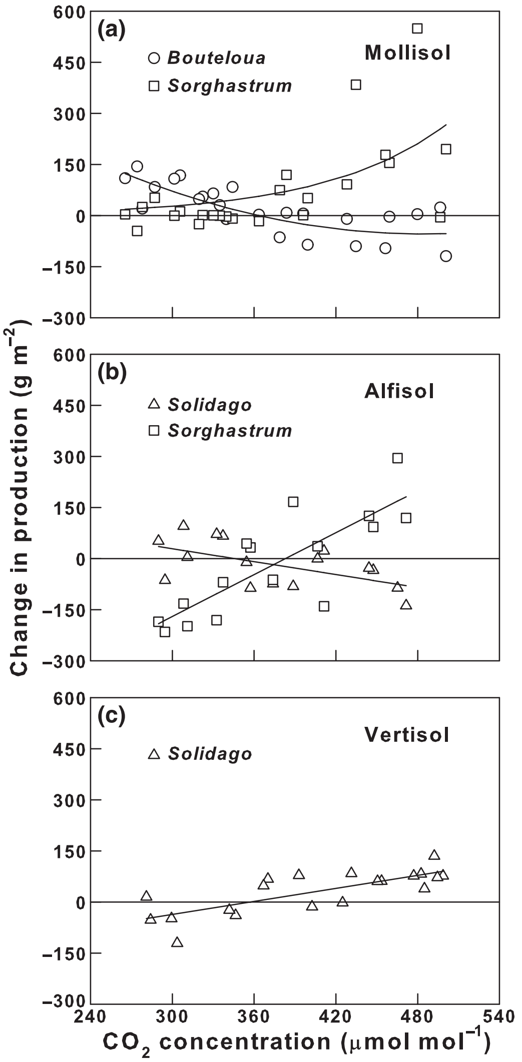

Fig. 3 Between-year changes in aboveground biomass production of dominant species grown in prairie assemblages along a subambient to elevated CO_2 gradient. Relationships between CO_2 and the 2007–2008 change in production of the C_4 perennial grasses *Bouteloua* (circles) and *Sorghastrum* (squares) on a Mollisol (a) were fit with quadratic and exponential regressions, respectively ($r^2 = 0.57$, $P < 0.0001$, $n = 24$, for each). Relationships between CO_2 and the 2006–2007 change in production of the C_4 perennial grass *Sorghastrum* (squares) and C_3 forb *Solidago* (triangles) on an Alfisol (b) and 2005–2006 change in production of *Solidago* on a Vertisol (c) were derived using linear regression ($r^2 = 0.65$, $P < 0.0001$ and $r^2 = 0.28$, $P < 0.02$, respectively, $n = 16$ and $r^2 = 0.56$, $P < 0.0001$, $n = 20$).

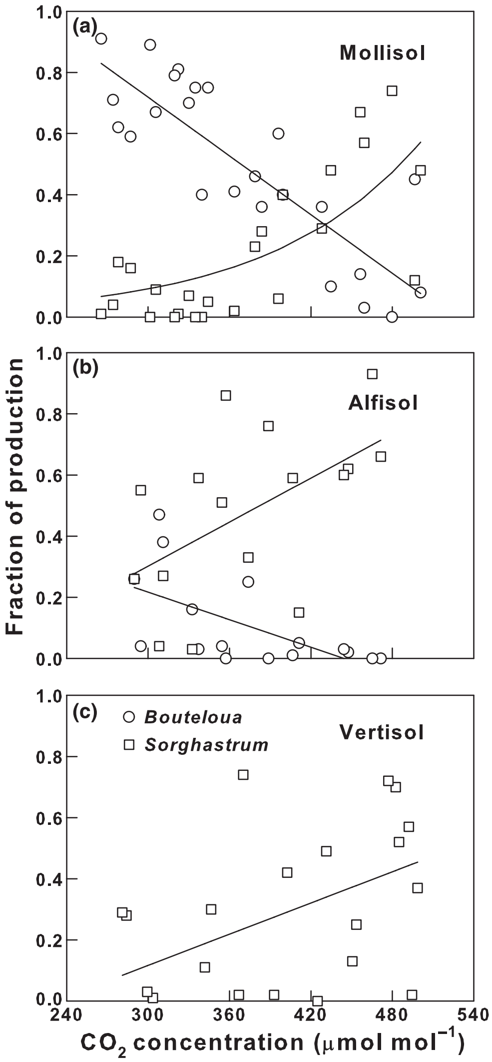

Fig. 4 The contribution of the C_4 grasses *Bouteloua* (circles) and *Sorghastrum* (squares) to aboveground production of tallgrass prairie vegetation during the third (2008) and fourth (2009) years of exposure along a subambient to elevated gradient in CO_2 concentration. Data from the 2 years were averaged at each CO_2 concentration for each species on each soil type (a–c). Lines are regression fits to averaged data for each soil type and species, *Sorghastrum* [$r^2 = 0.50$, $P < 0.0001$ (Mollisol), $r^2 = 0.24$, $P = 0.03$ (Alfisol), $r^2 = 0.21$, $P = 0.03$ (Vertisol)] and *Bouteloua* [$r^2 = 0.69$, $P < 0.0001$ (Mollisol), $r^2 = 0.32$, $P = 0.01$ (Alfisol)].

whereas the *Sorghastrum* fraction rose from 0.08 to 0.47 (Fig. 4a). *Bouteloua* and *Sorghastrum* contributed 0.45 and 0.20 proportion, respectively, of production on the Mollisol at the current ambient CO_2 concentration.

Alfisol. *Sorghastrum* was the dominant species on the Alfisol. Averaged across CO_2 treatments, *Sorghastrum* contributed 0.29–0.55 of aboveground production during 2006–2009. On average, production of *Sorghastrum* and total production of assemblages were greater at subambient than elevated CO_2 during the pre-treatment year of 2005 and first year of CO_2 treatment (Fig. S2). This pattern was reversed in 2007. *Sorghastrum* production declined at subambient CO_2 and increased at elevated CO_2. The change in *Sorghastrum* production between 2006 and 2007 was a positive linear function of CO_2 concentration, rising by >200 g m^{-2} per 100 μmol mol^{-1} increase in CO_2 (Fig. 3b). Production of the grass increased at elevated CO_2 partly at the expense of the dominant forb species, *Solidago*. The 2006 to 2007 change in *Solidago* production was a negative linear function of CO_2. *Bouteloua,* the dominant species on the Mollisol, contributed only 9% (0.09 fraction) of total production on the Alfisol across years.

Both *Sorghastrum* biomass and its contribution to total production on the Alfisol continued to rise at higher CO_2 concentrations in subsequent years (Table S1). As calculated from linear regressions, *Sorghastrum* production increased by 160, 219, and 239 g m^{-2} per 100 μmol mol^{-1} increase in CO_2 in 2007, 2008, and 2009, respectively (n = 16). The *Sorghastrum* fraction of aboveground production increased from an average of 0.25–0.75 over a 280–480 μmol mol^{-1} CO_2 gradient in 2008 and 2009 (Fig. 4b). *Bouteloua* production was negatively related to CO_2 in all years (Table S1). The *Bouteloua* fraction declined from a mean of 0.25–0.00 as CO_2 increased. *Bouteloua* and *Sorghastrum* contributed 0.11 and 0.48 proportion, respectively, of production on the Alfisol at the current CO_2 concentration.

Vertisol. CO_2 enrichment had relatively little effect on species abundances on the Vertisol compared to the other two soils. Production at subambient CO_2 averaged 87% (2006 and 2007) and 90% (2008 and 2009) of that at elevated concentrations (Fig. S2). Vegetation was co-dominated by the forb *Solidago* and two grasses *Bouteloua* and *Sorghastrum*. These three species contributed an average of 20%, 17%, and 35% of production, respectively, over the pre-treatment and four treatment years. Growth of *Solidago* and forbs as a group exhibited a strong positive correlation with CO_2 at the end of the first year of CO_2 treatment (r^2 = 0.65, P < 0.0001, n = 20; not shown). The change in *Solidago* production between the pre-treatment year (2005) and first year of CO_2 exposure (2006) was also a positive linear function of CO_2 concentration, rising by 64 g m^{-2} per 100 μmol mol^{-1} increase in CO_2 (Fig. 3c). The increase in forb growth in 2006 was followed by an equally dramatic decline in *Solidago* production at elevated CO_2 during the following year (not shown).

Of the remaining species, only *Sorghastrum* exhibited a production response to CO_2 in some years. Production of the tall-grass was a weak positive linear function of CO_2 in 2007, 2008, and 2009 (Table S1, n = 20). The *Sorghastrum* fraction of aboveground production increased from a mean of 0.08–0.42 over a 280–480 μmol mol^{-1} CO_2 gradient in 2008 and 2009 (Fig. 4c). *Bouteloua* and *Sorghastrum* contributed 0.27 and 0.26, respectively, of production on the Vertisol at the current CO_2 concentration.

Soil water content

We applied the same amount of water to each monolith each growing season but varied the timing of water input among years. Changing seasonal patterns of watering alone created large differences in temporal trends of soil water content (Fig. 5). Soil water fol-

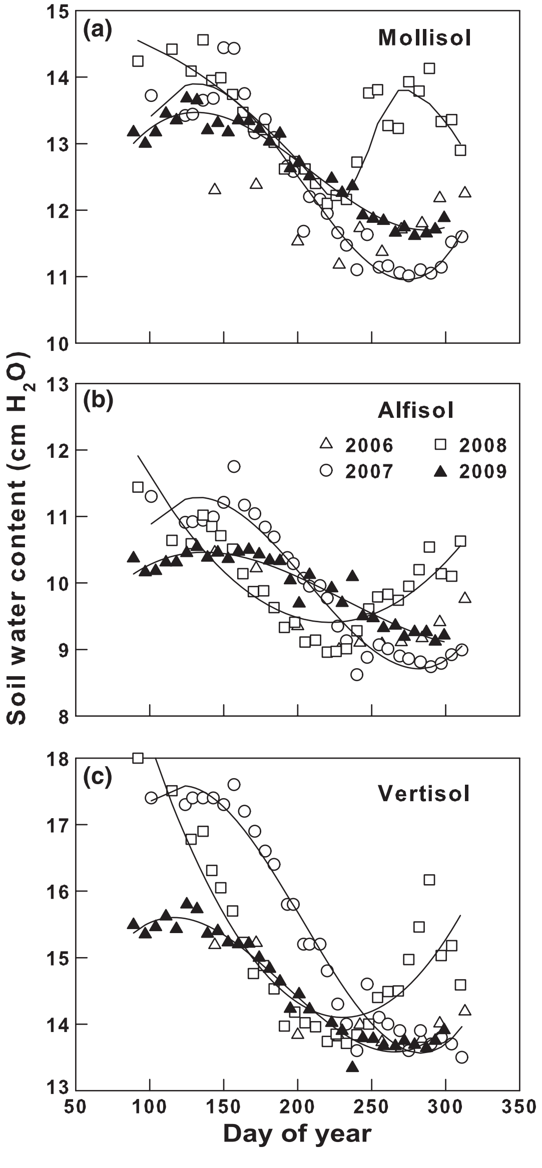

Fig. 5 Trends in soil water content (50–90 cm depth increment) during four seasons of CO_2 treatment (2006, open triangles; 2007, circles; 2008, squares; 2009, closed triangles) for each of three soil types (a–c). Measurements on each date were averaged across the subambient to elevated CO_2 gradient. Lines are regression fits to trends from 2007–2009. Note that the scale of the y-axis differs among panels.

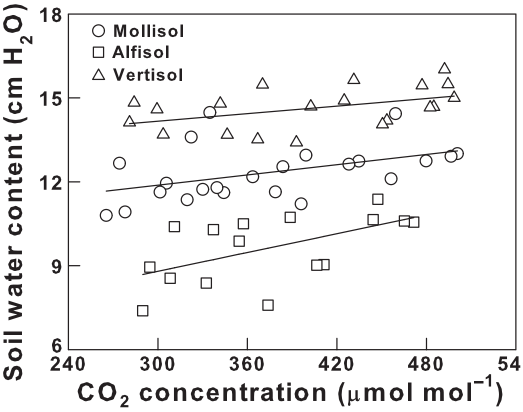

Fig. 6 Relationships between average values of soil water content (50–90 cm depth increment) and CO_2 concentration. Weekly measurements of soil water content were averaged over four growing seasons for each monolith along the CO_2 gradient. Lines are regression fits to 16–24 mean values per soil type, with slopes of 0.61, 1.12, and 0.45 cm H_2O per 100 μmol mol^{-1} increase in CO_2 for Mollisol (circles), Alfisol (squares), and Vertisol (triangles) soils (P = 0.03, 0.02, and 0.04, respectively).

lowed the seasonal pattern usually observed in central Texas during the 2 years (2007, 2009) in which irrigation was timed to mimic the typical seasonal pattern of precipitation. Soil water content was maximal during spring and declined to a minimum late in the growing season (September, October). By reversing the typical seasonal pattern of water input in 2008, we substantially increased soil water content during the latter part of the season.

CO_2 enrichment increased soil water content (Fig. 6). Slopes of soil water vs. CO_2 regressions differed significantly among soils ($F_{2,53}$ = 2.1, $P > 0.10$). Per unit of rise in CO_2, the increase in soil water content was greater by more than a factor of 1.8 and 2.4 on the Alfisol than Mollisol and Vertisol soils.

Xylem potentials

The mid-day xylem potential (Ψ) of *Sorghastrum* was positively and linearly correlated with the water content of each soil type (Fig. 7). The Ψ of *Sorghastrum* was more highly correlated with water content over the 50–90 cm depth increment of each soil type than with water content over the 0–50 cm increment (r^2 = 0.13–0.28). The increase in Ψ per unit of increase in soil water content was about 60% greater for the sandy Alfisol than clay soils. As calculated from regressions, the Ψ of *Sorghastrum* declined to −1.85 MPa at volumetric water contents of 9.5, 11.5, and 14.5 cm for the Alfisol, Mollisol, and Vertisol soils, respectively.

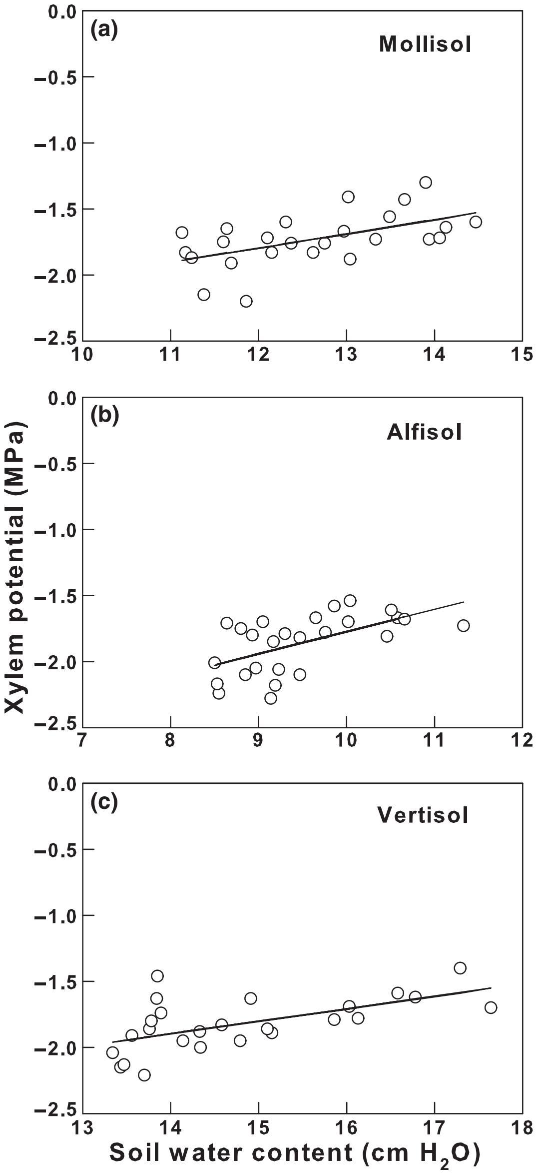

Fig. 7 Relationships between mid-day xylem potentials (Ψ) of the perennial grass *Sorghastrum* and soil water content over the 50–90 cm depth increment for each of three soil types (a–c). Xylem potentials and water content were averaged across subambient to elevated CO_2 concentrations for each of 25 measurement dates over 3 years. Lines are regression fits to mean values with slopes of 0.106, 0.168, and 0.094 MPa cm^{-1} of water for the Mollisol, Alfisol, and Vertisol soils (P = 0.004, 0.002, and 0.002, respectively). Note that the scale of the x-axis differs among panels.

Vegetation change and soil water

Mollisol. CO_2 effects on vegetation were explained largely by changes in soil water content and the frequency with which soil water dipped below threshold levels ($f\Theta_{WLT}$). Vegetation change on the Mollisol was greatest during 2007 and 2008 and was characterized by a strong decrease in *Bouteloua* and increase in *Sorghastrum* over the subambient to elevated CO_2 gradient. The 2006 to 2007 change in the proportional contribution of *Bouteloua* to production was a negative linear function of both CO_2 concentration and $f\Theta_{WLT}$ for 2007 (Fig. 8). Values of the $f\Theta_{WLT}$ decreased linearly at higher CO_2 in parallel with the 2006–2007 decrease in the contribution of *Bouteloua* to production. Change in *Bouteloua* thus was correlated with two predictor variables, CO_2 and $f\Theta_{WLT}$, which also were correlated (predictor variables were collinear). We distinguished the unique contribution of CO_2 to *Bouteloua* change by using the residuals from a regression of CO_2 concentration on $f\Theta_{WLT}$, rather than just CO_2 concentration, as the predictor variable. The statistical effect of CO_2 on the change in *Bouteloua* fraction in 2007 disappeared when we used this technique to account for collinearity between the $f\Theta_{WLT}$ and CO_2 (P = 0.14). The implication is that CO_2 enrichment limited the increase in *Bouteloua* biomass by reducing the fraction of time that soil water content fell below the stress threshold level. Change in *Bouteloua* fraction was not correlated with seasonal means of soil water content (P = 0.18). In contrast, the contribution of *Sorghastrum* to productivity decreased at subambient CO_2 and increased at elevated CO_2 between 2006 and 2007 (not shown). The 2006–2007 change in the *Sorghastrum* fraction was a positive linear function of CO_2 concentration (r^2 = 0.15, P = 0.03, n = 24; not shown), but was not correlated to either soil water content (P = 0.96) or $f\Theta_{WLT}$ (P = 0.20).

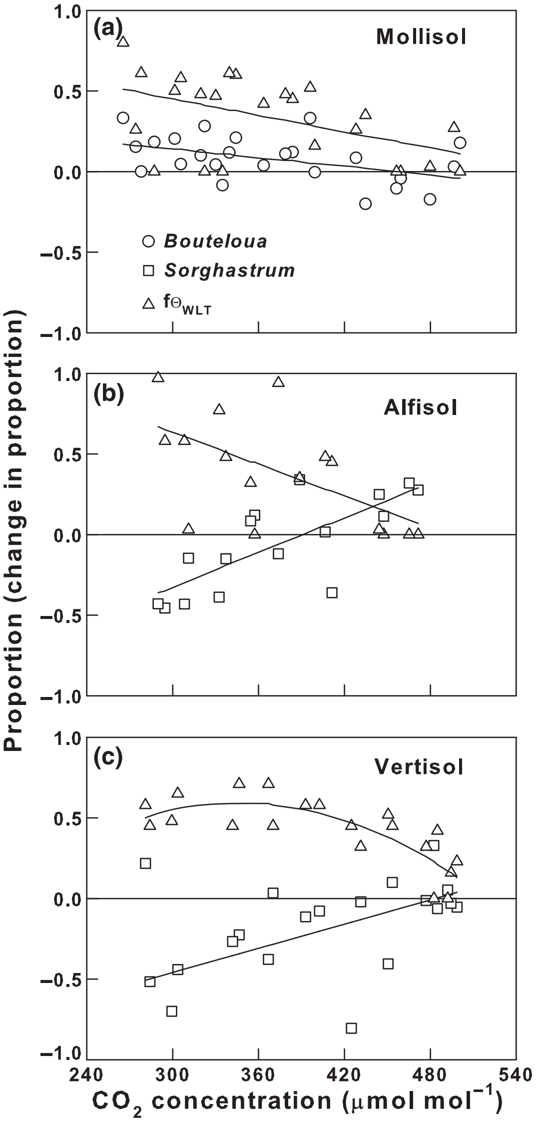

Fig. 8 Relationships between CO_2 concentration and the (1) change from 2006 to 2007 in the proportional contribution of *Bouteloua* (circles) or *Sorghastrum* (squares) to community biomass production and (2) proportion of soil water measurements for which the Ψ of *Sorghastrum* is estimated to have been ≤ -1.85 MPa ($f\Theta_{WLT}$; triangles). Lines are regression fits to the change in the proportional contribution of individual species to production of prairie assemblages (P = 0.03, 0.0006, 0.005) and the proportion of soil measurements with limiting water content (P = 0.01, 0.01, 0.06) for Mollisol, Alfisol, and Vertisol soils (a–c), respectively (n = 16–24).

The *Sorghastrum* fraction on the Mollisol soil increased sharply during 2008 at CO_2 levels >325 μmol mol^{-1}, whereas the *Bouteloua* fraction decreased at elevated CO_2 (not shown). These changes in the *Sorghastrum* and *Bouteloua* fractions were linearly correlated to both CO_2 concentration and aboveground production during the previous year (2007). Change in neither species was correlated with the $f\Theta_{WLT}$ or any of several indices of soil water content for 2008. There was no statistical effect of CO_2 on vegetation change in 2008 after accounting for collinearity between CO_2 and production (*Bouteloua*, P = 0.28; *Sorghastrum*, P = 0.13). We interpret this trend to mean that elevated CO_2 favored the tall-grass over mid-grass in 2008 by increasing productivity and possibly competition for light or other resources.

Alfisol. Vegetation change on the Alfisol was greatest during 2007. The contribution of *Sorghastrum* to production decreased at subambient CO_2 and increased at elevated CO_2 between 2006 and 2007 (Fig. 8). The change in *Sorghastrum* fraction from 2006 to 2007 was a positive linear function of CO_2 treatment and the mean of soil water content over 50–90 cm depth during the period from June through September in 2007 (r^2 = 0.47, P = 0.002, n = 16; not shown). Change in *Sorghastrum* was negatively correlated with $f\Theta_{WLT}$ (r^2 = 0.48, P = 0.002, n = 16). The *Sorghastrum* fraction increased during 2007 when fewer than 25% of weekly measurements of volumetric soil water content (50–90 cm depth) fell to <9.5 cm H_2O, but decreased when water limitation occurred more frequently. We found no statistical effect of CO_2 on the 2006–2007 change in *Sorghastrum* after accounting for collinearity between CO_2 and either the June–September mean of soil water content ($P = 0.12$) or $f\Theta_{WLT}$ ($P = 0.11$). These results imply that the *Sorghastrum* fraction increased at elevated CO_2 because CO_2 enrichment increased soil water content and reduced the frequency of water limitation.

Vertisol. Vegetation change on the Vertisol was best expressed during 2007 and resulted largely from the change in *Sorghastrum*. The 2006–2007 change in *Sorghastrum* fraction was a positive linear function of CO_2 treatment and a negative function of $f\Theta_{WLT}$ (Fig. 8). The $f\Theta_{WLT}$ increased to a maximum of about 0.6 at 340 μmol mol^{-1} then decreased at higher CO_2 concentrations. Change in *Bouteloua* fraction was not correlated to seasonal means of soil water content ($P = 0.35$). There was no statistical effect of CO_2 on the 2006–2007 change in *Sorghastrum* fraction after accounting for collinearity between CO_2 and $f\Theta_{WLT}$ ($P = 0.49$). CO_2 enrichment reduced the decline in *Sorghastrum*'s contribution to production in 2007, apparently by reducing the frequency of significant water limitation.

Discussion

We predicted that CO_2 enrichment would increase the contribution of C_3 perennial forbs to aboveground production of tallgrass prairie assemblages and favor tallgrass over mid-grass species by increasing soil water content. Increasing CO_2 from subambient to elevated concentrations favored forbs over C_4 grasses on only one of three soil types, a Mollisol. Grass production and the C_4 grass fraction of production increased dramatically across the CO_2 gradient on all soils, even as forb production declined. The prediction that CO_2 enrichment would favor taller grasses was realized for all soils, albeit to varying degrees. Aboveground production of the tallgrass *Sorghastrum* and the *Sorghastrum* fraction of production increased dramatically at elevated CO_2 on the Mollilsol and Alfisol, the two most coarse-textured of the soils studied. The increase in *Sorghastrum* at elevated CO_2 was accompanied by an offsetting and equally dramatic decline in production of the mid-grass *Bouteloua* that was most apparent on the Mollisol. Elevated CO_2 favored the tall-grass over mid-grass on the Mollisol apparently by intensifying competition for light or other resources. By contrast, CO_2 enrichment favored *Sorghastrum* over *Bouteloua* (Alfisol) or reduced the decline in *Sorghastrum* (Vertisol) largely by increasing soil water content and reducing the frequency with which soil water dipped below threshold levels at which mid-day Ψ of *Sorghastrum* reached -1.85 MPa. Increasing CO_2 by 250 μmol mol^{-1} above the pre-industrial concentration thus led to a shift in the composition of established vegetation that is similar in magnitude to vegetation differences observed between mid-grass and tallgrass prairies along a precipitation gradient in the central USA.

Contrary to the trend typically observed (Potvin & Vasseur, 1997; Leadley *et al.*, 1999; Niklaus *et al.*, 2001; Teyssonneyre *et al.*, 2002), CO_2 enrichment did not increase the ratio of forb to grass production (C_3/C_4 production) on two of three soil types. Grass production and the grass fraction increased with time on all soils and across CO_2 concentrations. In a well-watered pot experiment in which the CO_2 effect on soil water was reduced, Dijkstra *et al.* (2010) observed increased C_3 grass production at elevated CO_2, increased C_4 grass production with increased water, and mixed or no forb response. In our experiment, grass production rose sharply during 2008 when we reversed the typical seasonal pattern of water input by adding larger amounts of water in summer than in spring. The grass fraction of production increased from 0.72 to 0.86 and from 0.60 to 0.77 during 2008 on the Alfisol and Vertisol soils, for example. Skinner *et al.* (2002) found that summer irrigation increased the C_4 fraction of biomass in Wyoming grassland. Others also have noted that contribution of C_4 species to community production is positively correlated with the proportion or magnitude of precipitation during summer (Paruelo & Lauenroth, 1996; Von Fischer *et al.*, 2008). Forbs in our system may not have responded to elevated CO_2 because the primary benefit of CO_2 enrichment, improved water relations in mid- to late season, occurred following the period of peak growth for C_3 species while C_4 grasses were still active. Forbs in tallgrass prairie in Kansas, USA also responded little to elevated CO_2 (Owensby *et al.* 1999), perhaps for the same reason.

CO_2 enrichment changed relative abundances of C_4 grasses by slowing transpiration (Polley *et al.*, 2008) and increasing soil water content. Similarly, Yang *et al.* (2011) found that warming and water addition affected plant diversity in temperate steppe largely by changing soil water availability. The predominant shift in vegetation in our experiment, the large increase in the contribution of *Sorghastrum* to production at elevated CO_2, was most strongly expressed on the two soils on which the per-unit effect of CO_2 on water content was greatest (Mollisol, Alfisol). On these relatively coarse-textured soils, the increase in the tall-grass fraction was offset by a decline in the mid-grass fraction. This tradeoff between *Bouteloua* and *Sorghastrum* was most dramatic for the soil (Mollisol) on which *Bouteloua* production was greatest. More commonly, elevated CO_2 alters vegetation by differentially stimulating production among species, rather than by also inducing negative biomass responses among some dominant species (Leadley *et al.*, 1999; Morgan *et al.*, 2004). Species and community dynamics are mediated by the availability of plant-essential resources, such as water and N. Large changes in resource availability, whether caused by CO_2 enrichment, environmental variation, or other factors, may accentuate differences among species and lead to opposing changes in species abundances. For example, elevated CO_2 increased the density of an invasive annual grass, red brome (*Bromus madritensis* ssp. *rubens*), at the expense of total density of native species of annuals in a Mojave Desert community during a wet year (Smith *et al.*, 2000).

CO_2 enrichment led to opposing changes in the contributions of tall-grass and mid-grass species to community biomass partly by reducing the frequency with which soil water content fell below threshold levels of stress ($f\Theta_{WLT}$). *Sorghastrum's* contribution to production on each soil type decreased from 1 year to the next when soil water content dropped below the threshold level for >45% of the growing season. We envision the quantity of water in the rooting zone of plants as having both upper and lower stress thresholds for plant processes that differ among species, as suggested by the 'soil water bucket' model (Gordon & Famiglietti, 2004). When soil water availability is neither limiting nor excessive, plants that grow quickly or to greater height will usurp soil resources and light, and competitively displace plants that grow less rapidly. At these intermediate levels of water availability, community dynamics are determined by competition for light, soil N, or other resources. Indeed, our analysis implies that CO_2 enrichment favored the tall grass over mid-grass on the Mollisol in 2008 by increasing competition for light or other resources. Dijkstra *et al.* (2010) found that competitive winners and losers in five-species mixtures differed between elevated CO_2 and high-water treatments, implying that species responses to CO_2 were determined by competition for resources other than soil water in this relatively well-watered pot experiment. Water availability more directly mediates vegetation dynamics when soil water content falls below threshold levels that separate non-limiting from water-limiting states for one or more species. Species may exhibit opposing changes in production if threshold levels between limiting and non-limiting states differ among species. As used here, the concept of water stress thresholds implies a non-linear response of one or more species to a decrease in soil water availability. As illustrated in our data, the existence of thresholds implies that vegetation change cannot adequately be described by changes in means of soil water content alone.

The concept of water stress thresholds is of considerable heuristic value (e.g., Knapp *et al.*, 2008), but stress thresholds have proven difficult to quantify. We used the soil water content at which Ψ of a dominant grass declined to -1.85 MPa as the water stress threshold for vegetation change (Θ_{WLT}). Despite its utility in demonstrating a role of thresholds in vegetation change in this experiment, the Θ_{WLT} has limitations that, lacking sufficient data, could not be addressed. For example, the Θ_{WLT} was derived using plant and soil measurements averaged across CO_2 concentrations. Plants may respond differently to a given soil water content or potential when grown at different CO_2 concentrations (e.g., Polley *et al.*, 1996).

Increasing CO_2 from subambient to elevated concentrations led to vegetation change that is similar in character and magnitude to that observed along a west-east gradient of increasing precipitation in central Texas, USA, from Rolling Plains to Blackland Prairie. Grasslands in the Rolling Plains region in north-central to western Texas are the southern extension of mid-grass prairies of the Great Plains, whereas grasslands in the Blackland Prairie region in east-central Texas are southern extensions of the true prairie (Diamond, 2011). Important grasses in the Rolling Plains include *B. curtipendula*, *Sc. scoparium*, and *Hilaria belangeri*. Dominant grasses in the Blackland Prairie include *Sc. scoparium*, *S. nutans*, and *Sporobolus asper*. Mean annual precipitation increases from 605 to 945 mm along a west to east transect between cities located near the center of the Rolling Plains (Abilene) and Blackland Prairie (Hillsboro) regions of Texas.

Increasing CO_2 by 250 $\mu mol\ mol^{-1}$ above the preindustrial concentration thus resulted in a shift in the composition of established vegetation that is similar in character and magnitude to the change in vegetation observed along a geographic gradient between midgrass and tallgrass prairies. Our results imply that atmospheric CO_2 enrichment may alter the composition and even structure of grassland vegetation by reducing water limitation to plants.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Aboveground biomass production of perennial grasses (open circles) and forbs (open squares) grown in tallgrass prairie assemblages and of the two growth forms combined (total; closed circles) averaged across a subambient to elevated CO_2 gradient. Mean values (±SE) of production are shown for each of three soil types (a–c) during the pre-treatment year (2005) and 4 years of CO_2 exposure.

Figure S2. Aboveground biomass production of tallgrass prairie assemblages (total; circles) and of dominant grass species [*Bouteloua* (a) or *Sorghastrum* (b, c); squares] averaged across subambient (sub; open symbols) or elevated CO_2 levels (ele; closed symbols). Means (±SE) of production are shown for each of three soil types (a–c) during the pretreatment year (2005) and 4 years of CO_2 treatment.

Table S1. Statistically-significant regression analyses relating aboveground biomass production of dominant C_4 grasses, *Bouteloua curtipendula* and *Sorghastrum nutans*, to growth CO_2 concentration. Linear ($y = ax + b$), quadratic ($y = ax + cx^2 + b$), or exponential ($y = b \times e^{ax}$) functions were fit to relationships between aboveground production (y; g biomass m^{-2}) and CO_2 concentration (x; 250–500 μmol mol^{-1}) for the years 2006 through 2009. There were no significant relationships between production and CO_2 for the Mollisol or Vertisol soils in 2006 (P = 0.66–0.97) or for *Bouteloua* on the Vertisol (P = 0.53–0.74).