Increasing CO_2 from subambient to superambient concentrations alters species composition and increases above-ground biomass in a C_3/C_4 grassland

H. Wayne Polley[1], Hyrum B. Johnson[1] and Justin D. Derner[2]

[1]Grassland, Soil and Water Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Temple, TX 76502, USA; [2]High Plains Grasslands Research Station, U.S. Department of Agriculture, Agricultural Research Service, Cheyenne, WY 82009, USA

Author for correspondence:
Wayne Polley
Tel: +1 254 770 6629
Fax: +1 254 770 6561
Email: wpolley@spa.ars.usda.gov

Received: *01 May 2003*
Accepted: *20 July 2003*
doi: 10.1046/j.1469-8137.2003.00897.x

Summary

- The glacial-to-present increase in atmospheric CO_2 concentration is likely to have stimulated plant production, but experimental tests in natural ecosystems are lacking.
- We measured above-ground biomass production, plant nitrogen (N) accumulation, and species dynamics in a C_3/C_4 grassland exposed for 4 yr (1997–2000) to a continuous gradient in CO_2 from 200–560 µmol mol^{-1}.
- Biomass increased with CO_2 concentration in 1997–99. Biomass increases ranged between 121 and 161 g m^{-2} per 100 µmol mol^{-1} rise in CO_2 and were similar at subambient and superambient concentrations. Biomass responses to CO_2 were determined by different species or functional groups of species during different years. Increasing CO_2 accelerated a successional shift initiated by release from grazing in which C_3 forbs increased at the expense of a C_4 grass. Effects of CO_2 on tissue N concentration varied among species and functional groups, but CO_2 did not alter total N in above-ground tissues.
- Results imply that rising CO_2 has stimulated plant production and accelerated successional change and that grasslands will remain sensitive to rising CO_2 for several decades.

Key words: biomass production, C_3 species, C_4 grasses, CO_2 concentration, grassland, nitrogen concentration, nitrogen use efficiency, species composition.

Introduction

Atmospheric CO_2 concentration has nearly doubled since the last glaciation (from < 200 µmol mol^{-1}; Petit *et al.*, 1999) and may reach 550 µmol mol^{-1} during the next 50 yr (Alcamo *et al.*, 1996). Biological processes are fueled by carbon derived from CO_2, so the ongoing increase in CO_2 concentration almost surely has enhanced plant productivity and affected ecosystem processes. Indeed, the large proportional increase in CO_2 since glaciation has been implicated in a variety of changes including shifts in vegetation (Johnson *et al.*, 1993; Ehleringer *et al.*, 1997; Street-Perrott *et al.*, 1997), stimulation of forest production and tree turnover rates (Phillips & Gentry, 1994), and an increase in soil carbon sequestration (Gill *et al.*, 2002). Yet, direct evidence from intact ecosystems for most of these trends is lacking.

Most experiments at subambient CO_2 have been conducted with simplified plant communities under conditions favorable for plant growth (Baker *et al.*, 1990; Allen *et al.*, 1991; Dippery *et al.*, 1995). In more natural ecosystems, CO_2 effects are influenced by plant community composition and plant accumulation and utilization of N and other limiting elements. Effects of CO_2 enrichment on production may be small in communities that are dominated by C_4 species (Owensby *et al.*, 1999), but atmospheric change also may increase the abundance of species that are responsive to CO_2 (Stöcklin *et al.*, 1998; Niklaus *et al.*, 2001). Nitrogen limitation, by contrast, usually dampens plant sensitivity to CO_2 enrichment (Poorter & Pérez-Soba, 2001).

We exposed a C_3/C_4 grassland in central Texas, USA to a 200–560 µmol mol^{-1} gradient in CO_2 concentration for 4 yr following the exclusion of cattle to determine effects of prehistorical to predicted CO_2 levels on grassland productivity. Because primary production and its response to CO_2 are sensitive to plant N accumulation and species composition and dynamics, we measured N in above-ground tissues of dominant species and functional groups of plant species and assessed relationships between species dynamics and CO_2. Results from mostly greenhouse and growth chamber experiments indicate that plant growth is highly responsive at subambient CO_2 concentrations (Baker *et al.*, 1990; Allen *et al.*, 1991; Dippery *et al.*, 1995; Grünzweig & Körner, 2001) and that C_3 species are favored over C_4 species by increases in CO_2 at subambient concentrations (Johnson *et al.*, 1993). Consequently, we predicted that CO_2 enrichment would increase C_3 contribution to biomass production and consistently stimulate grassland biomass at subambient concentrations. Resource-based models of community composition predict that the species composition of plots differing in resource supply should become less similar or should diverge with time (Inouye & Tilman, 1988). We predicted therefore that the species composition of grassland plots exposed to different CO_2 concentrations would become increasingly dissimilar and that the magnitude of divergence in composition would increase with differences in CO_2 treatment. Consistent with results from other studies (Owensby *et al.*, 1993; Morgan *et al.*, 2001), we predicted that tissue N concentration would decline and that plant nitrogen use efficiency would increase as CO_2 concentration increased.

Materials and Methods

CO_2 chambers / Research site

We studied effects of atmospheric CO_2 enrichment on a C_3/ C_4 grassland in central Texas, USA (31°05′-N, 97°20′-W) with elongated field chambers that control CO_2 along continuous gradients from subambient to superambient concentrations (Johnson *et al.*, 2000). The site previously was cultivated, but has been managed as grassland and grazed by cattle for at least 50 yr. Cattle were excluded in 1992 before construction of chambers. Soils at the study site are classified as fine-silty, carbonatic, thermic Udorthentic Haplustolls. The surface 0.4 m of soil is composed mostly (55%) of clay. Annual precipitation at the site averages 879 mm (89-years average). Annual rainfall was greater than average in 1997, 1998, and 2000 (1143, 1043, and 903 mm), but was only 52% of the 89-yr mean during 1999 (461 mm).

The CO_2 facility consists of two transparent, tunnel-shaped chambers, each with 10 consecutive compartments that are 1 m wide and tall and 5 m long (Johnson *et al.*, 2000). Pure CO_2 is injected into one chamber during daylight to initiate a superambient CO_2 gradient (560–350 μmol mol^{-1}). Ambient air is introduced to the second chamber to initiate a subambient CO_2 gradient (365–200 μmol mol^{-1}). Night-time CO_2 concentrations are regulated at about 150 μmol mol^{-1} above daytime values along each chamber. Desired CO_2 concentration gradients are maintained by automatically varying the direction (daylight, night) and rate of air flow through chambers in response to changes in photosynthetic (daylight) or respiration rates (night). Air temperature and vapour pressure deficit are regulated near ambient values by cooling and dehumidifying air at 5-m intervals along chambers. Soil beneath chambers is separated from surrounding soil to a depth of 0.9 m with a rubber-coated fabric.

A continuous gradient in CO_2 from 560 to 200 μmol mol^{-1} was maintained on this grassland dominated by the C_4 perennial grass *Bothriochloa ischaemum* (L.) Keng and C_3 perennial forbs *Solanum dimidiatum* Raf. and *Ratibida columnaris* (Sims) D. Don during growing seasons (March–November) of 1997 through 2000. Johnson *et al.* (2000) described in detail regulation of CO_2 concentration and environmental parameters along chambers.

Irrigation equivalent to rainfall was applied to the chambered grassland on the day following precipitation through July 1999. We eliminated runoff when irrigating, so soil sometimes was wetter beneath than outside of chambers. To better approximate soil water content in surrounding grassland, the irrigation regime was altered in August 1999. Subsequently, the amount of water applied to the entire system was determined weekly by subtracting the water content of soil in the chamber compartment maintained at 360 μmol mol^{-1} from the mean soil water content (n = 5) measured along a 50-m-long transect in adjacent *Bothriochloa*-dominated grassland. Water addition was calculated from weekly measurements of volumetric soil water content to 1.35 m depth in the center of chamber compartments and along the unchambered transect with a neutron probe.

Sampling

Following imposition of CO_2 treatments, vegetation in the 0.5-m long and 1.0-m wide area immediately before and after each cooling coil was clipped monthly to ground level to minimize plant interference with air flow through coils, leaving 4 m^2 (4 m × 1 m) of vegetated area in each compartment for CO_2 experiments. Above-ground production was determined during each year of CO_2 treatment (1997–2000) by clipping all vegetation in the center 4-m^2 area of each chamber compartment to 5 cm height at the end of the growing season in early December. To estimate accumulation of N in actively growing above-ground tissues, we harvested two 0.5 m × 0.2 m (0.1 m^2) areas from each 4-m^2 area during June and October of each year. These harvests were timed to correspond with periods of peak biomass of early season (June) and late-season species (October) in this grassland. Harvests were systematically allocated among adjacent 0.5 m × 0.2 m quadrats that spanned the 1 m width of compartments and that were located at two positions, 1 m from the air entrance and 1 m from the air exit of each 5-m long compartment.

At each harvest, plants were sorted by species and weighed after oven drying for 72 h at 60°C. For some analyses, biomass also was calculated for each of five functional groups of species (C_4 grasses, C_3 grasses, C_3 annual forbs, C_3 perennial forbs, and legumes). To minimize effects of harvesting on element cycling, plant material that was removed in December was returned in January before the next growing season to the 1 m^2 area in compartments from which it was harvested. Before plant material was returned, it was shredded with a wood chipping machine to simulate effects of late-season mowing that commonly is applied to grasslands in central Texas.

Plant tissue (green + senescent) removed from the two 0.1 m^2 plots during June and October of each year was assigned to functional groups. Nitrogen accumulation of each functional group was estimated by multiplying the value of peak biomass for the group by the N concentration of biomass. The total of above-ground N accumulation per year for vegetation was estimated by summing these values of peak N across functional groups for each compartment along the CO_2 gradient. The nitrogen use efficiency (NUE) of vegetation was calculated by dividing above-ground biomass by N contained in live plus dead above-ground tissues (productivity per unit of N accumulation).

Photosynthetic photon flux density (light) was measured at the soil surface, at 0.5 m above the surface, and above the plant canopy in the center of each compartment. Light was measured at mid-day on single days in May and October of each year with a 1-m long sensor (SunScan, Delta-T Devices, Ltd) placed diagonally across each of two 1-m^2 plots per compartment.

Species composition

For each year, we calculated the 'similarity' in vegetation between pairs of chamber compartments using Sorensen's community coefficient (CC) weighted by biomass harvested in December (Barbour *et al.*, 1980).

Sorensen's CC = 2MC / (MA + MB),

(MA, summation of percentage of total biomass harvested from compartment A that is attributable to each species; MB, summation of percentage of total biomass harvested from compartment B that is attributable to each species; and MC, summation of minimum values of percentage of total biomass for each species common to compartments A and B.) Like other indices of community similarity, Sorensen's CC is a measurement of species shared between sampling units. Unlike the commonly used Jaccard's index as applied to weighted data, Sorensen's CC has the desirable property of ranging between 0 (complete difference) and 1 (identity). We tested for divergence in species composition along the CO_2 gradient by plotting values of Sorensen's CC calculated for all possible pairs of chamber compartments vs differences in CO_2 concentration. As an index of species shared by compartments, Sorensen's CC should decline as differences in CO_2 concentration increase if CO_2 treatments influence species composition and abundances.

Statistics

The relationship between CO_2 concentration during daylight and distance along both superambient and subambient chambers was slightly curvilinear (Johnson *et al.*, 2000). From these relationships, we calculated mean CO_2 concentration for each 5-m compartment along chambers. These CO_2 concentrations were used as the independent variable in regression analyses with biomass, N concentration and content, and parameters descriptive of species composition as dependent variables ($P < 0.05$ significance level). To accommodate a variety of possible response curves, we fit linear, hyperbolic, power, and logarithmic functions to data. The model with the greatest r^2 value was deemed the best fit. Differences among sampling years in light interception by plants were tested with single degree of freedom contrasts.

Results

Vegetation

Vegetation changed considerably during the 4 yr of this study, from dominance by C_4 grasses to codominance by C_4 grasses and C_3 perennial forbs (Fig. 1). Among individual species, *Bothriochloa* exhibited the most striking change. This C_4 grass alone comprised almost 60% of biomass harvested from chambers following the first season of CO_2 treatment, 1997. Contribution of *Bothriochloa* to end-of-season biomass declined to 24–25% in 1999 and 2000, partly because of an increase in abundances of taller perennial forbs *Ratibida columnaris*, *Solanum dimidiatum*, and *Solidago canadensis*. As abundances of forbs increased, light interception by taller plants also increased. Of total light intercepted by plants, the fraction intercepted above 0.5 m height was greater during the final 3 yr of the experiment than during the initial year of 1997 (linear contrasts, $P < 0.0001$, $n = 20$ values per year), whether measured in May (means = 0.05 and 0.24 in 1997 and across subsequent years) or October (means = 0.16 and 0.29 in 1997 and across subsequent years).

There was no relationship between Sorensen's CC, calculated using biomass per species of the total of 71 species encountered, and CO_2 during the 4 yr of this experiment (not shown). Species also were grouped by photosynthetic pathway (either C_3 or C_4) or into one of five functional groups before calculating Sorensen's CC between each pair of chamber compartments. Neither of these calculations of vegetation similarity was related to differences in CO_2 treatment (not shown). The absence of a relationship between Sorensen's CC and CO_2 across years indicates that CO_2 played little role in

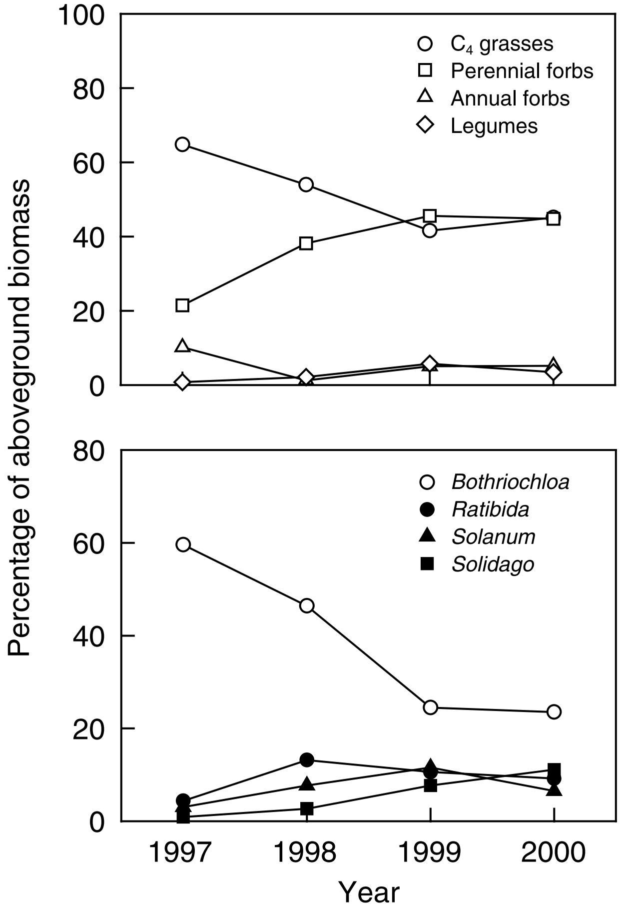

Fig. 1 Percentage contribution of each of four functional groups of species (upper panel) and of four dominant species (lower panel) to above-ground biomass harvested at the end of each season of CO_2 enrichment. Values are averages across 4-m^2 grassland plots along the CO_2 gradient (n = 20). C_3 grasses comprised < 3% of total biomass in each year, so these data were omitted to improve clarity.

species composition during the experiment. Heterogeneity in species mixtures increased with time, irrespective of CO_2 treatment. Averaged across pair-wise comparisons of chamber compartments, values of Sorensen's CC that were calculated using biomass per species declined from 0.53 in 1997 to 0.29 in 2000 (values were 0.48 in 1998 and 0.31 in 1999, n = 190).

Above-ground biomass

Averaged across CO_2 concentrations, above-ground biomass was greater during the final 3 yr of treatment (means = 1086, 962, 1131 g m^{-2} in 1998, 1999, and 2000; n = 20) than during the initial year of 1997 (mean = 737 g m^{-2}; n = 20). Above-ground biomass increased significantly from subambient to superambient CO_2 concentrations in 1997 and 1998 and increased marginally (P = 0.09) with greater CO_2 concentration in 1999 (Fig. 2). Biomass production was best described by a positive, linear function of CO_2 in 1997 and by curvilinear functions (logarithmic and hyperbolic) in 1998 and 1999. There was no relationship between above-ground biomass and CO_2 in 2000 (P = 0.20). Although there was considerable scatter in relationships between production and CO_2, mean responses of biomass to CO_2 often were dramatic. We estimate from regression, for example, that in 1997 above-ground production increased by 86% (from 510 to 949 g m^{-2}) as CO_2 rose from 210 to 550 µmol mol^{-1}. Across the full CO_2 gradient, biomass production increased by a mean of between 121 g m^{-2} (1999) and 161 g m^{-2} (1998) per 100 µmol mol^{-1} increase in CO_2 concentration.

Much of the increase in above-ground biomass during the initial year of CO_2 exposure (1997) occurred in *Bothriochloa* (Fig. 3). As estimated from regression, biomass of the dominant C_4 grass more than doubled with the 210–550 µmol mol^{-1} increase in CO_2 concentration. This increase in *Bothriochloa* biomass accounted for 75% of the stimulation of community biomass by higher CO_2 during 1997. In subsequent years, however, there was no relationship between production of the C_4 grass and CO_2 (not shown; P = 0.48, 0.60, and 0.48 in 1998, 1999, and 2000). Biomass of *Bothriochloa* declined precipitously during the 1999 season, by an average of 245 g m^{-2} across CO_2 treatments.

As biomass of the dominant grass declined, above-ground production of perennial forbs (including *Ratibida columnaris*, *Solanum dimidiatum*, and *Solidago canadensis*) increased from an average of 161 g m^{-2} in 1997 to greater than 400 g m^{-2} in subsequent years (means = 402, 441, 495 g m^{-2} in 1998, 1999, and 2000; n = 20). Biomass of these C_3 species was not related to CO_2 treatment during 1997 (P = 0.39), but increased marginally (P = 0.10) at higher CO_2 during 1998 when 2 outlier points were excluded and increased significantly with CO_2 concentration during 1999 (P = 0.016) and 2000 (P = 0.003; Fig. 3). In each of the final two years of the experiment, CO_2 effects were dramatic. Across the full CO_2 gradient, we estimate from the curvilinear functions fit to biomass-CO_2 relationships that production of perennial forbs increased by a factor of 4 in 1999 and of 7 in 2000. Indeed, forb biomass increased slightly more at higher CO_2 than did total biomass during 1999 and increased during 2000 despite the absence of a CO_2 effect on production of all species combined (Fig. 2). Clearly then, biomass of other species must have declined as CO_2 and forb biomass increased during 1999 and 2000. Much of this decline in biomass occurred in *Bothriochloa* (Fig. 4). During the final 3 yr of the experiment (1998–2000), above-ground biomass of this C_4 grass decreased more at elevated than at subambient concentrations.

Nitrogen

Nitrogen accumulation of vegetation was estimated for each year from values of peak N of 5 functional groups of species as determined from harvests of 0.1 m^2 plots. In no year did above-ground N accumulation change significantly with CO_2

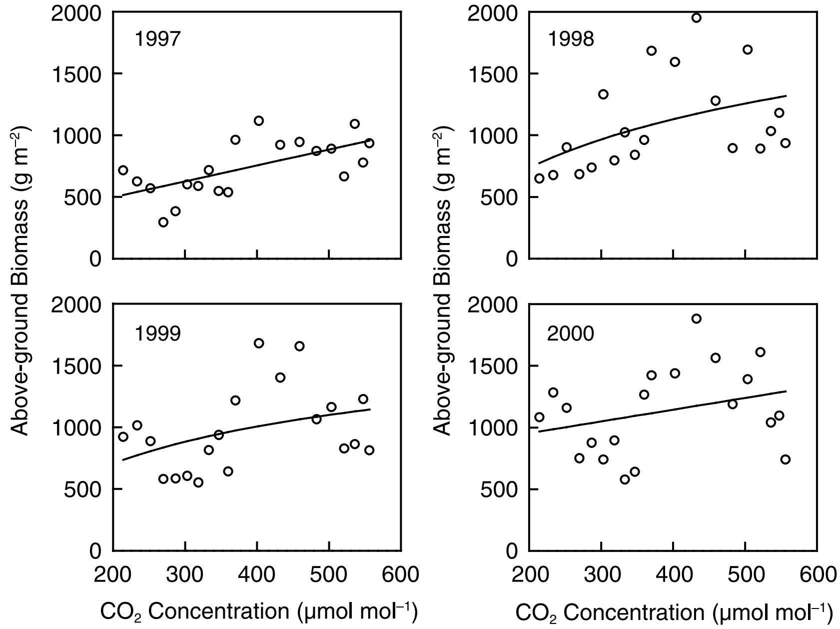

Fig. 2 Relationships between above-ground biomass and the mean daytime CO_2 concentration to which 4-m^2 grassland plots were exposed for four growing seasons ($n = 20$). Data were fit with a logarithmic function in 1998 (biomass = –2282.6 + 569.4 ∗ ln(CO_2), $r^2 = 0.20$, $P = 0.05$), with an hyperbolic function in 1999 (biomass = (1746.8 ∗ CO_2)/(294.4 + CO_2), $r^2 = 0.15$, $P = 0.09$) and with linear functions in 1997 (biomass = 239.14 + 1.29 ∗ CO_2, $r^2 = 0.41$, $P = 0.002$) and 2000 ($P = 0.20$).

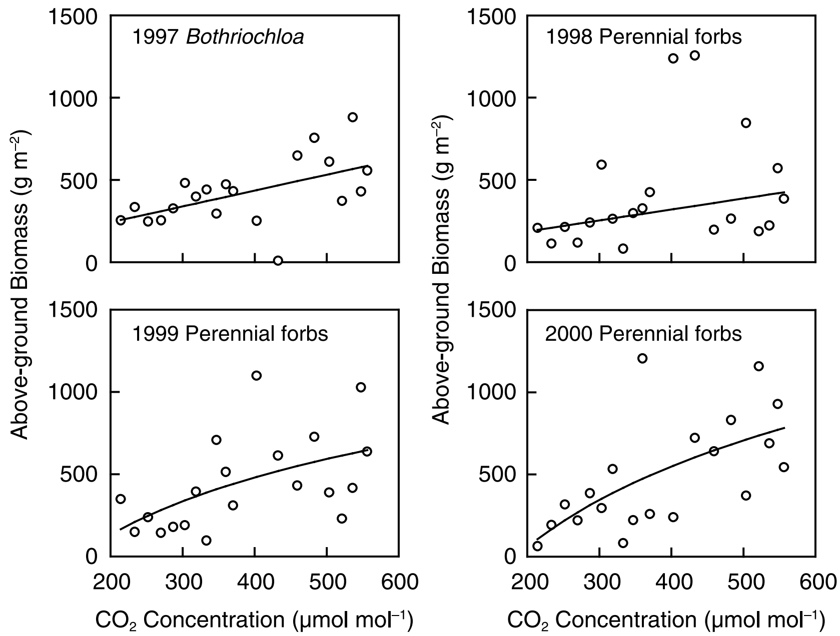

Fig. 3 Relationships between above-ground biomass production of *Bothriochloa*, the dominant C_4 grass, and of C_3 perennial forbs and the mean daytime CO_2 concentration to which 4-m^2 plots were exposed ($n = 20$). Data were fit with linear functions in 1997 (biomass = 49.44 + 0.96 ∗ CO_2, $r^2 = 0.29$, $P < 0.014$) and in 1998 following exclusion of two outlying points (biomass = 53.0 + 0.67 ∗ CO_2, $r^2 = 0.16$, $P = 0.10$, $n = 18$) and with logarithmic functions in 1999 (biomass = –2540.2 + 504.0 ∗ ln(CO_2), $r^2 = 0.28$, $P = 0.02$) and 2000 (biomass = –3695.6 + 708.4 ∗ ln(CO_2), $r^2 = 0.40$, $P = 0.003$). Biomass-CO_2 relationships were not significant for perennial forbs in 1997 ($P = 0.39$) or for *Bothriochloa* in 1998 ($P = 0.48$), 1999 ($P = 0.60$), or 2000 ($P = 0.48$). These data were omitted to improve clarity.

concentration (P ranged between 0.16 and 0.50; mean N ranged between 7.6 and 15.1 g N m^{-2}, $n = 20$).

Carbon dioxide enrichment frequently reduced tissue N concentration, but CO_2 effects varied among years and functional groups of species. As determined from regression, the N concentration of C_4 grasses (including *Bothriochloa*) declined by 27% in 1997 and 1998 as CO_2 concentration increased from 215 to 550 µmol mol^{-1} (Fig. 5). The N concentration of C_4 grasses as a group did not vary significantly with CO_2 treatment in either 1999 ($P = 0.67$) or 2000 ($P = 0.40$). Nitrogen concentration of the dominant C_4 grass *Bothriochloa* declined slightly more across subambient than superambient CO_2 concentrations in both 1998 and 1999 (Table 1), but was not significantly related to CO_2 treatment in 2000 ($P = 0.89$). Across measurements from 1998 and 1999, N concentration of perennial forbs declined more per unit increase in CO_2 over subambient than superambient concentrations (Fig. 5). The mean decrease in N concentration at subambient CO_2 was greater by more than an order of magnitude in perennial forbs than C_4 grasses. There was no relationship between N concentration of perennial forbs and CO_2 in either 1997 ($P = 0.86$) or 2000 ($P = 0.92$).

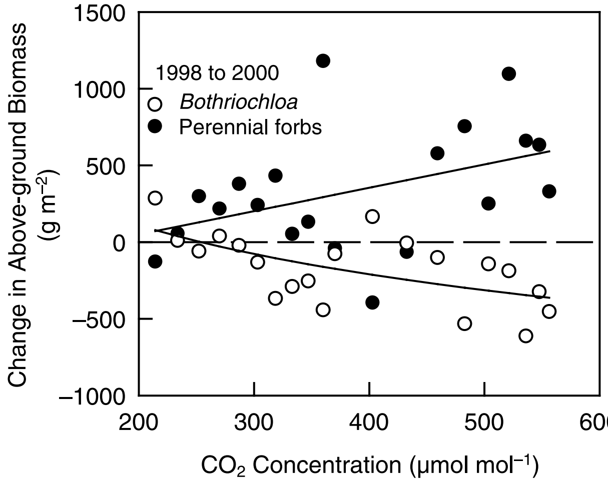

Fig. 4 Relationships between the change in above-ground biomass production of the C_4 grass *Bothriochloa* and of C_3 perennial forbs during 1998 through 2000 and the mean daytime CO_2 concentration to which 4-m^2 plots were exposed (n = 20). Data for perennial forbs were fit with a linear function (biomass = −253.58 + 1.52 ∗ CO_2, r^2 = 0.18, P = 0.06), and data for *Bothriochloa* was fit with a logarithmic function (biomass = 2562.0−462.6 ∗ ln(CO_2), r^2 = 0.35, P = 0.006).

The NUE of vegetation, defined as the ratio of above-ground biomass production to above-ground N content, increased with CO_2 concentration only in 1998 (Table 1; P = 0.36, 0.16, and 0.29 in 1997, 1999, and 2000). Although higher CO_2 frequently reduced the N concentration of dominant species, heterogeneity in species composition combined with the increased abundance of N-rich forbs at high CO_2 (Fig. 3) eliminated any CO_2 effect on NUE of the plant community during most years.

Discussion

Biomass production

Increasing CO_2 from prehistorical to predicted concentrations increased above-ground biomass of the C_3/C_4 grassland studied during the first 3 yr of the experiment, despite the absence of a CO_2 effect on plant N accumulation. Scatter in relationships between biomass and CO_2 limited our ability to define the shape of biomass-CO_2 relationships, but the average response of biomass to CO_2 in this intact grassland was similar at subambient and superambient concentrations. As expected, tissue N concentration often declined, but the decrease was not consistent across functional groups of species and varied among years for individual groups and species. Vegetation changed dramatically during the experiment. At the species level, the shift in plant composition and accompanying increase in compositional heterogeneity were not related to CO_2 treatment. Nor did CO_2 treatments change the relative contributions of C_3 and C_4 plants to biomass. But the predominant shift in vegetation during this experiment,

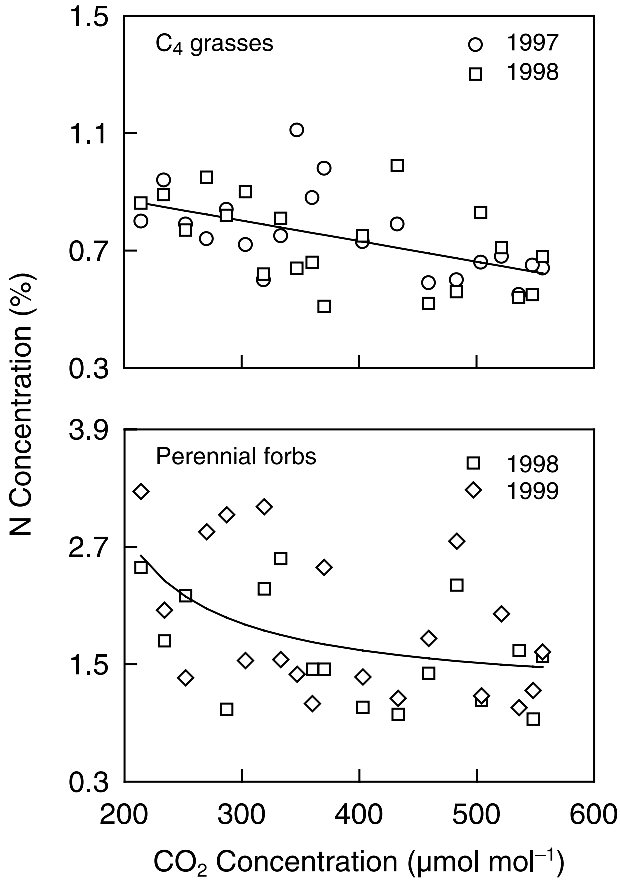

Fig. 5 The N concentration of above-ground tissues of C_4 grasses and of C_3 perennial forbs at peak biomass as a function of the CO_2 concentration at which plants were grown. Note that scale of the *y*-axis differs between upper and lower panels. Data from 1997 and 1998 for C_4 grasses were fit with a linear function ([N] = 1.0120−0.0007 ∗ CO_2, r^2 = 0.28, P = 0.0004, n = 39). Data from 1998 and 1999 for forbs were fit with a hyperbolic function ([N] = (1.15 ∗ CO_2)/(CO_2−119.29), r^2 = 0.23, P = 0.003, n = 36). Results of regression analyses on data from individual years are listed in Table 1. Nitrogen concentration did not change significantly with CO_2 concentration in 1999 or 2000 for C_4 grasses (means = 0.85% and 0.79%) or in 1997 or 2000 for perennial forbs (means = 1.45% and 1.54%).

from dominance by the C_4 grass *Bothriochloa* to codominance with C_3 perennial forbs, clearly was amplified by CO_2 enrichment.

The 350 μmol mol^{-1} increase in CO_2 concentration studied here is similar in magnitude to that employed in other CO_2 experiments on grasslands, but proportional increases in biomass during this experiment were larger than typically measured in grasslands. As calculated from regressions, for example, above-ground biomass rose by between 56% and 86% in 1997 through 1999 with the increase in CO_2 from 210 to 550 μmol mol^{-1}. Below-ground production was 50% greater at elevated than subambient concentrations (Gill *et al.*, 2002). Biomass responses to CO_2 enrichment vary from nil in alpine grassland (Schappi & Körner, 1996) to

Table 1 Results of significant regression analyses between above-ground N and CO_2 over a 200–560 µmol/mol^{-1} gradient (n = 20)

Year/ parameter	Model type	Slope or a value	Intercept or b value	r^2	P-value
1997					
[N] C_4 grasses	Linear	–0.0007	1.02	0.30	0.01
1998					
[N] C_4 grasses	Power	6.48	–0.37	0.29	0.01
[N] *Bothriochloa*	Hyperbolic	0.525	–64.00	0.14	0.05
[N] perennial forbs	Hyperbolic	1.11	–110.82	0.24	0.05
NUE	Linear	0.122	53.43	0.18	0.06
1999					
[N] *Bothriochloa*	Hyperbolic	0.83	–33.56	0.19	0.03
[N] perennial forbs	Linear	–0.003	3.09	0.21	0.04

Nitrogen use efficiency (NUE) is the ratio of above-ground biomass (determined by summing peak biomasses of 5 functional groups of species) to N content of that biomass. Linear ($y = ax + b$), hyperbolic [$y = ax/(b + x)$], or power [$y = ax^b$] functions were fit to relationships of two parameters [y; tissue N concentration (%) and NUE (g biomass g N^{-1})] to CO_2 concentration (x; µmol mol^{-1}). There were no significant relationships between N and CO_2 in 2000.

increases ranging between about 20% and 50% in annual grassland (Hungate *et al.*, 1997; Shaw *et al.*, 2002), calcareous grassland (Leadley *et al.*, 1999), tallgrass prairie (Owensby *et al.*, 1999), and shortgrass steppe (Morgan *et al.*, 2001). The rather large responses of above-ground biomass to CO_2 during initial years of this experiment apparently derived from large and usually linear increases in leaf and canopy photosynthesis (Anderson *et al.*, 2001; Mielnick *et al.*, 2001) and in leaf water use efficiency over subambient to superambient concentrations (Anderson *et al.*, 2001; Maherali *et al.*, 2002; Polley *et al.*, 2002).

Although constrained by scatter in biomass-CO_2 relationships, our results provide little evidence that above-ground biomass is more responsive to CO_2 at subambient than superambient concentrations. Biomass-CO_2 relationships were slightly curvilinear with greatest increase over subambient concentrations for the grassland community in 1998 and 1999 and for perennial forbs in 1999 and 2000. In other years, however, biomass was better described by a linear function of CO_2 concentration. Experimental evidence that plant production is more responsive to CO_2 at subambient than at superambient concentrations comes mostly from greenhouse or growth chamber studies that typically were of short duration or were conducted under conditions favorable for plant growth (Baker *et al.*, 1990; Allen *et al.*, 1991; Dippery *et al.*, 1995; Grünzweig & Körner, 2001). Conditions in natural and seminatural ecosystems frequently are more demanding of plants and may involve species change or other feedbacks between plants and the environment that dampen plant responses to CO_2 or contribute to temporal variability in CO_2 effects. In the C_3/C_4 grassland that we studied, biomass response to CO_2 apparently was influenced by one of these feedbacks, vegetation change. Nevertheless, increasing CO_2 from the pre-Industrial to current concentration (270–370 µmol/mol^{-1}) elicited a 20–22% increase in above-ground biomass during 1997 and 1998.

Carbon dioxide enrichment increased grassland production despite having no effect on N accumulation in above-ground tissues. Reduced N concentration is a common response to CO_2 enrichment (Cotrufo *et al.*, 1998; Körner, 2000). It is noteworthy therefore that in this experiment responses of N concentration to CO_2 varied among functional groups and years. Responses to CO_2 varied even within an individual species. The N concentration of *Bothriochloa*, for instance, declined as CO_2 increased during the first two years examined, but was not related to CO_2 treatment during a third year. Variability in response obviously complicates prediction and cautions against generalization.

Carbon dioxide enrichment increased biomass during the first 3 yr of this study despite temporal changes in species abundances and despite interannual differences in responses of species or groups of species to CO_2. Consequently, positive responses of biomass to CO_2 enrichment were determined by different species or by different functional groups of species during different years. Much of the increase in above-ground biomass during 1997 occurred in *Bothriochloa*. By 1999, CO_2 effects on grassland production were determined largely by the positive response of perennial forbs to CO_2.

Species composition

Species composition affects the response of plant production to CO_2 (Niklaus *et al.*, 2001; Reich *et al.*, 2001), but CO_2 enrichment, in turn, may influence the contribution of species to community biomass (Leadley *et al.*, 1999; Niklaus *et al.*, 2001). The predominant shift in vegetation during this experiment, greater production of perennial forbs at the expense of C_4 grasses, clearly was amplified by CO_2 enrichment across the full subambient to superambient gradient. Several studies have shown that CO_2 enrichment favors forbs over grasses (Potvin & Vasseur, 1997; Leadley *et al.*, 1999; Owensby *et al.*, 1999; Teyssonneyre *et al.*, 2002), although the trend is not universal (Morgan *et al.*, 2001). Superficially, this increase in forbs is consistent with a long-standing prediction that CO_2 enrichment favors C_3 over C_4 plants by preferentially stimulating photosynthesis and growth of C_3 species. But, relative abundances of C_3 and C_4 species did not respond predictably to CO_2 in this experiment, consistent with results from grassland studies at elevated CO_2 (Owensby *et al.*, 1999; Morgan *et al.*, 2001) but contrary to trends measured in salt marsh (Arp *et al.*, 1993) and in some reconstructed communities at subambient concentrations (Johnson *et al.*, 1993, but see Ward *et al.*, 1999). As evidenced by the increase in *Bothriochloa* biomass during 1997, possession of the C_4 metabolism does not preclude a CO_2 response even to elevated concentrations. Rather than simply reflecting CO_2 effects on C_3–C_4 balance, the increase in forb production probably was related to differences in plant morphology and growth habit (Bazzaz & McConnaughay, 1992), to release from grazing (Potvin & Vasseur, 1997), to differing sensitivities of grasses and of more deeply rooting forbs to droughts, or to some combination of these and other factors. Above-ground biomass of *Bothriochloa* decreased during 1998, a year with a 6-months drought during mid-season (Polley *et al.*, 2002), and declined precipitously during the drought year of 1999. Xylem potentials were more sensitive to changes in soil water in the grass than in a perennial forb (Polley *et al.*, 2002), suggesting that grasses were especially disadvantaged by drought. Perennial forbs increase in abundance following the release of *Bothriochloa*-dominated grassland from grazing (Wilsey & Polley, 2003). In this grassland as in tallgrass prairie (Owensby *et al.*, 1999), taller growth forms may eventually dominate ungrazed communities at ambient and higher CO_2 concentrations irrespective of physiological sensitivities to CO_2. That this shift from grasses to forbs was amplified as CO_2 increased indicates that CO_2 enrichment may speed successional changes in the composition of this grassland following release from grazing. Elevating CO_2 above the ambient concentration favored dicots over grasses in the pasture community studied by Potvin & Vasseur (1997), but this CO_2 effect slowed rather than accelerated successional change.

Conclusions

Our results provide the first evidence in an intact ecosystem for an increase in above-ground production and shift in plant composition over subambient to superambient CO_2 concentrations. Biomass increased despite temporal changes in the responses of biomass and tissue N to CO_2 among species and species groups. Increasing CO_2 accelerated a successional shift in vegetation from the dominant C_4 grass to perennial forbs. The continuous response to CO_2 of above-ground biomass, plant N concentration, and grass-forb relative abundances in this C_3/C_4 grassland indicates that grasslands may remain sensitive to CO_2 as concentration rises to twice the pre-Industrial level during coming decades.

Acknowledgements

Ron Whitis maintained CO_2 chambers. Anne Gibson, Katherine Jones, Chris Kolodziejczyk, and Kyle Tiner helped with harvests and processed samples, and Joaquin Sanabria contributed statistical guidance. William Dugas, Richard Gill, Robert Jackson, Hafiz Maherali, and Patricia Mielnick provided helpful comments on the manuscript.

References

Alcamo J, Kreileman GJJ, Bollen JC, van den Born GJ, Gerlagh R, Krol MS, Toet AMC, de Vries HJM. 1996. Baseline scenarios of global environmental change. *Global Environmental Change* **6**: 261–303.

Allen LH Jr, Bisbal EC, Boote KJ, Jones PH. 1991. Soybean dry matter allocation under subambient and superambient levels of carbon dioxide. *Agronomy Journal* **83**: 875–883.

Anderson LJ, Maherali H, Johnson HB, Polley HW, Jackson RB. 2001. Gas exchange and photosynthetic acclimation over subambient to elevated CO_2 in a C_3–C_4 grassland. *Global Change Biology* **7**: 693–707.

Arp WJ, Drake BG, Pockman WT, Curtis PS, Whigham DF. 1993. Interactions between C_3 and C_4 salt marsh plant species during four years of exposure to elevated atmospheric CO_2. *Vegetatio* **104/105**: 133–143.

Baker JT, Allen LH Jr, Boote KJ. 1990. Growth and yield responses of rice to carbon dioxide concentration. *Journal of Agricultural Science, Cambridge* **115**: 313–320.

Barbour MG, Burk JH, Pitts WD. 1980. *Terrestrial plant ecology*. Menlo Park, CA, USA: Benjamin/Cummings.

Bazzaz FA, McConnaughay KDM. 1992. Plant–plant interactions in elevated CO_2 environments. *Australian Journal of Botany* **40**: 547–563.

Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**: 43–54.

Dippery JK, Tissue DT, Thomas RB, Strain BR. 1995. Effects of low and elevated CO_2 on C_3 and C_4 annuals. I. Growth and biomass allocation. *Oecologia* **101**: 13–20.

Ehleringer JR, Cerling TE, Helliker BR. 1997. C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* **112**: 285–299.

Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB. 2002. Non-linear grassland responses to past and future atmospheric CO_2. *Nature* **417**: 279–282.

Grünzweig J, Körner C. 2001. Growth, water and nitrogen relations in grassland model ecosystems of the semi-arid Negev of Israel exposed to elevated CO_2. *Oecologia* **128**: 251–262.

Hungate BA, Holland EA, Jackson RB, Chapin FS III, Mooney HA, Field CB. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576–579.

Inouye RS, Tilman D. 1988. Convergence and divergence of old-field plant communities along experimental nitrogen gradients. *Ecology* **69**: 995–1004.

Johnson HB, Polley HW, Mayeux HS. 1993. Increasing CO_2 and plant–plant interactions: effects on natural vegetation. *Vegetatio* **104/105**: 157–170.

Johnson HB, Polley HW, Whitis RP. 2000. Elongated chambers for field studies across atmospheric CO_2 gradients. *Functional Ecology* **14**: 388–396.

Körner C. 2000. Biosphere responses to CO_2 enrichment. *Ecological Applications* **10**: 1590–1619.

Leadley PW, Niklaus PA, Stocker R, Körner C. 1999. A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia* **118**: 39–49.

Maherali H, Reid CD, Polley HW, Johnson HB, Jackson RB. 2002. Stomatal acclimation over a subambient to elevated CO_2 gradient in a C_3/C_4 grassland. *Plant, Cell & Environment* **25**: 557–566.

Mielnick PC, Dugas WA, Johnson HB, Polley HW, Sanabria J. 2001. Net grassland carbon flux over a subambient to superambient CO_2 gradient. *Global Change Biology* 7: 747–754.
Morgan JA, LeCain DR, Mosier AR, Milchunas DG. 2001. Elevated CO_2 enhances water relations and productivity and affects gas exchange in C_3 and C_4 grasses of the Colorado shortgrass steppe. *Global Change Biology* 7: 451–466.
Niklaus PA, Leadley PW, Schmid B, Körner C. 2001. A long-term field study on biodiversity–elevated CO_2 interactions in grassland. *Ecological Monographs* 71: 341–356.
Owensby CE, Coyne PI, Auen LM. 1993. Nitrogen and phosphorus dynamics of a tallgrass prairie ecosystem exposed to elevated carbon dioxide. *Plant, Cell & Environment* **16**: 843–850.
Owensby CE, Ham JM, Knapp AK, Auen LM. 1999. Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. *Global Change Biology* **5**: 497–506.
Petit JR, Jouzel J, Raynaud D *et al.* 1999. Climate and atmospheric history of the past 420 000 years from the Vostok ice core, Antarctica. *Nature* **399**: 429–436.
Phillips OL, Gentry AH. 1994. Increasing turnover through time in tropical forests. *Science* **263**: 954–958.
Polley HW, Johnson HB, Derner JD. 2002. Soil- and plant-water dynamics in a C_3/C_4 grassland exposed to a subambient to superambient CO_2 gradient. *Global Change Biology* **8**: 1118–1129.
Poorter H, Pérez-Soba M. 2001. The growth response of plants to elevated CO_2 under non-optimal environmental conditions. *Oecologia* **129**: 1–20.
Potvin C, Vasseur L. 1997. Long-term CO_2 enrichment of a pasture community: species richness, dominance, and succession. *Ecology* **78**: 666–677.
Reich PB, Knops J, Tilman D, Craine J, Ellsworth D, Tjoelker M, Lee T, Wedin D, Naeem S, Bahauddin D, Hendrey G, Jose S, Wrage K, Goth J, Bengston W. 2001. Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. *Nature* **410**: 809–812.
Schappi B, Körner C. 1996. Growth responses of an alpine grassland to elevated CO_2. *Oecologia* **105**: 43–52.
Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB. 2002. Grassland responses to global environmental changes suppressed by elevated CO_2. *Science* **298**: 1987–1990.
Stöcklin J, Schweizer K, Körner C. 1998. Effects of elevated CO_2 and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. *Oecologia* **116**: 50–56.
Street-Perrott FA, Huang Y, Perrott RA, Eglinton G, Barker P, Khelifa LB, Harkness DD, Olago DO. 1997. Impact of lower atmospheric carbon dioxide on tropical mountain ecosystems. *Science* **278**: 1422–1426.
Teyssonneyre F, Picon-Cochard C, Falcimagne R, Soussana J-F. 2002. Effect of elevated CO_2 and cutting frequency on plant community structure in a temperate grassland. *Global Change Biology* **8**: 1034–1046.
Ward JK, Tissue DT, Thomas RB, Strain BR. 1999. Comparative responses of model C_3 and C_4 plants to drought in low and elevated CO_2. *Global Change Biology* **5**: 857–867.
Wilsey BJ, Polley HW. 2003. Effects of seed additions and grazing history on diversity and productivity of subhumid grasslands. *Ecology* **84**: 920–931.