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CO_2 ENHANCES PRODUCTIVITY, ALTERS SPECIES COMPOSITION, AND REDUCES DIGESTIBILITY OF SHORTGRASS STEPPE VEGETATION

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Abstract. The impact of increasing atmospheric CO_2 concentrations has been studied in a number of field experiments, but little information exists on the response of semiarid rangelands to CO_2, or on the consequences for forage quality. This study was initiated to study the CO_2 response of the shortgrass steppe, an important semiarid grassland on the western edge of the North American Great Plains, used extensively for livestock grazing. The experiment was conducted for five years on native vegetation at the USDA-ARS Central Plains Experimental Range in northeastern Colorado, USA. Three perennial grasses dominate the study site, *Bouteloua gracilis*, a C_4 grass, and two C_3 grasses, *Pascopyrum smithii* and *Stipa comata*. The three species comprise 88% of the aboveground phytomass. To evaluate responses to rising atmospheric CO_2, we utilized six open-top chambers, three with ambient air and three with air CO_2 enriched to 720 μmol/mol, as well as three unchambered controls. We found that elevated CO_2 enhanced production of the shortgrass steppe throughout the study, with 41% greater aboveground phytomass harvested annually in elevated compared to ambient plots. The CO_2-induced production response was driven by a single species, *S. comata*, and was due in part to greater seedling recruitment. The result was species movement toward a composition more typical of the mixed-grass prairie. Growth under elevated CO_2 reduced the digestibility of all three dominant grass species. Digestibility was also lowest in the only species to exhibit a CO_2-induced production enhancement, *S. comata*. The results suggest that rising atmospheric CO_2 may enhance production of lower quality forage and a species composition shift toward a greater C_3 component.

Key words: Bouteloua gracilis*; C_3; C_4; carbon dioxide (CO_2); digestibility; forage quality; global change; grassland;* Pascopyrum smithii*; recruitment;* Stipa comata.

INTRODUCTION

Atmospheric CO_2 concentration has been rising steadily from ~280 μmol/mol at the start of the industrial revolution to >365 μmol/mol today, and is predicted to exceed 600 μmol/mol by the end of the century (IPCC 2001). Most plant species exhibit increased production when CO_2 concentration is increased above present ambient concentrations (Poorter 1993, Drake et al. 1996). This growth enhancement has been attributed to a direct stimulation in photosynthesis and/or to improved plant water relations, depending on the particular species and the environment (Poorter 1993, Drake 1996, Wand et al. 1999, Campbell et al. 2000). C_3 photosynthesis is limited by present-day ambient CO_2 concentrations while C_4 photosynthesis is nearly CO_2 saturated. Consequently, early predictions were that increasing atmospheric CO_2 concentrations would favor C_3 over C_4 species (Bazzaz 1990, Bowes 1993). However, subsequent research revealed that while CO_2-induced growth responses of C_3 species exceeded those in C_4 species, the differences were not as great as expected based on their photosynthetic pathway (Poorter 1993, Wand et al. 1999). In addition to its direct effect on photosynthesis, increasing CO_2 also induces stomatal closure and reduces transpirational water loss, thereby enhancing plant water use efficiency. This stomatal response occurs in most plant species, regardless of photosynthetic pathway, and appears to be the major reason growth responses to CO_2 do not differ any more than they do between photosynthetic classes (Owensby et al. 1993, 1996*b*, 1999, Hunt et al. 1996, Morgan et al. 2001*a*).

In native grasslands of North America like the subhumid tallgrass prairie of Kansas (Kirkham et al. 1991, Nie et al. 1992, Owensby et al. 1993, 1996*b*, 1999) or the semiarid shortgrass steppe of Colorado (Morgan et al. 2001*a*, *b*), enhanced production under elevated CO_2 atmospheres has been attributed primarily to improved

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water relations through stomatal closure and increased water use efficiency, and secondarily to increased photosynthesis. Improved water relations have also been reported to be the major cause of increased production in California annual grassland (Jackson et al. 1994, Chiariello and Field 1996) and in nutrient-poor calcareous Swiss grasslands (Niklaus et al. 1998, Volk et al. 2000) under elevated CO_2. Collectively, these findings suggest that this indirect effect of CO_2 on plant water relations is a potentially important aspect of grassland biomass response to CO_2, and that response may be especially strong in grasslands that are characteristically water limited (Campbell et al. 2000, Volk et al. 2000, Morgan et al. 2001*b*).

In addition to its effects on photosynthesis and water relations, CO_2 enrichment may enhance seedling recruitment (Edwards et al. 2001), which can significantly alter plant community composition as well as affecting its productivity. This can result from increases in seed number to CO_2 (Jablonski et al. 2002), or from the more indirect effects of CO_2 on the seedbed environment (Edwards et al. 2001), most importantly soil water content, light, and temperature, all of which can effect germination, emergence, and seedling survival (Wester 1995, Minnick and Coffin 1999). However, the significance of seedling recruitment responses to CO_2 is community dependent, as plant communities differ in the occurrence and importance of reproductive vs. vegetative recruitment.

Today's remaining native grasslands are used extensively for livestock grazing. As such, forage quality and seasonality of its production can be as critical as production in determining the value of these lands. There have been reports that elevated CO_2 concentration may sometimes reduce forage quality (Owensby et al. 1996*a*, Wand et al. 1999, Campbell et al. 2000), primarily by lowering forage nitrogen concentration (e.g., Owensby et al. 1996*a*). Perhaps more significant are the theorized shifts in vegetation composition predicted in future CO_2-enriched environments, with some scenarios suggesting a movement toward plant communities of lower forage quality (Polley et al. 1997, Campbell et al. 2000). Collectively, these reports suggest that rising atmospheric CO_2 concentration has the potential to cause significant alterations in grassland structure and function, with some outcomes leading to grasslands that are more productive but less useful for domestic livestock grazing.

In 1997, open-top chambers were installed on the Colorado shortgrass steppe to evaluate the responses of this grassland to elevated CO_2. Morgan et al. (2001*a*) reported that two years of CO_2 enrichment in this field experiment had enhanced mid and total season production of shortgrass steppe from 26% to 47%, with no production differences detected between C_3 and C_4 species. Similar results had been reported in an earlier growth chamber study involving monocultures of the same shortgrass steppe species (Hunt et al. 1996). This paper evaluates the effects of five years of CO_2 enrichment on aboveground productivity of the same field experiment (Morgan et al. 2001*a*), and examines production responses across all species, by functional groups (C_3 grasses, C_4 grasses, and herbaceous dicots) and by site codominant species (*Pascopyrum smithii* [Rybd.] A. Love, C_3; *Stipa comata* Trin and Rupr, C_3; *Bouteloua gracilis* [H.B.K.] Lag, C_4). Based on our experience with these species and other work indicating that altered water relations, and not photosynthetic pathway, tend to dominate plant responses to CO_2 in dry ecosystems, we hypothesized that production of the shortgrass steppe would continue to be enhanced in CO_2-enriched atmospheres, and that the three dominant grass species and three functional groups would respond similarly to CO_2. We assumed that recruitment from seed would not be an important feature of the system response to CO_2 over its 5-yr duration, due in large part to the difficulty in reseeding the dominant, *B. gracilis*, into disturbed sites (Hyder et al. 1971). A second objective was to determine whether growth under elevated CO_2 would alter forage digestibility of codominant species, as it had in the tallgrass prairie where lower forage N concentration was cited as a contributive factor (Owensby et al. 1996*a*). Since N is cycled tightly in the shortgrass steppe, with much of it tied up in microbial biomass (Woodmansee et al. 1978), we hypothesized that forage quality would decline in the shortgrass steppe under elevated CO_2 due to the inability of those soils to provide additional N to keep pace with CO_2-induced growth responses (Hunt et al. 1998), and would be reflected in reductions in in vitro dry-matter digestibility for all species. We also hypothesized that the decline in forage quality would be similar across species, based on the assumption of similar species production responses to CO_2 enrichment.

MATERIALS AND METHODS

Site and treatment descriptions

The experiment was conducted at the USDA-ARS Central Plains Experimental Range (CPER), 40°50′ N, 104°43′ W at the northern limit of the shortgrass steppe, a semiarid grassland on the western edge of the North American Great Plains used extensively for livestock grazing (Lauenroth and Milchunas 1991). The CPER is ~56 km northeast of Fort Collins, Colorado, USA. Long-term (52 yr) mean annual precipitation averages 321 ± 98 mm (1 SD), with the majority occurring during May, June, and July. Mean air temperatures are 15.6°C in summer and 0.6°C in winter with July maximum temperatures averaging 30.6°C. Dominant species at the experimental site are *Bouteloua gracilis* (comprising 45% of the vegetation at the site in 1996), a warm-season C_4 grass, and two cool-season C_3 grasses, *Pascopyrum smithii* (18% in 1996) and *Stipa comata* (25% in 1996). These three species comprise ~88% of the aboveground phytomass, with the remaining 12% contributed by 33 other species, most of which are perennial C_3 and C_4 grasses, and a few herbaceous dicots. The soil at the experimental site is a Remmit fine sandy loam (Ustollic carnborthids). This sandy soil holds 18% water at field capacity (−0.01 MPa), and 4% at the permanent wilting point (−1.5 MPa; Klute 1986).

The experiment was established in 1996 on a native rangeland pasture that had been previously grazed by cattle at a light to moderate intensity (~30% annual forage removal) for over 50 years. The effect of elevated CO_2 on this native ecosystem was investigated using open-top chambers (4.5 m diameter, ~3.8 m height, enclosing 15.5 m^2 ground area, or 60.5 m^3 volume). A portion of the pasture was initially divided into three blocks, and three 15.5 m^2 circular plots per block were randomly selected as the experimental treatment plots: an ambient chamber CO_2 treatment (360 ± 20 μmol/mol), an elevated chamber CO_2 treatment (720 ± 20 μmol/mol), and an unchambered control plot of equal ground area, which was used to asses the chamber effect.

Baseline plant and soils data were collected in 1996, prior to imposing the CO_2 treatments, to characterize the initial state of the system. From late March until mid October in 1997–2001, open-top chambers were placed on two plots in each of the three blocks (six total). Carbon dioxide fumigation proceeded in the elevated chamber CO_2 treatment as soon as the chambers were placed on the plots each spring, and continued until they were removed in the autumn when vegetation was dormant. The chamber tops had a small opening for exiting air, and precipitation that fell on the chamber tops was collected into reservoirs and immediately pumped back onto the plots with an automated sprinkler system. The precipitation catchment system was not 100% efficient, so water that sprinkled back onto chamber plots was monitored with a meter, and weekly additions of water were added to chambered plots to keep equal water additions the same as unchambered plots. Air temperatures at canopy height (15 cm) and soil temperatures at 10 cm depth were monitored every minute in all plots using thermocouples (thin wire for air, heavy gauge for soil), and hourly averages, plus 15 min averages of photosynthetically active radiation (PAR) measured above the plant canopy with quantum sensors (LI-COR LI-190SA) were recorded with a computer-based data acquisition system (Keithley Metrabyte WORKHORSE, Keithley Instrument, Taunton, Massachusetts, USA). More details on chamber design and operation may be found elsewhere (Morgan et al. 2001*a*).

Phytomass harvests

All aboveground plant biomass data presented herein were from samples collected in the southern half of the chambers. During the period of peak standing phytomass (late July), a metal wire grid containing fifty-six 40.5 × 15.3 cm quadrats (3.46 m^2 total) was placed over the south half of each plot and vegetation in every other quadrat (28 quadrats) was clipped to the crown, separated by species, dried at 60°C, and weighed. This defoliation protocol, which removed 50% of the green vegetation and represents defoliation by cattle that is intermediate between moderate and heavy grazing practices (Klipple and Costello 1960, Milchunas et al. 1988, 1995), was incorporated into the CO_2-treatment experimental design primarily as a means to represent the nominal grazing conditions for these grasslands. No attempt was made to simulate the hoof action that can be important in the incorporation of plant residues into the soil (Schuman et al. 1999). However, the stocking density is so light on the shortgrass steppe (1 steer/6.4 ha during the 6-mo grazing season) that the consequences of hoof action would be minimal in a 5-yr study. Further, the return of plant residues to belowground processes involves nutrient cycles with time constants considerably longer than the 5-yr duration of this study (Morgan 2002). We therefore assumed that such simulated livestock grazing over the short time period of five years in this study was an adequate representation of the grazed condition.

In autumn, aboveground phytomass was harvested in all 56 quadrats; weights from the July harvest were combined with this latter one to determine total seasonal productivity for the experimental area. The pattern was reversed the following year; quadrats that had not been clipped the previous summer were clipped at peak standing phytomass, and all quadrats harvested in the autumn. Annual alternation of the small grids that received or did not receive the defoliation during a particular growing season allowed for cycles of "rest-from-defoliation." The October final harvest involved only senescent shoot material. Phytomass in the northern half of the chambers was defoliated at ~2 cm height in October so that plant cover throughout the chamber was comparable at the beginning and end of each growing season. The northern half of the chamber was used for trace gas flux and other measurements, which are reported elsewhere (Mosier et al. 2002).

Culm counts of Pascopyrum smithii

Every year at peak standing phytomass, total numbers of *P. smithii* culms were counted in the 56 quadrats in the southern half of each plot where phytomass harvests were conducted. Heavy grazing of the shortgrass steppe leads to increased relative abundance of *B. gracilis*, with C_3 grasses like *P. smithii* decreasing (Hart 2001), so we monitored culm numbers of *P. smithii* to determine whether the defoliation regime was affecting stand density of this species. *Pascopyrum smithii* is rhizomatous, and vegetative expansions or contractions are reflected in changes in culm numbers. We did not monitor *B. gracilis* since it is a sod-forming grass, and difficult to quantify visually.

Plant counts of Stipa comata

Data analysis near the end of the study indicated a strong and exclusive aboveground phytomass response of *S. comata* to growth under elevated CO_2. We therefore decided to count plant numbers of this species by basal size classes within a few weeks after spring green-up following the final year of CO_2 enrichment (2001) to determine if seedling recruitment had been a factor in the production response of *S. comata. Stipa comata* is a bunchgrass, and propagates vegetatively by tillering. Seedlings start as one culm, adding more tillers as they grow. Older plants have more tillers, and consequently, more basal area. Our assumption was that differences in recruitment would be reflected in plant number and basal size class differences, with more plants, particularly in the smaller basal area classes, being diagnostic of more recruitment. On 10 June 2002, approximately three weeks after spring green-up (which occurred late due to an extended drought), individual plant counts of *S. comata* were made in each experimental plot where phytomass harvests had been conducted, by size class (basal diameters of <2 mm, 2–10 mm, 11–20 mm, 21–30 mm, 31–40 mm, 41–50 mm, 51–60 mm, and 61–110 mm). Basal diameters were determined on each plant counted from caliper measurements performed in two dimensions, and the readings were averaged.

Soil water

Soil water was measured at depths of 0–15 cm (Time Domain Reflectometry or TDR), and 15–45, 45–75, and 75–100 cm (Neutron Probe, Troxler Model 4301, Troxler Electronics, Research Triangle Park, North Carolina, USA) on a weekly basis during the growing season. A neutron probe and a TDR probe were installed on the periphery of the plant biomass sampling area near the center of each of the nine experimental plots to measure the soil water content of the treated area. The TDR probes were utilized in the top 15 cm since neutron probes are inaccurate near the soil surface. Both methodologies were calibrated against soil at the experimental site for soil water content.

In vitro dry-matter digestibility

The ruminant in vitro dry-matter digestibility (IVDMD) technique simulates microbial breakdown of forage in the rumen followed by lower gut digestion. The first stage of the two-stage process (Tilly and Terry 1963) involves rumen pumping an animal to obtain a microbial population, straining, filtering, and adding this to a buffer solution to produce a uniform inoculate, and delivering standard quantities of inoculate to forage subsamples (and blanks) in test tubes in water baths maintained at 38.5°C and under anaerobic conditions. Rumen material was collected from a fistulated cow maintained on an exclusively grass/hay diet for one week prior to collection, with feed removed 12 h and water removed 3 h prior to rumen pumping. The grass/hay diet yields a microbial population adapted to fibrous forage, and removal of water and feed prior to pumping yields a more uniform material. Precautions in handling rumen fluid necessary to maintain microbial activity, and methods of preparing and delivering the inoculum follow those described in the 25-strain-layer method of Milchunas and Baker (1982: strain-layer method). Microbial digestion in the tubes is terminated after 48 h, and an additional 48 h of digestion in acid-pepsin simulates lower gut processes. There are good relationships between IVDMD and in vivo digestible dry matter, in vivo digestible energy, and animal weight gain for various ruminant species (Van Soest 1994).

Statistics

CO_2 treatments were analyzed for their effects on soil water content, aboveground phytomass, IVDMD, and plant and culm counts using the SAS PROC MIXED analysis (SAS Institute, Cary, North Carolina, USA). "Year" was used as a repeated-measure variable, "block" was specified as a random effect (thereby removing the variability due to blocking), and block × CO_2 treatment was used as the error term for CO_2 treatment comparisons. Where significant treatment effects were detected, treatment comparisons were conducted utilizing the Tukey's means comparison test at the 0.05 level of confidence, unless otherwise indicated. The 1996 baseline plant data revealed a trend for a difference in species composition in the plots prior to any CO_2 treatment. Therefore, the 1996 data were used as a covariate in the PROC MIXED and Tukey's analysis of the species and functional group data. Covariance analysis resulted in transformed means, so the original, unadjusted means are presented in the figures. Treatment variances of the plant count data were decidedly unequal and residuals were not normally distributed, so analysis of variance was conducted using a natural log transform of the plant count data, which improved the variances and distribution of the residuals. Means of the nontransformed plant count data are given for plant count data.

RESULTS

Chamber conditions

Canopy height air temperatures and soil temperatures were respectively 2.6°C and 1.25°C higher inside than outside the chambers when averaged across all years. In the first year of the study (1997), more precipitation fell on the unchambered plots in a 2-wk period in early summer before we realized that the precipitation catchment system was not 100% efficient, so unchambered plots benefited from slightly more water that year (Morgan et al. 2001*a*). The Lexan walls passed 92–94% of the incident PAR. However, periodic measurements of PAR inside and outside of the chambers indicated an

TABLE 1. Treatment variable probabilities for aboveground phytomass data obtained from five years (1997–2001) of ambient and elevated CO_2 (360 and 720 µmol/mol) in open-top chambers and unchambered control plots on the shortgrass steppe for eastern Colorado, USA.

Source of variation	df	*P*	
		Mid season	Whole season
CO_2 treatment	2	0.0001	0.0001
Year	4	0.0001	0.0001
CO_2 × year	8	0.1907	0.2194

Notes: Results are given for a mid-season (late July) and end-of-the-season harvest. The block effect (not shown) was not significant.

~28% lower PAR in chambered plots, due mostly to shading by chamber framework.

Total aboveground phytomass

Analysis of variance conducted over the five years of CO_2 enrichment (1997–2001) indicated total aboveground phytomass was significantly affected ($P < 0.0001$) by CO_2 treatment and year (Table 1). Elevated CO_2 enhanced aboveground phytomass by 35% at the summer harvest and 41% for total annual production compared to that harvested in the ambient chambers (Fig. 1). Thirty-six percent more phytomass was harvested in ambient chambers compared to control plots (Fig. 1).

A significant year effect in aboveground phytomass (Table 1) was due to lower productivity in 2000 and 2001 (annual only) compared to the other three years (Fig. 2). The year 2000 was a relatively dry one in which only 247 mm of precipitation fell during the growing season, most of which fell in the mid to late summer months, when production tends to be lower than in the late spring. Although precipitation was greater in 2001, soil water stores never recharged (data not shown). Amounts of precipitation were above the long-term growing season average of 280 mm for the other three years (1997–1999), accounting for their higher productivity (Fig. 2).

Functional group comparisons

All main effects, one of the two-way interactions, and the three-way interaction involving functional groups (C_3 grasses, C_4 grasses, herbaceous dicots), CO_2 treatment, and years were highly significant in the statistical analysis of summer phytomass data ($P \leq 0.01$; Table 2). Therefore, means comparisons were conducted within each year for CO_2 treatments, within each of the three functional groups.

For C_3 grasses, a significant increase in aboveground phytomass under CO_2 enrichment was detected in 1997 and 1998 at the mid-season harvest (Fig. 3). No CO_2 treatment effects on production were observed for the C_4 grasses, although production was greater in chambered compared to control plots in 1997 and 1998. Production of herbaceous dicots was enhanced significantly in elevated CO_2 open-top chambers only in 1998.

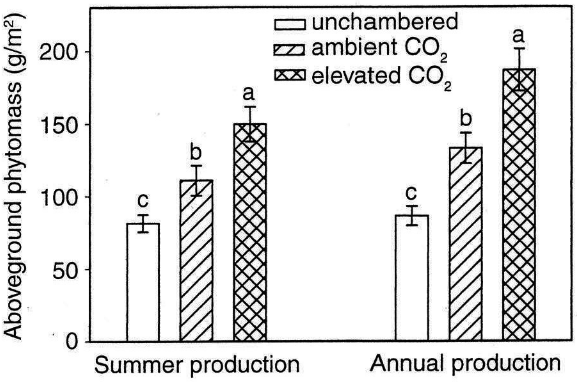

FIG. 1. Mean phytomass at the summer harvest and over the entire season (averaged over five years) in ambient and elevated CO_2 (360 and 720 µmol/mol) open-top chambers and in unchambered controls on the shortgrass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

Species comparisons

Significant main effects were observed in the analysis of summer phytomass data for dominant site species (*B. gracilis*, *P. smithii*, and *S. comata*), CO_2 treatment, and years (Table 2). Two-way interactions between CO_2 and year, species and year, and a strong trend ($P < 0.10$) between species and CO_2 treatment were also observed, but there was no significant three-way interaction. Therefore, means comparisons were conducted according to the two-way interactions.

When evaluated over the five years of CO_2 enrichment, aboveground phytomass was stimulated only for the C_3 grass *S. comata*, with plants grown under ele-

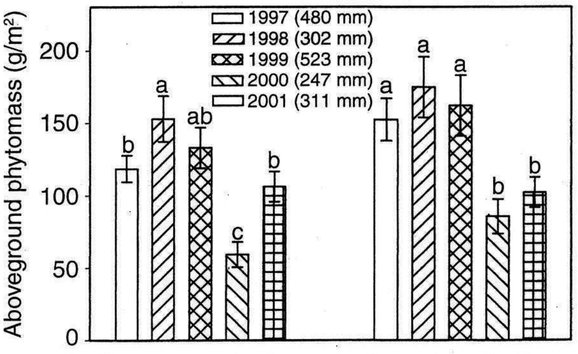

FIG. 2. Mean phytomass at the summer harvest and over the entire season (averaged over the three treatments) during five years on the shortgrass steppe of eastern Colorado. Growing season precipitation for each year is in parenthesis. Means within a harvest with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

TABLE 2. Treatment variable probabilities for aboveground phytomass (harvested during the summer) from five years (1997–2001) of ambient and elevated CO_2 (360 and 720 μmol/mol) in open-top chambers and unchambered control plots on the shortgrass steppe of eastern Colorado, USA.

Source of variation	df	P
Functional group		
CO_2 treatment	2	0.0001
Functional group	2	0.0001
Year	4	0.0001
CO_2 treatment × functional group	4	0.5051
CO_2 treatment × year	8	0.1324
Functional group × year	8	0.0001
CO_2 treatment × functional group × year	16	0.0092
Species		
CO_2 treatment	2	0.0017
Species	2	0.0001
Year	4	0.0001
CO_2 treatment × species	4	0.0978
CO_2 treatment × year	8	0.0156
Species × year	8	0.0001
CO_2 × species × year	16	0.5339

Notes: The three functional groups are C_3 grasses, C_4 grasses, and herbaceous dicots. The three species are *Bouteloua gracilis, Pascopyrum smithii*, and *Stipa comata.* Aboveground phytomass data from the pretreatment year 1996 were used as covariates in the analysis. The block effect (not shown) was not significant.

vated CO_2 having 84% greater phytomass at mid season compared to ambient chamber plants (Fig. 4). No CO_2-induced production increase was observed for the other codominant C_3 grass, *P. smithii*, or for the C_4 grass, *B. gracilis.* Although total aboveground productivity was significantly higher in ambient vs. unchambered plots (Fig. 1), no significant chamber effect was detected when evaluating the three main grass species.

The interaction between year and CO_2 treatment (Table 2) was mostly due to differences between the control plots and the elevated CO_2 chambers in 1997, and also to strong trends among the chambered treatments that varied among years, but which were all in the direction of increased production in elevated CO_2 chambers (data not shown).

Mid-year aboveground phytomass varied among the species, with production being greatest in 1997 for *B. gracilis*, while production in the two C_3 grasses peaked in 1998 and 1999 (Fig. 5). Productivity of all three grass species dropped drastically in 2000 due to the drought. Productivity of the two C_3 grasses remained relatively low in 2001, but increased from the previous year for *B. gracilis.*

Culm/plant counts

The yearly summer culm count of *P. smithii* revealed no treatment differences throughout the experiment, nor was there a decline in culm density over time due to the defoliation (data not shown). However, plant counts made of *S. comata* at the conclusion of the study revealed significant treatment effects for CO_2, with higher total numbers of plants in elevated compared to ambient chambers ($P = 0.007$; Fig. 6), especially in the small-sized plants (CO_2 × size class, $P = 0.005$). The smaller size classes were comprised of relatively young plants, generally consisting of only one to several culms. Collectively, these data indicate increased recruitment of *S. comata* plants under elevated CO_2. A significant chamber effect was also noted, with higher numbers of plants in ambient chambers compared to control plots in the smaller sized classes.

In vitro dry-matter digestibility

Measurements of in vitro digestibility conducted on summer-harvested shoots in 1998 and 1999 indicated that growth at elevated CO_2 reduced digestibility by an average of 16% (Fig. 7) in *S. comata*, *B. gracilis*, and *P. smithii* ($P < 0.0001$). Analysis of variance conducted among species also suggested that *S. comata* was lower in digestibility than the other two grass species ($P < 0.05$).

Soil water

Soil water contents measured to a depth of one meter and averaged over the entire study were 12.1 ± 0.6% in elevated CO_2 chambers, significantly greater ($P < 0.05$) than the 10.7 ± 0.5% soil water content measured in ambient chambers. While these seasonally averaged differences in soil water content do not appear great, they are significant as they include sustained periods in which substantial differences were maintained for long periods. For instance, Morgan et al. (2001*a*) observed higher soil water contents in the elevated CO_2 chambers from late summer 1997 through most of 1998. Similar results were seen in subsequent years (LeCain et al. 2003).

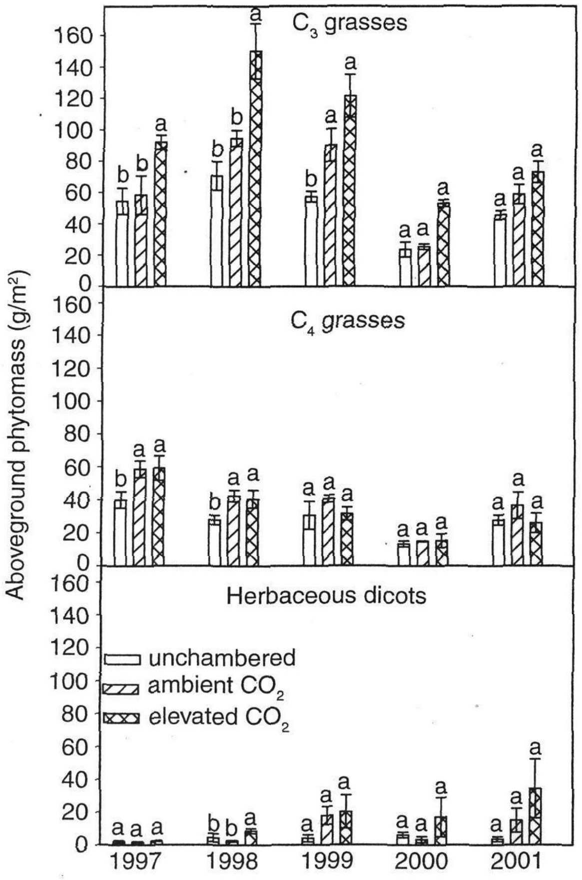

FIG. 3. Mean phytomass of the major functional groups at the summer harvest in ambient and elevated CO_2 (360 and 720 µmol/mol) open-top chambers and in unchambered controls on the shortgrass steppe of eastern Colorado. Means within a year with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

Soil water content was 12.7 ± 0.6% in the unchambered control plots, also greater than that measured in the ambient plots.

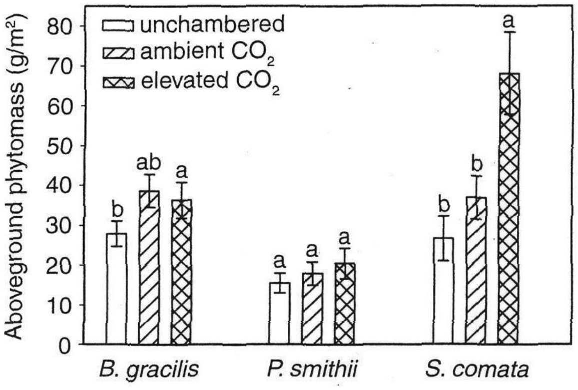

FIG. 4. Mean phytomass of the three main species at the summer harvest (averaged over five years) in ambient and elevated CO_2 (360 and 720 µmol/mol) open-top chambers and in unchambered controls on the shortgrass steppe of eastern Colorado. Means within a species with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

DISCUSSION

Phytomass

The results of this field study clearly indicate that productivity in the shortgrass steppe of northern Colorado is consistently and strongly responsive to a doubling of CO_2 concentration above present ambient levels. Periodic measurements of soil and plant water traits indicated higher soil water contents and leaf water potentials of dominant shortgrass steppe grasses in elevated compared to ambient CO_2 open-top chambers (Morgan et al. 2001*a*, LeCain et al. 2003). Although photosynthesis was often stimulated under elevated CO_2, this response appeared more related to improved soil–plant water relations than to any direct stimulation of photosynthesis (Morgan et al. 2001*a*, LeCain et al. 2003). These results are consistent with the notion that, in water-limited grasslands, soil water savings and higher water use efficiency from CO_2-induced stomatal closure may be the dominant force behind growth enhancements under elevated CO_2 (Kirkham et al. 1991, Nie et al. 1992, Jackson et al. 1994, Drake et al. 1996, Owensby et al. 1996*b*, Sindhøj et al. 2000, Volk et al. 2000, Morgan et al. 2001*a, b*).

In contrast to results reported here in the Colorado shortgrass steppe, plant biomass responses to CO_2 in another native Great Plains grasslands, the Kansas tallgrass prairie, were evident in dry rather than wet years (Owensby et al. 1996*b*). Similarly, increased production under CO_2 enrichment in Swiss calcareous grasslands was relatively greater as soil water became more

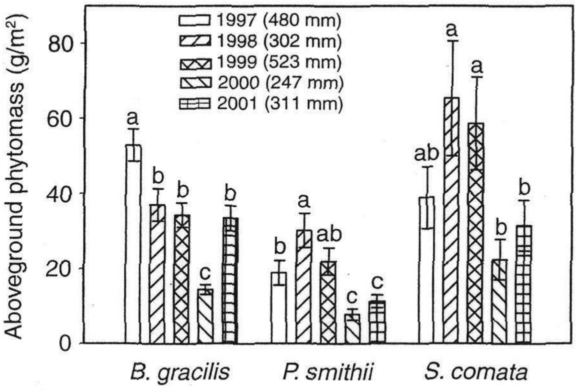

FIG. 5. Mean phytomass of the three main species at the summer harvest (averaged over the three treatments) during five years on the shortgrass steppe of eastern Colorado. Growing season precipitation for each year is in parenthesis. Means within a species with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

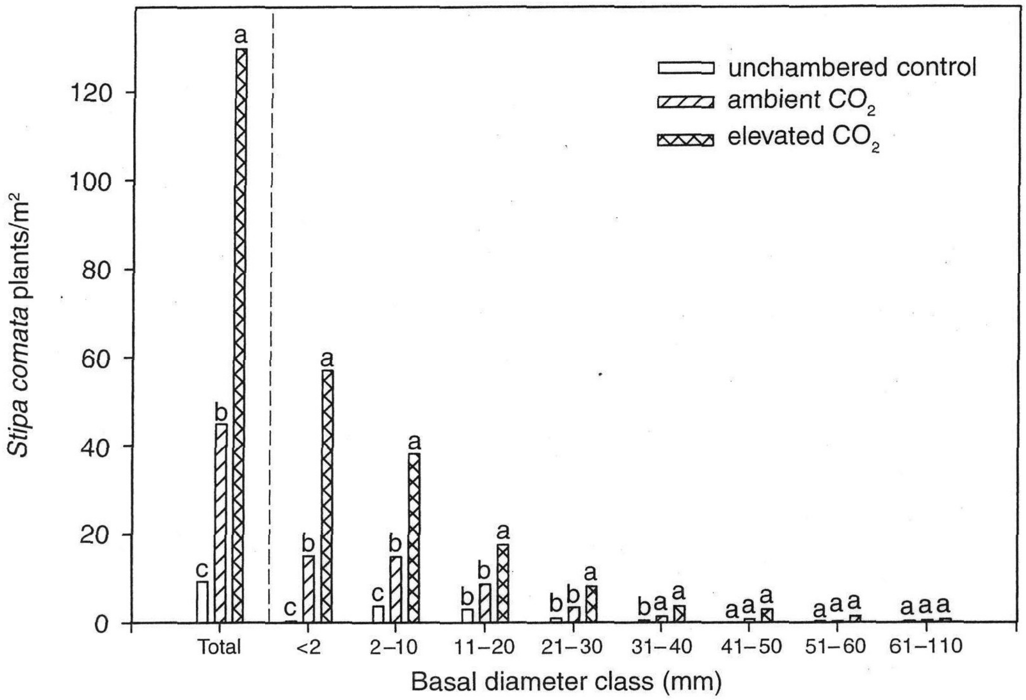

FIG. 6. Total number of *Stipa comata* plants per square meter and in different size classes in ambient and elevated CO_2 (360 and 720 μmol/mol) open-top chambers and in unchambered controls on the shortgrass steppe of eastern Colorado. Means within a size class with different letters are significantly different using Tukey's hsd test ($P < 0.10$).

limiting (Niklaus et al. 1998, Volk et al. 2000). Such results indicate a potentially strong interactive effect of soil water on CO_2 production responses. However, despite years with variable precipitation (Fig. 2), no year × CO_2 interaction for phytomass was detected in our study. Growing season precipitation amounts ranged from 12% below the 52-yr average in the driest year (2000) to 87% greater than average in the wettest year (1999). We suspect that the consistent CO_2-induced production responses in the shortgrass steppe are due in large part to its semiarid environment, which offers more opportunity for CO_2-related improvements in plant water relations than would occur in the semihumid tallgrass prairie or in other more mesic grasslands.

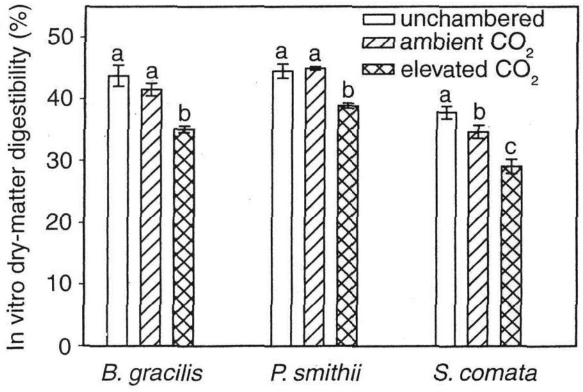

FIG. 7. In vitro dry-matter digestibility of the three main species (averaged over two years) in ambient and elevated CO_2 (360 and 720 μmol/mol) open-top chambers and in unchambered controls on the shortgrass steppe of eastern Colorado. Means within a species with different letters are significantly different using Tukey's hsd test ($P < 0.05$); error bars represent ±1 SE.

Functional group/species responses

As a group, the C_3 grasses were most responsive to CO_2, showing significantly higher production in only two years, and trends suggested production enhancement from CO_2 enrichment in the other three years of the study. C_4 grasses were unresponsive to CO_2, and herbaceous dicots responded with higher production under elevated CO_2 only in 1998. These results differ from our analyses of this same experiment after just two years of CO_2 enrichment in which we detected a 37% increase in aboveground phytomass, but could not yet detect a different response among the C_3 and C_4 grasses (Morgan et al. 2001*a*). At first glance, these results would appear to support, at least in part, the hypothesis that C_3 plants will respond more favorably to rising CO_2 concentrations compared to C_4 species (Bazzaz 1990). However, a closer examination of the species responses suggests that the patterns of CO_2 responses may go beyond photosynthetic pathway.

Of the two dominant C_3 species, only *Stipa comata* exhibited higher aboveground phytomass in response to elevated CO_2. Thus, our hypothesis of equal species response to CO_2 enrichment was rejected. *P. smithii*, which has been evaluated in monocultures in several previous studies and found to be responsive to CO_2 (Hunt et al. 1996, Read and Morgan 1996, Morgan et al. 1998), showed no change in aboveground phytomass under elevated CO_2 in the field. Extensive gas exchange studies have shown that leaves of *P. smithii* grown at elevated CO_2 exhibit strong and consistent downward acclimation of photosynthesis, often to the extent that photosynthesis rates are similar in plants grown in ambient and elevated CO_2 (Morgan et al. 1994*a*, Read et al. 1997). The same was observed for leaves measured in this field study (Morgan et al. 2001*a*, LeCain et al. 2003), suggesting that little or no direct photosynthetic gain was realized by *P. smithii* plants growing in elevated CO_2 open-top chambers. We have considerably less leaf gas exchange data for *S. comata*, so cannot assess its potential for a direct photosynthetic response to elevated CO_2. However, intensive water relations measurements conducted throughout the experiment indicate significant and consistent increases in leaf water potential were observed for both species under elevated CO_2 (Morgan et al. 2001*a*, LeCain et al. 2003).

These are the first results to report differential species responses to CO_2 in the shortgrass steppe, and they differ considerably from prior reports in which we indicated that elevated CO_2 enhanced photosynthesis (Morgan et al. 1994*a*) and growth (Morgan et al. 1994*b*, Hunt et al. 1996) and improved plant water relations (Morgan et al. 1998) in both *P. smithii* and *B. gracilis*. These previous experiments were conducted in environmental growth chambers with monocultures of *P. smithii* and *B. gracilis*, with plants grown in reconstituted or native soil contained in columns. While such experiments allow the genetic expression of plant species, they do not necessarily reflect responses that may develop in more complex plant communities where interspecific competition for resources or microclimatic feedbacks often define the outcome of environmental perturbations. The results of the present study are especially intriguing as they clearly indicate beneficial effects of elevated CO_2 on plant water relations in the three dominant species (Morgan et al. 2001*a*, LeCain et al. 2003), yet aboveground phytomass was enhanced in only one of these species.

The inclusion of *S. comata* in this study was fortuitous, as it provides a clear example of a species response to CO_2 that would not have been predicted based on photosynthetic class assignment alone. Our early CO_2 enrichment studies focused on *Bouteloua gracilis* and *P. smithii*, the former because it is the dominant species of the shortgrass steppe, and a C_4 grass, and the latter as a representative of several codominant C_3 grasses. We assumed that seed recruitment would be a minor feature of this study since the community dominant, *B. gracilis*, recovers slowly after disturbance, often taking several decades for stand density to return to predisturbance levels (Lauenroth and Coffin 1992). Further, an earlier growth chamber CO_2 enrichment study indicated no significant effect of elevated CO_2 on seed production of *B. gracilis*, and the growth response of *P. smithii* to elevated CO_2 appeared to be driven primarily by rhizomatous growth (Hunt et al. 1996). However, *S. comata* appears to be an opportunist, increasing under disturbance or when soil water is more plentiful. We noticed that numbers of *S. comata* plants increased over the five years of our experiment in the field where our open-top chambers were installed, and suspect that high rainfall in the early years of our experiment contributed to seedling recruitment of this species at the site. Similarly, higher soil water content in the elevated CO_2 chambers may have enhanced recruitment over that occurring in the ambient chambers (Morgan et al. 2001*a*, LeCain et al. 2003). The exact mechanism of this recruitment is unknown as we did not monitor plant developmental and recruitment responses, nor do we know the extent to which this enhanced recruitment in the elevated CO_2 chambers varied among the study years. As a cool-season grass, *S. comata* commences growth in early spring, flowers in early summer, and releases its seeds in mid to late summer (Coupland 1950). Thus, a CO_2-enriched atmosphere could have benefited one or more processes related to the recruitment of new individuals through higher soil water content. It is also possible that CO_2 may have directly stimulated seedling growth or enhanced water use efficiency. While we can't determine the relative importance of individual plant production vs. the recruitment response to CO_2, both were likely important.

Chamber effects

The chamber effect, which led to increased plant production and number, deserves comment. Although PAR was reduced 28% in the chambers, production was greater inside than outside chambers, suggesting that chamber light levels were apparently not important in chamber-related production responses. The chamber effect was likely driven more by the 2.6°C increase in air temperature and 1.25°C increase in soil. We reported the chamber production response previously (Morgan et al. 2001*a*), and attributed it to warmer chamber temperatures, which resulted in earlier spring green-up and may have enhanced N mineralization, leading to more available soil N, thereby enhancing production (Rustad et al. 2001). Higher plant production in the ambient chambers likely increased transpiration, leading to the lower soil water content compared to the control plots.

It seems likely that the chamber production response may also have been due to the increased recruitment that occurred in the chambered plots. Chambers covered the plots throughout the entire growing season (7.5 mo), which included all phases of vegetative and reproductive growth and seed dispersal. The chamber effects on recruitment could have involved any of a potentially large number of plant responses, from plant developmental reactions resulting from warmer chamber temperatures to seed dispersal and related matters caused by the physical barriers of the chambers. Faster plant development from warmer temperatures could have led to earlier flowering and seed formation, thereby altering the environmental conditions at critical reproductive times for plants inside compared to outside of chambers. It is also possible that the chambers might have affected seed dispersal and the plot seed bank. *Stipa comata* seeds are heavy and fall close to their respective reproductive culms, so the initial deposition of seeds would likely have been little affected by the chambers. But the chambers could have excluded small mammals that might have been important in seed predation, thereby resulting in an abundant seed bank inside the chambers. While small mammal seed predation activity occurs mostly overwinter on the shortgrass steppe (French et al. 1976), a period in which chambers had been removed from the plots, it is conceivable that the exclusion of seed predation during the growing summer months could have resulted in a larger seed bank inside than outside of chambers, particularly in years with abundant seed production.

Chamber effects and other artificial aspects of global change studies, particularly the instantaneous change in environmental conditions required to induce the global change treatments (Morgan 2002), make simple extrapolation of such results to future environments complicated. While comparison of the ambient and elevated CO_2 chamber results of the present study clearly demonstrate that both growth and recruitment of *S. comata* were enhanced in the shortgrass steppe by an increase in CO_2 concentration, it is important to keep in mind that these responses apply to chamber conditions in a study in which CO_2 was instantaneously doubled for five years. Thus, these findings present a potential that will likely be realized differently in different years and environments, and will evolve over time as global change is experienced in an incremental rather than sudden fashion. The strong responses observed in this study over a relatively short time period of only five years suggests the recruitment and growth responses will be featured prominently in the decades and centuries ahead as atmospheric CO_2 concentrations continue to rise.

Implications for Great Plains grasslands

The major grassland types in the Great Plains are formed in large part due to a precipitation gradient which extends west to east (Sims 1991, Coffin and Lauenroth 1996). Its most arid regions are in the south and central areas of the Great Plains, where a rain shadow created by the Rocky Mountains limits precipitation on its western boundary and results in the semiarid shortgrass steppe. Moving east and out of the rain shadow, precipitation gradually increases and vegetation changes first into the southern mixed-grass prairie, and eventually into subhumid tallgrass prairie on its eastern boundaries in midwestern states. Northern regions of the Great Plains are almost all northern mixed-grass prairie. The shortgrass steppe and mixed-grass prairies share many of the same species, but the mixed-grass prairie has a higher percentage of cool-season, mid grasses like *S. comata* (Coupland 1991, Lauenroth and Milchunas 1991). The results of our study, which show increased recruitment and greater production of a C_3 shortgrass steppe species that is relatively more important in the mixed-grass prairie, represent the first empirical evidence that rising CO_2 concentrations may have the potential to shift present boundaries between these two important Great Plains grassland types. The final outcome of any such potential shifts will certainly involve interactions with altered temperature and precipitation patterns, both of which are predicted to have strong impacts on both production and shifts in C_3/C_4 dominance in the Great Plains (Coffin and Lauenroth 1996).

Another interpretation of the strong *S. comata* response is that rising CO_2 and accompanying climate change may simply increase the relative presence of this grass species in much of the shortgrass steppe. This apparent shift in favor of *S. comata* is important, as *S. comata* is more susceptible to drought than the current shortgrass steppe dominant, *B. gracilis*. Weaver and Albertson (Allen-Diaz 1995) reported that fully four-fifths of the remaining vegetation in the shortgrass steppe following the drought of the dust bowl days in the 1930s was comprised of just two species, *B. gracilis*, and another warm-season grass, *Buchloe dactyloides*, with *B. gracilis* being the more drought tolerant. That remaining cover of warm-season grasses was vitally important in providing minimal forage during the drought, and a base of plants from which the shortgrass steppe recovered when normal precipitation patterns resumed in the early 1940s. If the increased competitiveness of *S. coma*ta seen in the elevated CO_2 chambers eventually leads to a reduction in *B. gracilis* in future CO_2-enriched environments, the susceptibility and severity of drought in the shortgrass steppe may increase.

The reduction in digestibility of the dominant perennial grasses of this ecosystem supports our hypothesis that elevated CO_2 would lead to lower forage quality, and means that cattle will have to consume relatively more forage compared to grasses grown under present CO_2 atmospheres to achieve comparable weight gain. Reduced digestibility of shortgrass steppe grasses in the CO_2-enriched chambers was likely due to lower N concentration (Drake et al. 1996, Owensby et al. 1996*a*, Campbell et al. 1997, Polley et al. 2000, Morgan et al. 2001*a*). In this grassland, the additive effect of CO_2 on tissue quality within a species and the effect on species compositional change, with relatively more production under elevated CO_2 being contributed by the least digestible grass, *S. comata*, both act to reduce forage quality, with the latter having the greater negative effect. Animal selection for the higher quality species could further drive the system toward an even less palatable state. The decline in plant N concentration is a fairly common response to CO_2 enrichment. Thus, we concur with Owensby et al. (1996*a*) that continued CO_2 enrichment of the earth's atmosphere appears likely to reduce forage quality in many of the world's grasslands, and will likely reduce animal performance.

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