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

Jack A. Morgan*,§, Arvin R. Mosier†, Daniel G. Milchunas‡,‡‡, Daniel R. LeCain*,
Jim A. Nelson* and William J. Parton‡‡

*USDA-ARS, Rangeland Resources Research Unit, 1701 Centre Ave., Fort Collins, CO, 80526; †USDA-ARS, Soil-Plant-Nutrient Research Unit, Federal Bldg., 301 S. Howes, Fort Collins, CO, 80522; ‡Dept. of Rangeland Ecosystem Science, Natural Resources Bldg., Colorado State University, Ft. Collins, CO, 80523 and ‡‡Natural Resources Ecology Lab, Natural and Environmental Sciences Bldg., Colorado State University, Ft. Collins, CO 80523

§Corresponding author: J.A. Morgan; Phone (970) 498-4216; FAX (970) 482-2909; email morgan@lamar.colostate.edu

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 semi-arid rangelands to CO_2, and even less on consequences for forage quality. This study was initiated to study the CO_2 response of the shortgrass steppe, an important semi-arid 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 north-eastern Colorado. Three perennial grasses dominate the study site, *Bouteloua gracilis* (H.B.K.) Lag., a C_4 grass, and two C_3 grasses, *Pascopyrum smithii* (Rybd.) A. Love and *Stipa comata* Trin and Rupr. 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, three with air CO_2-enriched to 720 μL L^{-1}, and three unchambered controls. We found that elevated CO_2 enhanced production of the shortgrass steppe throughout the study, with 38% 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 towards a composition more typical of the mixed-grass prairie. Growth under elevated CO_2 reduced the digestibility of all three dominant grass species, and was 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, more drought sensitive forage in the shortgrass steppe.

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] has been rising steadily from approximately 280 μL L^{-1} at the start of the industrial revolution to over 365 μL L^{-1} today, and is predicted to exceed 600 μL L^{-1} by the end of the century (IPCC, 2001). Most plant species exhibit increased production when [CO_2] is increased above present ambient concentrations (Drake et al., 1996; Poorter 1993). 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 (Campbell et al., 2000; Drake 1996a; Poorter 1993; Wand et al., 1999). In native grasslands of North America like the sub-humid tallgrass prairie of Kansas (Kirkham et al., 1991; Nie et al., 1992; Owensby et al., 1993, 1996b, 1999), California annual grassland (Chiariello and Field, 1996; Jackson et al., 1994), or the semi-arid shortgrass steppe of Colorado (Morgan et al., 2001a), enhanced production under elevated CO_2 atmospheres has been attributed primarily to improved water relations through stomatal closure and increased water use efficiency, and secondarily to increased photosynthesis. The fact that photosynthetic responses have not been primarily featured in the responses of grasslands to elevated CO_2 may explain, in part, the apparent lack of the predicted advantage for native C_3 grasses under elevated CO_2 compared to C_4 species (Hunt et al., 1996; Morgan et al., 2001a; Owensby et al., 1993, 1996b, 1999). 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, the water relations benefit of elevated CO_2 is realized by most plant species, independent of photosynthetic pathway, which tends to minimize differences in species responses to CO_2 in water limited systems. Further, the potential for increased plant production from elevated [CO_2] may be greater in grasslands that are characteristically water-limited (Campbell et al., 2000; Morgan et al., 2001b; Volk et al., 2000).

In addition to relatively direct effects of CO_2 on photosynthesis and improved water relations, CO_2 enrichment may sometimes enhance seedling recruitment (Edwards et al., 2001). This can result from direct effects of CO_2 on reproductive attributes like seed number and quality or from the effects of CO_2 on seed-bed environment, most importantly soil water content, light and temperature. Recruitment success depends also on potentially

complicated interactions with CO_2-induced responses of competitors, like enhanced growth, which can increase the canopy and leave fewer spaces for seedling establishment (Edwards et al., 2001). Recruitment may be especially important as a mechanism for CO_2-induced plant community changes.

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

In 1996, open-top chambers were installed on the Colorado shortgrass steppe to evaluate the responses of this grassland to elevated CO_2. Morgan et al. (2001a) reported that two years of CO_2 enrichment 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. This paper evaluates the effects of five years of CO_2 enrichment on aboveground productivity of this same experiment, and evaluates production responses across all species, by functional groups (C_3 grasses, C_4 grasses and forbs) and by site co-dominant species (*Pasycopyrum smithii*, C_3; *Stipa comata*, C_3; *Bouteloua gracilis*, C_4). Based on earlier growth chamber work in which we observed similar and significant CO_2-induced enhancements in productivity of shortgrass steppe C_3 and C_4 grasses, grown in monoculture (Hunt et al., 1996), we predicted no functional group or species differences to growth under elevated CO_2 in the shortgrass steppe. We assumed that recruitment from seed would not be an important feature of the system response to CO_2, due in large part to the difficulty in re-seeding the dominant, *B. gracilis*, into disturbed sites (Hyder et al., 1971). We evaluated in vitro dry matter digestibility of co-dominant species, and predicted it would decline across species based on the findings of Owensby et al. (1996a) in the tallgrass prairie.

MATERIALS AND METHODS

Site and Treatment Descriptions. The experiment was conducted at the USDA-ARS Central Plains Experimental Range (CPER), lat. 40° 50' N. long. 104° 43' W at the northern limit of the shortgrass steppe, a semi-arid grassland on the western edge of the North American Great Plains used extensively for livestock grazing (Lauenroth and Milchunas 1991). The CPER is about 56 km north-east of Fort Collins, CO. Long-term (55 yr) mean annual precipitation averages 320 mm, 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 maximum July temperatures averaging 30.6 °C. Dominant species at the experimental site are *Bouteloua gracilis* (H.B.K.) Lag., a warm season C_4 grass, and two cool season C_3 grasses, *Pascopyrum smithii* (Rybd.) A. Love and *Stipa comata* Trin and Rupr. These three species comprise approximately 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 forbs. The soil at the experimental site is a Remmit fine sandy loam (Ustollic carnborthids). This sandy soil holds 18% water at field capacity, and 4% at the permanent wilting point.

The experiment was established in 1996 on a native rangeland pasture which had been previously grazed by cattle at a light to moderate intensity (about 30% annual forage removal). The effect of elevated CO_2 on this native ecosystem was investigated using open top chambers (4.5 m diameter, enclosing 15.5 m^2). A portion of the pasture was initially divided into three blocks, and three 15.5 m^2 circular plots per block were randomly chosen as experimental plots. 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). Baseline plant and soils data were collected in 1996, prior to imposing the CO_2 treatments, to characterize the initial state of the system. In the fall of 1996, one chamber was randomly assigned an ambient CO_2 treatment (360$\pm$20 Φmol mol^{-1}), the other an elevated CO_2 treatment (720$\pm$20 Φmol mol^{-1}). From 1997 through 2001, carbon dioxide fumigation proceeded 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. Each block had an unchambered plot of equal ground area, which was used to monitor the effect of the chamber. Details on chamber

design and operation may be found elsewhere (Morgan et al., 2001a).

Phytomass Harvests. Aboveground plant biomass harvests were conducted in the southern half of the chambers. During the period of peak standing crop (late July), a metal wire grid containing fifty-six 40.5 X 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^o C and weighed. This defoliation protocol, which removed 50% of the green vegetation and represents defoliation by cattle that is between moderate and heavy grazing practices, was incorporated into the CO_2-treatment experimental design primarily as a means to represent the nominal grazing conditions for these grasslands (Klipple and Costello 1960; Milchunas et al., 1988, 1995). In autumn, aboveground phytomass was harvested in all 56 quadrats to estimate total seasonal productivity for the experimental area. The pattern was reversed the following year; quadrats which had not been clipped the previous summer were clipped at peak standing crop, 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, and so did not elicit a physiological response. Phytomass in the northern half of the chambers was defoliated at about 2 cm height in October so that plant cover throughout the chamber was comparable at the beginning and end of each growing season.

Culm and Plant Counts. Every year at peak standing crop, total numbers of *P. smithii* culms were counted in each plot. 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 effecting stand density of this species.

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 size classes within a few weeks after spring green-up following the final year of CO_2 enrichment (2001) to determine if seedling recruitment was a factor in the production response of *S. comata*. On June 10, 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 the phytomass sampling area of each experimental plot, 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 averaging the readings.

Soil Water. Soil water was measured weekly using a Troxler model 4301 neutron probe (Troxler Electronics Lab., Research Triangle Park, NC, USA) which had been calibrated against soil from the experimental site. Soil moisture content was converted to the total mm of water in the upper meter of the soil profile.

In Vitro Dry Matter Digestibility. Inoculum for IVDDM was collected from a fistulated cow maintained on an exclusively grass-hay diet for one week prior to collection, with feed removed 12 hrs prior to rumen-pumping. 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). IVDDM was run according to the two-stage, 96 hr, Tilly and Terry (Tilley and Terry 1963) method with modifications and quality controls described in Milchunas and Baker (1982).

Statistics. CO_2 treatments were analyzed for their effects on soil water content and aboveground productivity using the SAS PROC MIXED analysis (SAS Institute Inc., Cary, NC, USA), with year represented as a repeated measure. 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. Treatment variances of the plant count data were decidedly unequal and residuals were not normally distributed. Therefore, analysis of variance was conducted using a natural log transform of the plant count data, which improved the variances and distribution of the residuals.

RESULTS

Precipitation and Soil Water. Growing season (March 20 - October 10) precipitation amounts for 1997-2001 were respectively 480, 302, 523, 247 and 311 mm, compared to the long-term average of 280 mm for the site. Soil water contents (SWC) averaged 12.1% in elevated CO_2 chambers over the entire study, significantly greater ($P<0.05$) than the 10.7% soil water content measured in ambient chambers. While these seasonally averaged differences in SWC do not appear great, they are significant as they include periods in which there were no treatment differences in SWC, as well as sustained periods in which substantial differences were maintained for long periods (LeCain et al., in review).

Soil water content was 12.7% in the non-chambered control plots, also greater than that measured in the ambient plots. Higher plant production in the ambient chambers (below) likely increased transpiration sufficiently to reduce soil water content relative to that in the control plots.

Phytomass. The summer phytomass data were obtained by clipping only half of the plots to simulate the approximate 50% forage removal of recommended grazing practices for the shortgrass steppe, while the autumn harvest (for calculating total annual production) removed all remaining vegetation in previously clipped and non-clipped sections of the plots (see Materials and Methods for more detail). 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 of the plots by 33% compared to that harvested in the ambient chambers at mid-season (Fig. 1). Similar results were found for total seasonal phytomass production. These results are consistent with earlier reports of strong productivity responses of shortgrass steppe vegetation to elevated CO_2 (Hunt et al., 1996; Morgan et al., 1998, 2001a). 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 250 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. Rainfall was plentiful in the spring of 2001, but was

inconsequential late-season (LeCain et al., in review), accounting for the low annual productivity in 2001 (Fig. 2). Amounts of precipitation were above the long-term growing season average of 280 mm for the other three years, accounting for their higher productivity.

Functional Group Comparisons. The summer phytomass data were harvested and processed by species and functional groups (C_3 grasses, C_4 grasses, forbs). The statistical analysis evaluated CO_2 treatment, functional groups, years, and all two-way and the one three-way interaction. All main effects, one of the two-way interactions, and the three-way interaction were all highly significant ($P \leq 0.01$; 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). Trends suggesting higher productivity for CO_2 enriched C_3 grasses were observed in 1999-2001. 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 forbs was enhanced significantly in elevated CO_2 OTCs only in 1998.

Species Comparisons. A third analysis of variance was conducted to investigate CO_2 treatment responses of the three dominant species of the site, *B. gracilis* (C_4 comprised 45% of the vegetation at the site in 1996), *P. smithii* (C_3, 18% in 1996), and *S. comata* (C_3, 25% in 1996). The remaining approximate 30 species of the experimental site are mostly perennial C_3 and C_4 grasses and some forbs, and their relative contribution to production varied from year to year. Analysis of variance conducted over all five years of the study indicated significant effects of CO_2, species, and year on productivity (Table 3). Significant 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 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 production was stimulated only for the C_3 grass *S. comata*, with plants grown under elevated CO_2 having 84% greater phytomass at mid-season compared to ambient chamber plants (Fig. 4). Surprisingly, no CO_2 induced production increase was observed for the other co-dominant C_3 grass, *P. smithii*, nor 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 3) 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 productivity 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 the C_4, *B. gracilis*.

Culm/Plant Counts. The yearly summer culm counts 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 ($P=0.02$), with higher numbers of plants in elevated compared to ambient chambers, especially in the small-sized plants, indicating increased recruitment from seed due to growth under elevated CO_2 (Fig. 6). A 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*. These data also show that *S. comata* is lower in digestibility than the other two grass species. The decline in digestibility from control conditions tended to be greater ($P=0.10$) for *S. comata* than for the other species.

DISCUSSION

Production. 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 parameters indicated higher soil water contents and leaf water potentials of dominant shortgrass steppe grasses in elevated compared to ambient OTCs (LeCain et al., in review; Morgan et al., 2001a). 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 in grasslands due to elevated CO_2 (Drake et al., 1996a; Jackson et al., 1994; Kirkham et al., 1991; Morgan et al., 2001a,b; Nie et al., 1992; Owensby et al., 1996a; Sindhoj et al., 2000; Volk et al., 2000). However, in a Nevada arid desert, CO_2-induced growth enhancements of vegetation were evident only in years with above-average rainfall (Smith et al., 2000). Further, Hunt et al. (1996) observed that production responses of shortgrass steppe vegetation were greatest at intermediate levels of water stress compared to well-watered or highly water-stressed conditions. These latter findings indicate a critical level of soil water availability that is required to elicit CO_2-induced production responses, below which little or no significant response to CO_2 occur. That level may have to be fairly low in the shortgrass steppe since in the present study we could detect no interaction of year with the CO_2-induced growth enhancement, despite the fact that precipitation amounts were 22% below normal in 2000. Collectively, these findings would suggest that in modeling ecosystem responses to rising atmospheric CO_2, the relative production responses will tend to be inversely proportional to the available soil water supply. But as soil water declines, this trend will reverse and CO_2-induced production responses will decline with lower soil water until a threshold is reached at which no production responses to higher CO_2 are realized due to extreme water shortage. The absolute amount of available soil water required for this threshold will likely vary among ecosystems, especially those systems that differ substantially in annual precipitation since plants will have very different adaptabilities to soil water dynamics.

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 forbs 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 detect a different response among the C_3 and C_4 grasses (Morgan et al., 2001a). 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 *S. comata* exhibited higher aboveground phytomass in response to elevated CO_2. *P. smithii*, which has been evaluated in monocultures in several previous studies and found to be responsive to CO_2 (Hunt et al., 1996; Morgan et al., 1998; Read and Morgan, 1996), 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 exhibited strong and consistent down-ward acclimation of photosynthesis, often to the extent that photosynthesis rates are similar in ambient and elevated CO_2-grown plants (Morgan et al., 1994a; Read et al., 1997). The same was observed for leaves measured in this field study (LeCain et al., in review; Morgan et al., 2001a), suggesting that little or no direct photosynthetic gain was realized by *P. smithii* plants growing in the elevated CO_2 OTCs. Although we have considerably less information on the photosynthetic responses of *S. comata*, water relations appeared to be the primary driver behind CO_2-induced responses in this species as well (LeCain et al., in review).

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., 1994a) and growth (Hunt et al., 1996; Morgan et al., 1994b) 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 plants, they do not necessarily reflect responses that may develop in more complex plant communities where competition for resources or microclimatic feed-backs often define the outcome of environmental perturbations.

The inclusion of *S. comata* in this study was fortuitous, as it provided 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 *B. 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 co-dominant C_3 grasses. We assumed that seed recruitment would be a minor feature of this study since the system dominant, *B. gracilis*, recovers slowly after disturbance, often taking several decades for stand density to return to pre-disturbance 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 in number 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 (personal observations), and suspect that high rainfall in the early years of our experiment contributed to seedling recruitment of this species at the site. Extending that thinking to our CO_2 enrichment experiment, higher soil water content in the elevated CO_2 chambers may have enhanced recruitment over that occurring in the ambient chambers. While we can't determine the relative importance of individual plant production vs. the recruitment response to CO_2, both were likely important. Increased plant numbers alone would be insufficient to account for CO_2-induced production since water limitations restrict production in this semi-arid grassland. Higher water use efficiency from elevated CO_2 no doubt contributed to the production response of *S. comata*.

The chamber response, which tends to increase production and plant number, deserves comment. We reported the production response previously (Morgan et al., 2001a), and attributed it to warmer chamber temperatures, which results in earlier spring green-up and may enhance N mineralization, leading to more available soil N, thereby enhancing production (Rustad et al., 2001). However, it seems likely that the production response may also be linked to the increased recruitment which occurred in the chambered plots. It is difficult to interpret the chamber effect on recruitment since the placement of chambers on the ambient plots during the growing season and the exclusion of chambers from the control plots means that very different patterns of seed availability and dispersal prevailed between chambered and non-chambered plots. The fact that the chamber effect for *S. comata* plant count

numbers was, with one exception, limited to plants with a basal diameter less than 11 mm suggests that the response occurred late in the study. However, as mentioned earlier, increased recruitment alone is likely insufficient to account for large production responses in the shortgrass steppe, where annual production is strongly regulated by seasonal variations in water and temperature.

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 (Coffin and Lauenroth, 1996; Sims, 1991). It's 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 semi-arid 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 sub-humid tallgrass prairie on its eastern boundaries in mid-western 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*. 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). In fact, we suspect that the significant year by species response in the present study (Table 3) was driven in part by year-to-year variation in precipitation and temperature patterns. Patterns of high and low production years for the two C_3 grasses were similar, and they contrasted some from the C_4 response (Figure 5). However, we were unable to relate those different growth patterns with the precipitation and temperature data collected.

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. A reduction in *B. gracilis* in future CO_2-enriched environments could increase the susceptibility and severity of drought in the shortgrass steppe.

The reduction in digestibility of the dominant perennial grasses of this ecosystem 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 (Campbell et al., 1997; Drake et al., 1996; Morgan et al., 2001a; Owensby et al., 1996; Polley et al., 2000). 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 towards 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. (1996a) 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.

Acknowledgements. We thank Dean Ackerman, Barry Weaver, Dennis Mueller, Scott Andre, Doug Whitt, Lisa Auen and Clenton Owensby for chamber design and construction, and Mary Ashby, Jeff Thomas, Mary Smith, Susan Crookall, Larry Tisue for technical assistance. Mark West was instrumental in statical analyses. This research was supported, in part, from funding received from the NSF-TECO award IBN-9524068, NSF award DEB-9708596, and from the NSF Shortgrass Steppe LTER Project DEB-9350273.

LITERATURE CITED

Allen-Diaz, B. 1995. Rangelands in a changing climate: impacts, adaptations, and mitigation. Pages 130-158 *in* R.T. Watson, M.C. Zinyowera, R.H. Moss and D.J. Dokken (editors), Climate Change 1995 - Impacts, Adaptations and Mitigation of Climate Change. Scientific-Technical Analyses, Cambridge University Press, NY.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO2 levels. Annual Review of Ecological Systems **21**:167-196.

Bowes, G. 1993. Facing the inevitable: plants and increasing atmospheric CO2. Annual review of Plant Physiology and Plant Molecular Biology **44**:309-332.

Campbell, B.D., D.M. Stafford-Smith and GCTE Pastures and Rangelands Network Members. 2000. A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. Agriculture Ecosystems & Environment **82**: 39-55.

Campbell, B.D., D.M. Stafford-Smith and G.M. McKeon. 1997. Elevated CO_2 and water supply interactions in grasslands: a pastures and rangelands management perspective. Global Change Biology **3**: 177-187.

Chiariello, N.R. and C.B. Field. 1996. Annual grassland responses to elevated CO_2 in multiyear community microcosms. Pages 139-157 in C. Körner and F.A. Bazzaz (editors) Carbon Dioxide, Populations, and Communities. Academic Press, Inc., San Diego, CA.

Coffin, D.P. and W.K. Lauenroth. 1996. Transient responses of North-American Grasslands to change in climate. Climatic Change **34**:269-278.

Drake, B.G., Gonzalez,-Meler, M.A., Long S.P. 1996. More efficient plants: A consequence of rising atmospheric CO_2? Ann. Rev. Plant Physiology and Molecular Biology **48**:607-637.

Edwards, G.R., H. Clark, P.C.D. Newton. 2001. The effects of elevated CO_2 on seed production and seedling recruitment in a sheep-grazed pasture. Oecologia **127**: 383-394.

Hart, R.H. 2001. Plant biodiversity on shortgrass steppe after 55 years of zero, light, moderate, or heavy cattle grazing. Plant Ecology **155**:111-118.

Hunt, H.W., Elliott, E.T., Detling, J.K., Morgan, J.A., Chen, D-X. 1996. Responses of a C_3 and C_4 perennial grass

to elevated CO_2 and climate change. Global Change Biology **2**: 35-47.

Hyder, D.N., A.C. Everson, and R.E. Bement. 1971. Seedling morphology and seeding failures with blue grama. Journal of Range Management **24**:287-292.

IPCC. 2001. The carbon cycle and atmospheric carbon dioxide. Pages 183-237 *in* J.T. Houghton, Y. Ding, D.J. Griggs, M. Noguer, P.J. van der Linden, X. Dai, K. Maskett, and C.A. Johnson (editors), Climate Change 2001: The Scientific Basis. Cambridge Univ. Press, Cambridge, UK.

Jackson, R.B., Sala, O.E., Field C.B., Mooney, H.A. 1994. CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. Oecologia **98**: 257-262.

Kirkham, M.B., H. He, T.P. Bolger, D.J. Lawler and E.T. Kanemasu. 1991. Leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. Crop Science **31**: 1589-1594.

Klipple, G.E. and D.F. Costello. 1960. Vegetation and cattle response to different intensities of grazing on shortgrass ranges on the central Great Plains. USDA Tech. Bull. 1216, USDA, Washington, DC.

Lauenroth, W.K., and D.P. Coffin. 1992. Belowground processes and the recovery of semiarid grasslands from disturbance. Pages 131-150 *in* M.K. Wali (editor) Ecosystem Rehabilitation, volume 2: Ecosystem analysis and synthesis. SPB Academic Publishing, The Hague, The Netherlands.

Lauenroth, W.K. and D.G. Milchunas. 1991. Short-grass steppe. Pages 183-226 *in* R.T. Coupland (editor) Ecosystems of the World 8A: Natural Grasslands. Elsevier, Amsterdam.

LeCain, D.R., J.A. Morgan, A.R. Mosier and J.A. Nelson. Soil and plant water relations, not photosynthetic pathway, primarily influence photosynthetic responses in a semi-arid ecosystem under elevated CO_2. (*in review*, New Phytologist)

Milchunas, D.G. and D.L. Baker. 1982. In vitro digestion - sources of within- and between-trial variability. Journal of Range Management **35**: 199-203.

Milchunas, D.G., W.K. Lauenroth and O.E. Sala. 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. American Naturalist **132**:87-106.

Milchunas, D.G., A.S. Varnamkhasti, W.K. Lauenroth and H. Goetz. 1995. Forage quality in relation to long-term grazing history, current-year defoliation, and water resource. Oecologia **101**:366-374.

Morgan, J.A., H.W. Hunt, C.A. Monz and D.R. LeCain. 1994a. Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4). Plant, Cell and Environment **17**:1023-1033.

Morgan, J.A., W.G. Knight, L.M. Dudley and H.W. Hunt. 1994b. Enhanced root system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic *Bouteloua gracilis* subjected to CO_2 enrichment. Plant and Soil **165**:139-146.

Morgan, J.A., D.R. LeCain, A.R. Mosier and D.G. Milchunas. 2001a. 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.

Morgan, J.A., D.R. LeCain, J.J. Read, H.W. Hunt and W.G. Knight. 1998. Photosynthetic pathway and ontogeny affect water relations and the impact of CO_2 on *Bouteloua gracilis* (C_4) and *Pascopyrum smithii* (C_3). Oecologia **114**: 483-493.

Morgan, J.A., P.C.D. Newton, J. Nösberger and C.E. Owensby. 2001b. The influence of rising atmospheric CO_2 on grassland ecosystems. Pages 973-980 *in* Proceedings of the XIX Intn. Grassland Congress. Brazilian Society of Animal Husbandry, Piracicaba, SP, Brazil.

Nie, D., H. He, G. Mo, M.B. Kirkham and E.T. Kanemasu. 1992. Canopy photosynthesis and evapotranspiration of rangeland plants under doubled carbon dioxide in closed-top chambers. Agricultural and Forest Meteorology **61**: 205-217.

Owensby, C.E., R.M. Cochran and L.M. Auen. 1996a. Effects of elevated carbon dioxide on forage quality for ruminants. Pages 363-371 *in* C. Körner and F. Bazzaz (editors) Carbon Dioxide, Populations and Communities. Physiological Ecology Series, Academic Press.

Owensby, C.E., P.I. Coyne, J.M. Ham, L.M. Auen, A.K. Knapp. 1993. Biomass production in a tallgrass prairie ecosystem exposed to ambient and elevated CO_2. Ecological Applications **3**: 644-653.

Owensby, C.E., J.M. Ham, A. Knapp, C.W. Rice, P.I. Coyne and L.M. Auen. 1996b. Ecosystem-level responses of tallgrass prairie to elevated CO2. Pages 147-162 *in* C. Körner and F. Bazzaz (editors) Carbon Dioxide, Populations and Communities. Physiological Ecology Series, Academic Press.

Owensby, C.E., J.M. Ham, A.K. Knapp and L.M. Auen. 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.

Polley, H.W., H.S. Mayeux, H.B. Johnson and Tischler, C.R. 1997. Viewpoint: atmospheric CO_2, soil water, and shrub/grass ratios on rangelands. Journal of Range Management **5**0: 278-284.

Polley, H.W., J.A. Morgan, B.D. Campbell and M. Stafford-Smith. 2000. Crop ecosystem responses to climatic change: rangelands. Pages 293-314 *in* K.R. Reddy and H.F. Hodges (editors). Climate Change and Global Crop Productivity. CABI Publishing, NY.

Poorter, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO2 concentration. Vegetatio **104/105**: 77-97.

Read, J.J. and J.A. Morgan. 1996. Growth and partitioning in *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) as influenced by carbon dioxide and temperature. Ann. Bot. **77**:487-496.

Read, J.J., J.A. Morgan, N.J. Chatterton, and P.A. Harrison. 1997. Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) at different carbon dioxide concentrations and temperatures. Annals of Botany **79**:197-206.

Rustad, L.E., J.L. Campbell, G.M. Marion, R.J. Norby, M.J. Mitchell, A.E. Hartley, J.H.C. Cornelissen, J. Gurevitch, and GCTE-NEWS. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia **126**:543-562.

Sims, P.L. 1991. Grasslands. Pages 265-286 *in* M.G. Barbour and W.D. Billings (editors), North American Terrestrial Vegetation, Cambridge University Press, Cambridge.

Sindhoj, E., A-C. Hansson, O. Andren, T. Katterer, M. Marissink and R. Petterson. 2000. Root dynamics in a semi-arid grassland in relation to atmospheric carbon dioxide enrichment, soil water, and shoot biomass. Plant and Soil **223**: 253-263.

Sionit, N. and D.T. Patterson. 1984. Responses of C_4 grasses to atmospheric CO_2 enrichment. I. Effect of irradiance. Oecologia **65**: 30-34.

Smith, S.D., T.E. Huxman, S.F. Zitzer, T.N. Charlet, D.C. Houseman, J.S. Coleman, L.K. Fenstermaker, J.R. Seemann Jr. and R.S. Nowak. 2000. Elevated CO_2 increases productivity and invasive species success in an arid ecosystem. Nature **408**: 79-82.

Tilley, J.M.A. and R.A. Terry. 1963. A two-stage technique for in vitro digestion of forage crops. Journal of the British Grassland Society **18**: 401-411.

Volk, M., P.A. Niklaus and C. Korner. 2000. Soil moisture effects determine CO_2 responses of grassland species. Oecologia **125**:380-388.

Wand, S.J.E. 1999. Ecophysiological responses of *Themeda triandra* Forsk. and other southern African C_4 grass species to increases in atmospheric CO_2 concentrations. DS Thesis, University of Cape Town, South Africa.

Wand, S.J.E, G.F. Midgley, M.H. Jones, P.S. Curtis. 1999. Responses of wild C_4 and C_3 grass (Poaceae) species to elevated CO_2 concentration: a meta-analytic test of current theories and perceptions. Global Change Biology **5**:723-741.

Table1. Treatment variable probabilities for aboveground production data from five years (1997-2001) of CO_2 treatment (ambient (360 µl l^{-1}), elevated (720 µl l^{-1}), and unchambered plots) in open-top-chambers on the shortgrass steppe of eastern Colorado, USA. Results are given for a mid-season (late July) and end of the season harvest.

	Production	
source of variation	mid-season	whole season
CO_2 treatment	0.0001	0.0001
year	0.0001	0.0001
CO_2 * year	0.1907	0.2194

Table 2. Treatment variable probabilities for aboveground phytomass (harvested at peak standing crop) from five years (1997-2001) of CO_2 treatment (ambient (360 µl l^{-1}), elevated (720 µl l^{-1}), and unchambered plots) in open-top-chambers on the shortgrass steppe of eastern Colorado, USA. The three functional groups are C_3 grasses, C_4 grasses and forbs. Results are given for the mid-season (late July) harvest.

Source of variation	Probability
CO_2 treatment	0.0001
Functional group	0.0001
Year	0.0001
CO_2 treatment * Functional group	0.5051
CO_2 treatment * Year	0.1324
Functional group * Year	0.0001
CO_2 treatment * Functional group * Year	0.0092

Table 3. Treatment variable probabilities for aboveground phytomass (harvested at peak-standing crop) from five years (1997-2001) of CO_2 treatment (ambient (360 µl l^{-1}), elevated (720 µl l^{-1}), and unchambered plots) in open-top-chambers on the shortgrass steppe of eastern Colorado, USA. The species are *Bouteloua gracilis*, *Pascopyrum smithii* and *Stipa comata*.

Source of variation	Aboveground Phytomass
CO_2 treatment	0.0017
Species	0.0001
Year	0.0001
CO_2 trt. * Species	0.0978
CO_2 trt. * Year	0.0156
Species * Year	0.0001
CO_2 trt * Species * Year	0.5339

FIGURE CAPTIONS

Figure 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 m^{-2} s^{-1}) open-top-chambers and unchambered controls on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

Figure 2. Mean phytomass at the summer harvest and over the entire season (averaged over the three treatments) during five years on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

Figure 3. Mean phytomass of the major functional groups at the summer harvest in ambient and elevated CO_2 (360 and 720 μmol m^{-2} s^{-1}) open-top-chambers and unchambered controls on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

Figure 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 m^{-2} s^{-1}) open-top-chambers and unchambered controls on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

Figure 5. Mean phytomass of the three main species, at the summer harvest (averaged over the three treatments), during five years on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

Figure 6. Numbers of *Stipa comata* plants m^{-2} for total plants and different size classes of plants in ambient and elevated CO_2 (360 and 720 μmol m^{-2} s^{-1}) open-top-chambers and unchambered controls on the short-grass steppe of

eastern Colorado. Means within a harvest with different letters are significantly different using t-test ($P < 0.05$).

Figure 7. In vitro digestible dry matter of the three main species (averaged over two years) in ambient and elevated CO_2 (360 and 720 μmol m^{-2} s^{-1}) open-top-chambers and unchambered controls on the short-grass steppe of eastern Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

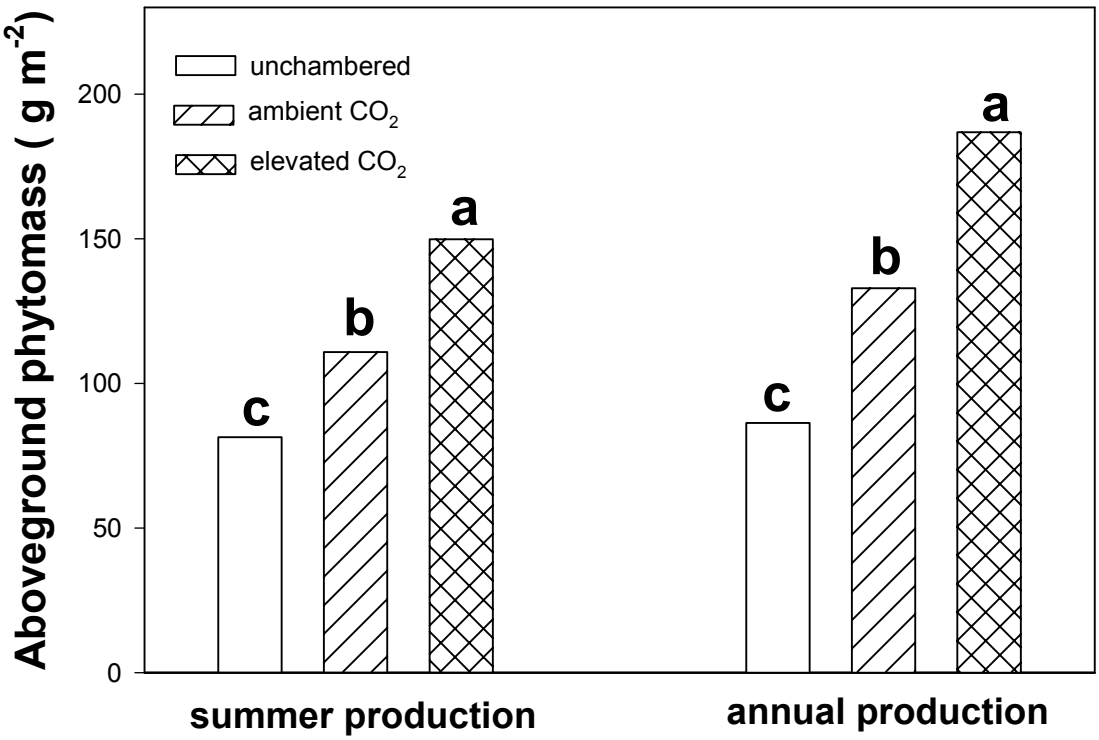

Aboveground phytomass (g m-2)
unchambered
ambient CO2
elevated CO2
200
150
100
50
0
c
b
a
c
b
a
summer production
annual production

Fig. 1

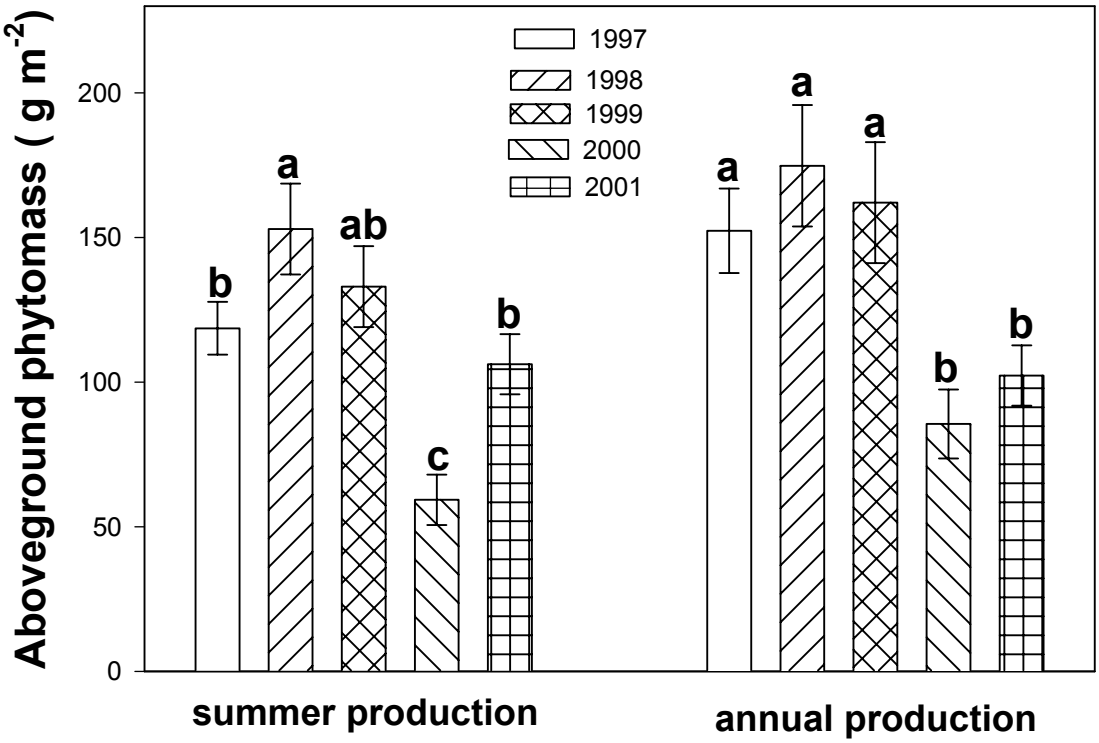
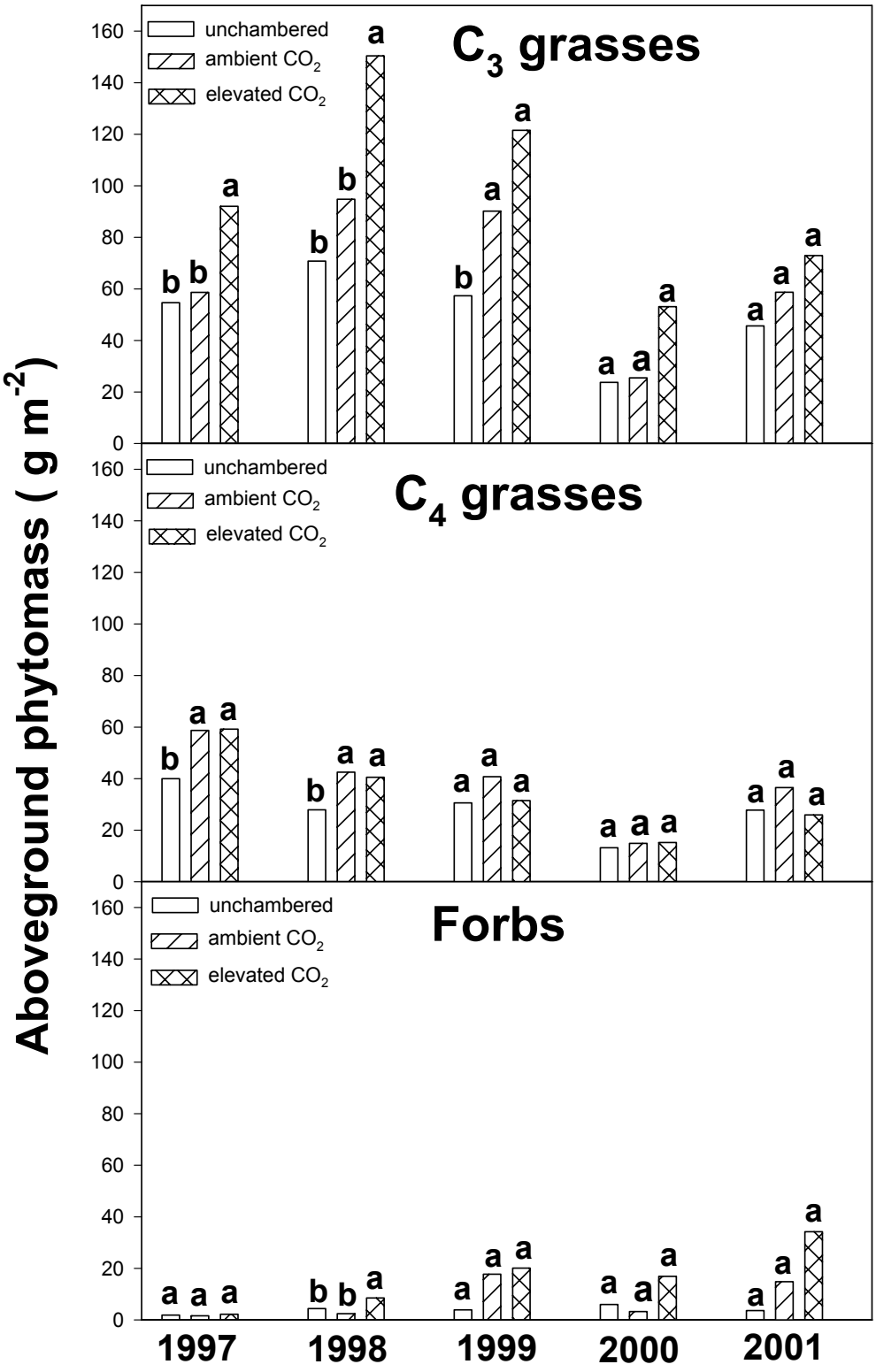

Aboveground phytomass (g m-2)
200
150
100
50
0
1997
1998
1999
2000
2001
a
ab
b
c
b
a
a
a
b
b
summer production
annual production

Fig. 2

Fig. 3


C3 grasses
C4 grasses
Forbs
unchambered
ambient CO2
elevated CO2
Aboveground phytomass (g m-2)
0
20
40
60
80
100
120
140
160
1997
1998
1999
2000
2001

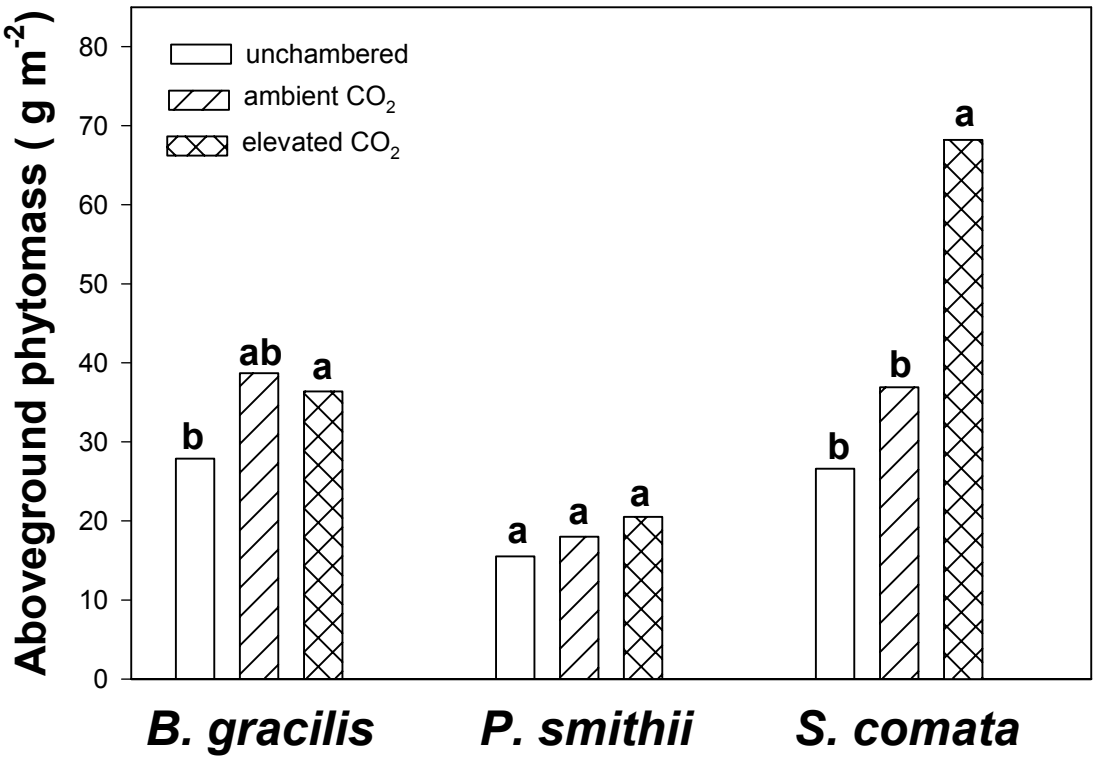

Aboveground phytomass (g m-2)
unchambered
ambient CO2
elevated CO2
0
10
20
30
40
50
60
70
80
b
ab
a
a
a
a
b
b
a
B. gracilis
P. smithii
S. comata

Fig. 4

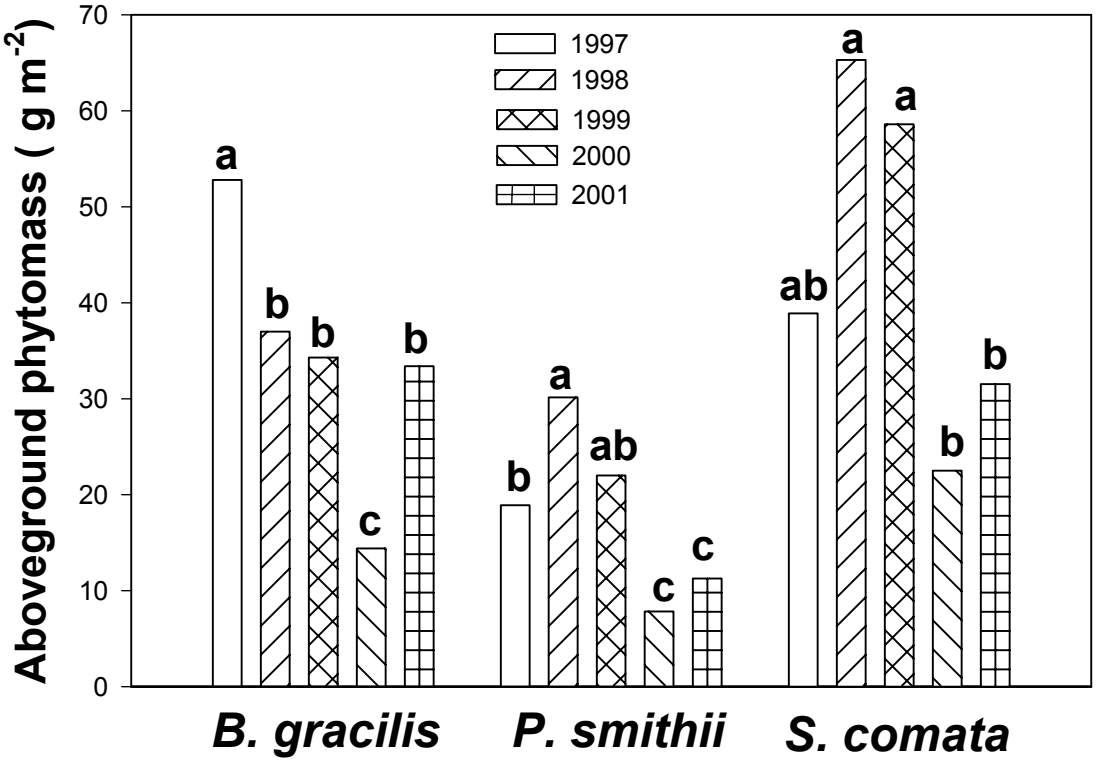

Aboveground phytomass (g m-2)
70
60
50
40
30
20
10
0
1997
1998
1999
2000
2001
a
b
b
c
b
b
a
ab
c
c
ab
a
b
b
B. gracilis
P. smithii
S. comata

Fig. 5

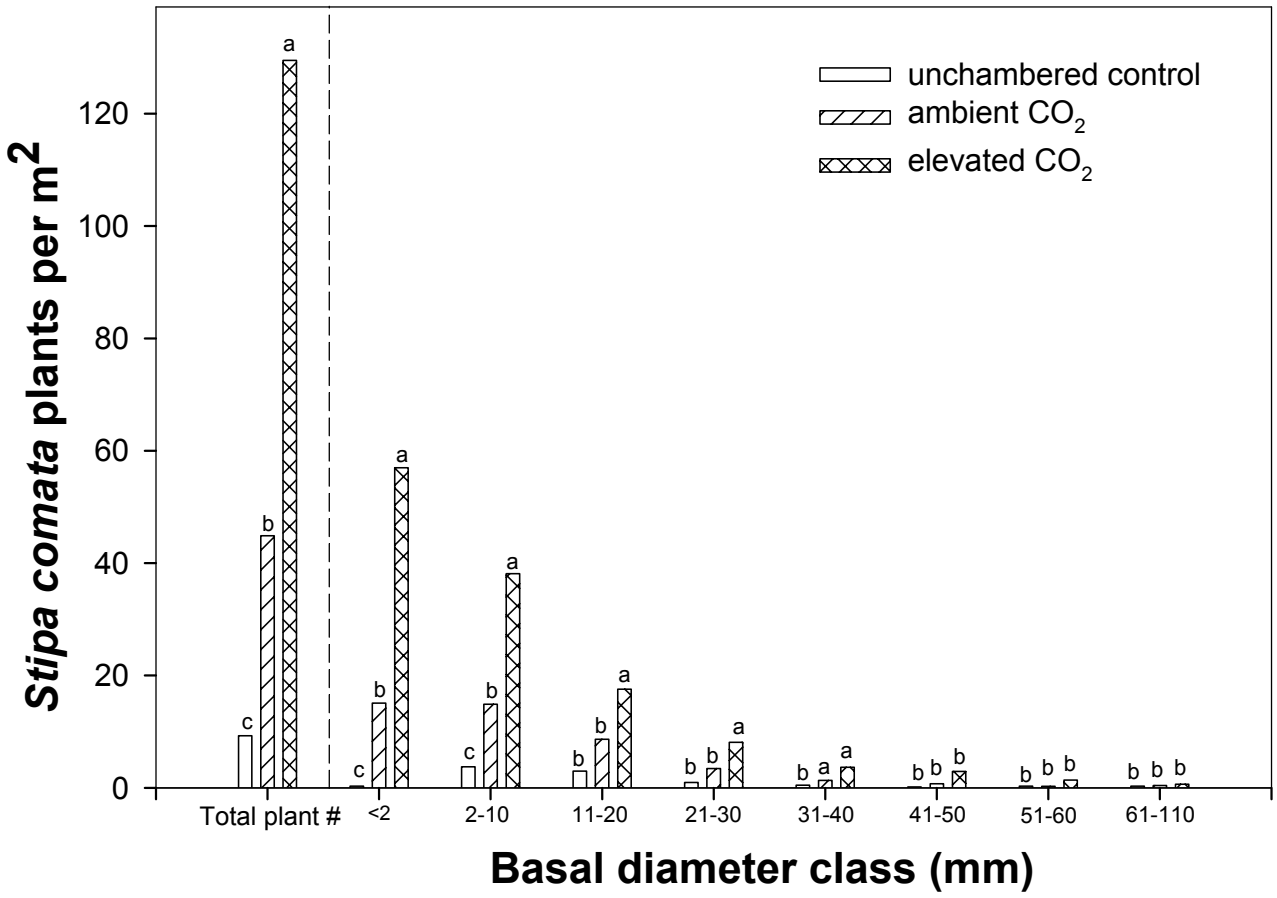

unchambered control
ambient CO2
elevated CO2
Stipa comata plants per m2
0
20
40
60
80
100
120
Total plant #
<2
2-10
11-20
21-30
31-40
41-50
51-60
61-110
Basal diameter class (mm)

Fig. 6

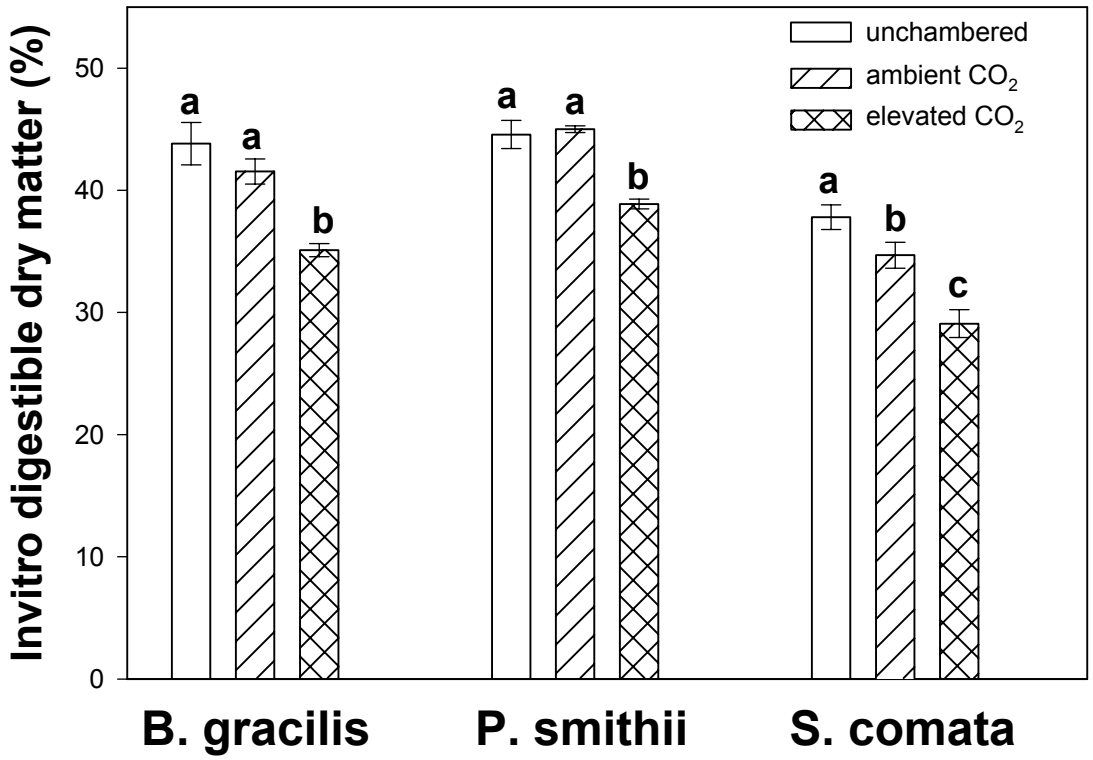

Invitro digestible dry matter (%)
0
10
20
30
40
50
unchambered
ambient CO2
elevated CO2
a
a
b
a
a
b
a
b
c
B. gracilis
P. smithii
S. comata

Fig. 7