

**CO₂ 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

1 **ABSTRACT**

2 The impact of increasing atmospheric CO₂ concentrations has been studied in a number of field experiments, but
3 little information exists on the response of semi-arid rangelands to CO₂, and even less on consequences for forage
4 quality. This study was initiated to study the CO₂ response of the shortgrass steppe, an important semi-arid
5 grassland on the western edge of the North American Great Plains used extensively for livestock grazing. The
6 experiment was conducted for five years on native vegetation at the USDA-ARS Central Plains Experimental Range
7 in north-eastern Colorado. Three perennial grasses dominate the study site, *Bouteloua gracilis* (H.B.K.) Lag., a C₄
8 grass, and two C₃ grasses, *Pascopyrum smithii* (Rybd.) A. Love and *Stipa comata* Trin and Rupr. The three species
9 comprise 88% of the aboveground phytomass. To evaluate responses to rising atmospheric CO₂, we utilized six
10 open-top chambers, three with ambient air, three with air CO₂-enriched to 720 μL L⁻¹, and three unchambered
11 controls. We found that elevated CO₂ enhanced production of the shortgrass steppe throughout the study, with 38%
12 greater aboveground phytomass harvested annually in elevated compared to ambient plots. The CO₂-induced
13 production response was driven by a single species, *S. comata*, and was due in part to greater seedling recruitment.
14 The result was species movement towards a composition more typical of the mixed-grass prairie. Growth under
15 elevated CO₂ reduced the digestibility of all three dominant grass species, and was lowest in the only species to
16 exhibit a CO₂-induced production enhancement, *S. comata*. The results suggest that rising atmospheric CO₂ may
17 enhance production of lower quality, more drought sensitive forage in the shortgrass steppe.

18 **Key Words:** *Bouteloua gracilis*, C₃, C₄, Carbon dioxide, CO₂, Digestibility, Forage quality, Global change,
19 Grassland, *Pascopyrum smithii*, Recruitment, *Stipa comata*.

INTRODUCTION

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3 Atmospheric [CO₂] has been rising steadily from approximately 280 μL L⁻¹ at the start of the industrial
4 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).
5 Most plant species exhibit increased production when [CO₂] is increased above present ambient concentrations
6 (Drake et al., 1996; Poorter 1993). This growth enhancement has been attributed to a direct stimulation in
7 photosynthesis and/or to improved plant water relations, depending on the particular species and the environment
8 (Campbell et al., 2000; Drake 1996a; Poorter 1993; Wand et al., 1999). In native grasslands of North America like
9 the sub-humid tallgrass prairie of Kansas (Kirkham et al., 1991; Nie et al., 1992; Owensby et al., 1993, 1996b,
10 1999), California annual grassland (Chiariello and Field, 1996; Jackson et al., 1994), or the semi-arid shortgrass
11 steppe of Colorado (Morgan et al., 2001a), enhanced production under elevated CO₂ atmospheres has been
12 attributed primarily to improved water relations through stomatal closure and increased water use efficiency, and
13 secondarily to increased photosynthesis. The fact that photosynthetic responses have not been primarily featured in
14 the responses of grasslands to elevated CO₂ may explain, in part, the apparent lack of the predicted advantage for
15 native C₃ grasses under elevated CO₂ compared to C₄ species (Hunt et al., 1996; Morgan et al., 2001a; Owensby et
16 al., 1993, 1996b, 1999). C₃ photosynthesis is limited by present-day ambient CO₂ concentrations while C₄
17 photosynthesis is nearly CO₂-saturated. Consequently, early predictions were that increasing atmospheric CO₂
18 concentrations would favor C₃ over C₄ species (Bazzaz, 1990; Bowes, 1993). However, the water relations benefit
19 of elevated CO₂ is realized by most plant species, independent of photosynthetic pathway, which tends to minimize
20 differences in species responses to CO₂ in water limited systems. Further, the potential for increased plant
21 production from elevated [CO₂] may be greater in grasslands that are characteristically water-limited (Campbell et
22 al., 2000; Morgan et al., 2001b; Volk et al., 2000).

23 In addition to relatively direct effects of CO₂ on photosynthesis and improved water relations, CO₂
24 enrichment may sometimes enhance seedling recruitment (Edwards et al., 2001). This can result from direct effects
25 of CO₂ on reproductive attributes like seed number and quality or from the effects of CO₂ on seed-bed environment,
26 most importantly soil water content, light and temperature. Recruitment success depends also on potentially

1 complicated interactions with CO₂-induced responses of competitors, like enhanced growth, which can increase the
2 canopy and leave fewer spaces for seedling establishment (Edwards et al., 2001). Recruitment may be especially
3 important as a mechanism for CO₂-induced plant community changes.

4 Today's remaining native grasslands are used extensively for livestock grazing. As such, forage quality
5 and seasonality of its production are just as critical as production in determining the value of these lands. There
6 have been reports that elevated [CO₂] may sometimes reduce forage quality (Campbell et al., 2000; Owensby et al.,
7 1996a; Wand et al., 1999), primarily by lowering forage N concentration. Perhaps more significant are the theorized
8 shifts in vegetation composition predicted in future CO₂-enriched environments, with some scenarios suggesting a
9 movement towards plant communities of lower forage quality (Campbell et al., 2000; Polley et al., 1997).

10 Collectively, these reports suggest that rising atmospheric [CO₂] has the potential to cause significant alterations in
11 grassland structure and function, with some outcomes leading to botanically more productive but less useful
12 grasslands.

13 In 1996, open-top chambers were installed on the Colorado shortgrass steppe to evaluate the responses of
14 this grassland to elevated CO₂. Morgan et al. (2001a) reported that two years of CO₂ enrichment had enhanced mid-
15 and total season production of shortgrass steppe from 26 to 47%, with no production differences detected between
16 C₃ and C₄ species. This paper evaluates the effects of five years of CO₂ enrichment on aboveground productivity of
17 this same experiment, and evaluates production responses across all species, by functional groups (C₃ grasses, C₄
18 grasses and forbs) and by site co-dominant species (*Pascopyrum smithii*, C₃; *Stipa comata*, C₃; *Bouteloua gracilis*,
19 C₄). Based on earlier growth chamber work in which we observed similar and significant CO₂-induced
20 enhancements in productivity of shortgrass steppe C₃ and C₄ grasses, grown in monoculture (Hunt et al., 1996), we
21 predicted no functional group or species differences to growth under elevated CO₂ in the shortgrass steppe. We
22 assumed that recruitment from seed would not be an important feature of the system response to CO₂, due in large
23 part to the difficulty in re-seeding the dominant, *B. gracilis*, into disturbed sites (Hyder et al., 1971). We evaluated
24 in vitro dry matter digestibility of co-dominant species, and predicted it would decline across species based on the
25 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 C₄ grass, and two cool season C₃ 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 C₃ and C₄ grasses, and a few forbs. The soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthids). 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 CO₂ on this native ecosystem was investigated using open top chambers (4.5 m diameter, enclosing 15.5 m²). A portion of the pasture was initially divided into three blocks, and three 15.5 m² 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 CO₂ treatments, to characterize the initial state of the system. In the fall of 1996, one chamber was randomly assigned an ambient CO₂ treatment (360±20 Φmol mol⁻¹), the other an elevated CO₂ treatment (720±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

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

2 **Phytomass Harvests.** Aboveground plant biomass harvests were conducted in the southern half of the
3 chambers. During the period of peak standing crop (late July), a metal wire grid containing fifty-six 40.5 X 15.3 cm
4 quadrats (3.46 m² total) was placed over the south half of each plot and vegetation in every other quadrat (28
5 quadrats) was clipped to the crown, separated by species, dried at 60° C and weighed. This defoliation protocol,
6 which removed 50% of the green vegetation and represents defoliation by cattle that is between moderate and heavy
7 grazing practices, was incorporated into the CO₂-treatment experimental design primarily as a means to represent
8 the nominal grazing conditions for these grasslands (Klippel and Costello 1960; Milchunas et al., 1988, 1995). In
9 autumn, aboveground phytomass was harvested in all 56 quadrats to estimate total seasonal productivity for the
10 experimental area. The pattern was reversed the following year; quadrats which had not been clipped the previous
11 summer were clipped at peak standing crop, and all quadrats harvested in the autumn. Annual alternation of the
12 small grids that received or did not receive the defoliation during a particular growing season allowed for cycles of
13 'rest-from-defoliation'. The October final harvest involved only senescent shoot material, and so did not elicit a
14 physiological response. Phytomass in the northern half of the chambers was defoliated at about 2 cm height in
15 October so that plant cover throughout the chamber was comparable at the beginning and end of each growing
16 season.

17 **Culm and Plant Counts.** Every year at peak standing crop, total numbers of *P. smithii* culms were
18 counted in each plot. Heavy grazing of the shortgrass steppe leads to increased relative abundance of *B. gracilis*,
19 with C₃ grasses like *P. smithii* decreasing (Hart, 2001), so we monitored culm numbers of *P. smithii* to determine
20 whether the defoliation regime was effecting stand density of this species.

21 Data analysis near the end of the study indicated a strong and exclusive aboveground phytomass response
22 of *S. comata* to growth under elevated CO₂. We therefore decided to count plant numbers of this species by size
23 classes within a few weeks after spring green-up following the final year of CO₂ enrichment (2001) to determine if
24 seedling recruitment was a factor in the production response of *S. comata*. On June 10, 2002, approximately three
25 weeks after spring green-up (which occurred late due to an extended drought), individual plant counts of *S. comata*
26 were made in the phytomass sampling area of each experimental plot, by size class (basal diameters of < 2 mm, 2-

1 10 mm, 11-20 mm, 21-30 mm, 31-40 mm, 41-50 mm, 51-60 mm and 61-110 mm). Basal diameters were
2 determined on each plant counted from caliper measurements performed in two dimensions, and averaging the
3 readings.

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

7 **In Vitro Dry Matter Digestibility.** Inoculum for IVDDM was collected from a fistulated cow maintained
8 on an exclusively grass-hay diet for one week prior to collection, with feed removed 12 hrs prior to rumen-pumping.
9 Precautions in handling rumen fluid necessary to maintain microbial activity, and methods of preparing and
10 delivering the inoculum follow those described in the 25-strain-layer method of Milchunas and Baker (1982).
11 IVDDM was run according to the two-stage, 96 hr, Tilly and Terry (Tilley and Terry 1963) method with
12 modifications and quality controls described in Milchunas and Baker (1982).

13 **Statistics.** CO₂ treatments were analyzed for their effects on soil water content and aboveground
14 productivity using the SAS PROC MIXED analysis (SAS Institute Inc., Cary, NC, USA), with year represented as a
15 repeated measure. Where significant treatment effects were detected, treatment comparisons were conducted
16 utilizing the Tukey's means comparison test at the 0.05 level of confidence unless otherwise indicated. Treatment
17 variances of the plant count data were decidedly unequal and residuals were not normally distributed. Therefore,
18 analysis of variance was conducted using a natural log transform of the plant count data, which improved the
19 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 CO₂ 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 CO₂ enrichment (1997 - 2001) indicated total aboveground phytomass was significantly affected ($P < 0.0001$) by CO₂ treatment and year (Table 1).

Elevated CO₂ 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 CO₂ (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

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

4 **Functional Group Comparisons.** The summer phytomass data were harvested and processed by species
5 and functional groups (C₃ grasses, C₄ grasses, forbs). The statistical analysis evaluated CO₂ treatment, functional
6 groups, years, and all two-way and the one three-way interaction. All main effects, one of the two-way interactions,
7 and the three-way interaction were all highly significant ($P \leq 0.01$; Table 2). Therefore, means comparisons were
8 conducted within each year for CO₂ treatments, within each of the three functional groups.

9 For C₃ grasses, a significant increase in aboveground phytomass under CO₂ enrichment was detected in
10 1997 and 1998 at the mid-season harvest (Fig. 3). Trends suggesting higher productivity for CO₂ enriched C₃
11 grasses were observed in 1999-2001. No CO₂ treatment effects on production were observed for the C₄ grasses,
12 although production was greater in chambered compared to control plots in 1997 and 1998. Production of forbs was
13 enhanced significantly in elevated CO₂ OTCs only in 1998.

14 **Species Comparisons.** A third analysis of variance was conducted to investigate CO₂ treatment responses
15 of the three dominant species of the site, *B. gracilis* (C₄ comprised 45% of the vegetation at the site in 1996), *P.*
16 *smithii* (C₃, 18% in 1996), and *S. comata* (C₃, 25% in 1996). The remaining approximate 30 species of the
17 experimental site are mostly perennial C₃ and C₄ grasses and some forbs, and their relative contribution to
18 production varied from year to year. Analysis of variance conducted over all five years of the study indicated
19 significant effects of CO₂, species, and year on productivity (Table 3). Significant two-way interactions between
20 CO₂ and year, species and year, and a strong trend ($P < 0.10$) between species and CO₂ treatment were observed, but
21 there was no significant three-way interaction. Therefore, means comparisons were conducted according to the two-
22 way interactions.

23 When evaluated over the five years of CO₂ enrichment, aboveground production was stimulated only for
24 the C₃ grass *S. comata*, with plants grown under elevated CO₂ having 84% greater phytomass at mid-season
25 compared to ambient chamber plants (Fig. 4). Surprisingly, no CO₂ induced production increase was observed for
26 the other co-dominant C₃ grass, *P. smithii*, nor for the C₄ grass, *B. gracilis*. Although total aboveground

1 productivity was significantly higher in ambient vs. unchambered plots (Fig. 1), no significant chamber effect was
2 detected when evaluating the three main grass species.

3 The interaction between year and CO₂ treatment (Table 3) was mostly due to differences between the
4 control plots and the elevated CO₂ chambers in 1997, and also to strong trends among the chambered treatments that
5 varied among years, but which were all in the direction of increased production in elevated CO₂ chambers (data not
6 shown).

7 Mid-year productivity varied among the species, with production being greatest in 1997 for *B. gracilis*,
8 while production in the two C₃ grasses peaked in 1998 and 1999 (Fig. 5). Productivity of all three grass species
9 dropped drastically in 2000 due to the drought. Productivity of the two C₃ grasses remained relatively low in 2001,
10 but increased from the previous year for the C₄, *B. gracilis*.

11 **Culm/Plant Counts.** The yearly summer culm counts of *P. smithii* revealed no treatment differences
12 throughout the experiment, nor was there a decline in culm density over time due to the defoliation (data not shown).
13 However, plant counts made of *S. comata* at the conclusion of the study revealed significant treatment effects for
14 CO₂ (P=0.02), with higher numbers of plants in elevated compared to ambient chambers, especially in the small-
15 sized plants, indicating increased recruitment from seed due to growth under elevated CO₂ (Fig. 6). A chamber
16 effect was also noted, with higher numbers of plants in ambient chambers compared to control plots in the smaller
17 sized classes.

18 **In Vitro Dry Matter Digestibility.** Measurements of in vitro digestibility conducted on summer-harvested
19 shoots in 1998 and 1999 indicated that growth at elevated CO₂ reduced digestibility by an average of 16% (Fig. 7) in
20 *S. comata*, *B. gracilis* and *P. smithii*. These data also show that *S. comata* is lower in digestibility than the other two
21 grass species. The decline in digestibility from control conditions tended to be greater (P=0.10) for *S. comata* than
22 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 CO₂ 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 CO₂-induced stomatal closure may be the dominant force behind growth enhancements in grasslands due to elevated CO₂ (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, CO₂-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 CO₂-induced production responses, below which little or no significant response to CO₂ 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 CO₂-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 CO₂, 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 CO₂-induced production responses will decline with lower soil water until a threshold is reached at which no production responses to higher CO₂ 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 C₃ grasses were most responsive to CO₂, showing significantly higher production in only two years, and trends suggested production enhancement from CO₂

1 enrichment in the other three years of the study. C₄ grasses were unresponsive to CO₂, and forbs responded with
2 higher production under elevated CO₂ only in 1998. These results differ from our analyses of this same experiment
3 after just two years of CO₂ enrichment in which we detected a 37% increase in aboveground phytomass, but could
4 not detect a different response among the C₃ and C₄ grasses (Morgan et al., 2001a). At first glance, these results
5 would appear to support, at least in part, the hypothesis that C₃ plants will respond more favorably to rising CO₂
6 concentrations compared to C₄ species (Bazzaz, 1990). However, a closer examination of the species responses
7 suggests that the patterns of CO₂ responses may go beyond photosynthetic pathway.

8 Of the two dominant C₃ species, only *S. comata* exhibited higher aboveground phytomass in response to
9 elevated CO₂. *P. smithii*, which has been evaluated in monocultures in several previous studies and found to be
10 responsive to CO₂ (Hunt et al., 1996; Morgan et al., 1998; Read and Morgan, 1996), showed no change in
11 aboveground phytomass under elevated CO₂ in the field. Extensive gas exchange studies have shown that leaves of
12 *P. smithii* grown at elevated CO₂ exhibited strong and consistent down-ward acclimation of photosynthesis, often to
13 the extent that photosynthesis rates are similar in ambient and elevated CO₂-grown plants (Morgan et al., 1994a;
14 Read et al., 1997). The same was observed for leaves measured in this field study (LeCain et al., in review; Morgan
15 et al., 2001a), suggesting that little or no direct photosynthetic gain was realized by *P. smithii* plants growing in the
16 elevated CO₂ OTCs. Although we have considerably less information on the photosynthetic responses of *S. comata*,
17 water relations appeared to be the primary driver behind CO₂-induced responses in this species as well (LeCain et
18 al., in review).

19 These are the first results to report differential species responses to CO₂ in the shortgrass steppe, and they
20 differ considerably from prior reports in which we indicated that elevated CO₂ enhanced photosynthesis (Morgan et
21 al., 1994a) and growth (Hunt et al., 1996; Morgan et al., 1994b) and improved plant water relations (Morgan et al.,
22 1998) in both *P. smithii* and *B. gracilis*. These previous experiments were conducted in environmental growth
23 chambers with monocultures of *P. smithii* and *B. gracilis*, with plants grown in reconstituted or native soil contained
24 in columns. While such experiments allow the genetic expression of plants, they do not necessarily reflect responses
25 that may develop in more complex plant communities where competition for resources or microclimatic feed-backs
26 often define the outcome of environmental perturbations.

1 The inclusion of *S. comata* in this study was fortuitous, as it provided a clear example of a species response
2 to CO₂ that would not have been predicted based on photosynthetic class assignment alone. Our early CO₂
3 enrichment studies focused on *B. gracilis* and *P. smithii*, the former because it is the dominant species of the
4 shortgrass steppe, and a C₄ grass, and the latter as a representative of several co-dominant C₃ grasses. We assumed
5 that seed recruitment would be a minor feature of this study since the system dominant, *B. gracilis*, recovers slowly
6 after disturbance, often taking several decades for stand density to return to pre-disturbance levels (Lauenroth and
7 Coffin, 1992). Further, an earlier growth chamber CO₂ enrichment study indicated no significant effect of elevated
8 CO₂ on seed production of *B. gracilis*, and the growth response of *P. smithii* to elevated CO₂ appeared to be driven
9 primarily by rhizomatous growth (Hunt et al., 1996). However, *S. comata* appears to be an opportunist, increasing
10 in number under disturbance or when soil water is more plentiful. We noticed that numbers of *S. comata* plants
11 increased over the five years of our experiment in the field where our open-top chambers were installed (personal
12 observations), and suspect that high rainfall in the early years of our experiment contributed to seedling recruitment
13 of this species at the site. Extending that thinking to our CO₂ enrichment experiment, higher soil water content in
14 the elevated CO₂ chambers may have enhanced recruitment over that occurring in the ambient chambers. While we
15 can't determine the relative importance of individual plant production vs. the recruitment response to CO₂, both
16 were likely important. Increased plant numbers alone would be insufficient to account for CO₂-induced production
17 since water limitations restrict production in this semi-arid grassland. Higher water use efficiency from elevated
18 CO₂ no doubt contributed to the production response of *S. comata*.

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

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

5 **Implications for Great Plains Grasslands.** The major grassland types in the Great Plains are formed in
6 large part due to a precipitation gradient which extends west to east (Coffin and Lauenroth, 1996; Sims, 1991). It's
7 most arid regions are in the south and central areas of the Great Plains, where a rain shadow created by the Rocky
8 Mountains limits precipitation on its western boundary and results in the semi-arid shortgrass steppe . Moving east
9 and out of the rain shadow, precipitation gradually increases and vegetation changes first into the southern mixed
10 grass prairie, and eventually into sub-humid tallgrass prairie on its eastern boundaries in mid-western states.
11 Northern regions of the Great Plains are almost all northern mixed-grass prairie. The shortgrass steppe and mixed-
12 grass prairies share many of the same species, but the mixed-grass prairie has a higher percentage of cool-season,
13 mid-grasses like *S. comata*. The results of our study, which show increased recruitment and greater production of a
14 C₃ shortgrass steppe species that is relatively more important in the mixed-grass prairie, represent the first empirical
15 evidence that rising CO₂ concentrations may have the potential to shift present boundaries between these two
16 important Great Plains grassland types. The final outcome of any such potential shifts will certainly involve
17 interactions with altered temperature and precipitation patterns, both of which are predicted to have strong impacts
18 on both production and shifts in C₃/C₄ dominance in the Great Plains (Coffin and Lauenroth, 1996). In fact, we
19 suspect that the significant year by species response in the present study (Table 3) was driven in part by year-to-year
20 variation in precipitation and temperature patterns. Patterns of high and low production years for the two C₃ grasses
21 were similar, and they contrasted some from the C₄ response (Figure 5). However, we were unable to relate those
22 different growth patterns with the precipitation and temperature data collected.

23 Another interpretation of the strong *S. comata* response is that rising CO₂ and accompanying climate
24 change may simply increase the relative presence of this grass species in much of the shortgrass steppe. This
25 apparent shift in favor of *S. comata* is important, as *S. comata* is more susceptible to drought than the current
26 shortgrass steppe dominant, *B. gracilis*. Weaver and Albertson (Allen-Diaz 1995) reported that fully four-fifths of

1 the remaining vegetation in the shortgrass steppe following the drought of the dust bowl days in the 1930s was
2 comprised of just two species, *B. gracilis*, and another warm-season grass, *Buchloe dactyloides*, with *B. gracilis*
3 being the more drought tolerant. That remaining cover of warm-season grasses was vitally important in providing
4 minimal forage during the drought, and a base of plants from which the shortgrass steppe recovered when normal
5 precipitation patterns resumed in the early 1940s. A reduction in *B. gracilis* in future CO₂-enriched environments
6 could increase the susceptibility and severity of drought in the shortgrass steppe.

7 The reduction in digestibility of the dominant perennial grasses of this ecosystem means that cattle will
8 have to consume relatively more forage compared to grasses grown under present CO₂ atmospheres to achieve
9 comparable weight gain. Reduced digestibility of shortgrass steppe grasses in the CO₂-enriched chambers was
10 likely due to lower N concentration (Campbell et al., 1997; Drake et al., 1996; Morgan et al., 2001a; Owensby et al.,
11 1996; Polley et al., 2000). In this grassland, the additive effect of CO₂ on tissue quality within a species and the
12 effect on species compositional change, with relatively more production under elevated CO₂ being contributed by
13 the least digestible grass, *S. comata*, both act to reduce forage quality, with the latter having the greater negative
14 effect. Animal selection for the higher quality species could further drive the system towards an even less palatable
15 state. The decline in plant N concentration is a fairly common response to CO₂ enrichment. Thus, we concur with
16 Owensby et al. (1996a) that continued CO₂ enrichment of the earth's atmosphere appears likely to reduce forage
17 quality in many of the world's grasslands, and will likely reduce animal performance.

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19
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- 14

1 Table1. Treatment variable probabilities for aboveground production data from five years (1997-2001) of CO₂
2 treatment (ambient (360 µl l⁻¹), elevated (720 µl l⁻¹), and unchambered plots) in open-top-chambers on the
3 shortgrass steppe of eastern Colorado, USA. Results are given for a mid-season (late July) and end of the season
4 harvest.

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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

1 Table 2. Treatment variable probabilities for aboveground phytomass (harvested at peak standing crop) from five
2 years (1997-2001) of CO₂ treatment (ambient (360 μl l⁻¹), elevated (720 μl l⁻¹), and unchambered plots) in open-top-
3 chambers on the shortgrass steppe of eastern Colorado, USA. The three functional groups are C₃ grasses, C₄ grasses
4 and forbs. Results are given for the mid-season (late July) harvest.

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6 Source of variation	Probability
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9 CO ₂ treatment	0.0001
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10 Functional group	0.0001
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11 Year	0.0001
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12 CO ₂ treatment * Functional group	0.5051
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13 CO ₂ treatment * Year	0.1324
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14 Functional group * Year	0.0001
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15 CO ₂ treatment * Functional group * Year	0.0092
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1 Table 3. Treatment variable probabilities for aboveground phytomass (harvested at peak-standing crop) from five
2 years (1997-2001) of CO₂ treatment (ambient (360 μl l⁻¹), elevated (720 μl l⁻¹), and unchambered plots) in open-top-
3 chambers on the shortgrass steppe of eastern Colorado, USA. The species are *Bouteloua gracilis*, *Pascopyrum*
4 *smithii* and *Stipa comata*.

6	Source of variation	Aboveground
7		Phytomass
10	CO ₂ treatment	0.0017
11	Species	0.0001
12	Year	0.0001
13	CO ₂ trt. * Species	0.0978
14	CO ₂ trt. * Year	0.0156
15	Species * Year	0.0001
16	CO ₂ trt * Species * Year	0.5339

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FIGURE CAPTIONS

Figure 1. Mean phytomass at the summer harvest and over the entire season (averaged over five years) in ambient and elevated CO₂ (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 CO₂ (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 CO₂ (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 CO₂ (360 and 720 μmol m⁻² s⁻¹) open-top-chambers and unchambered controls on the short-grass steppe of

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

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3 Figure 7. In vitro digestible dry matter of the three main species (averaged over two years) in ambient and elevated

4 CO₂ (360 and 720 $\mu\text{mol m}^{-2} \text{s}^{-1}$) open-top-chambers and unchambered controls on the short-grass steppe of eastern

5 Colorado. Means within a harvest with different letters are significantly different using Tukey's test ($P < 0.05$).

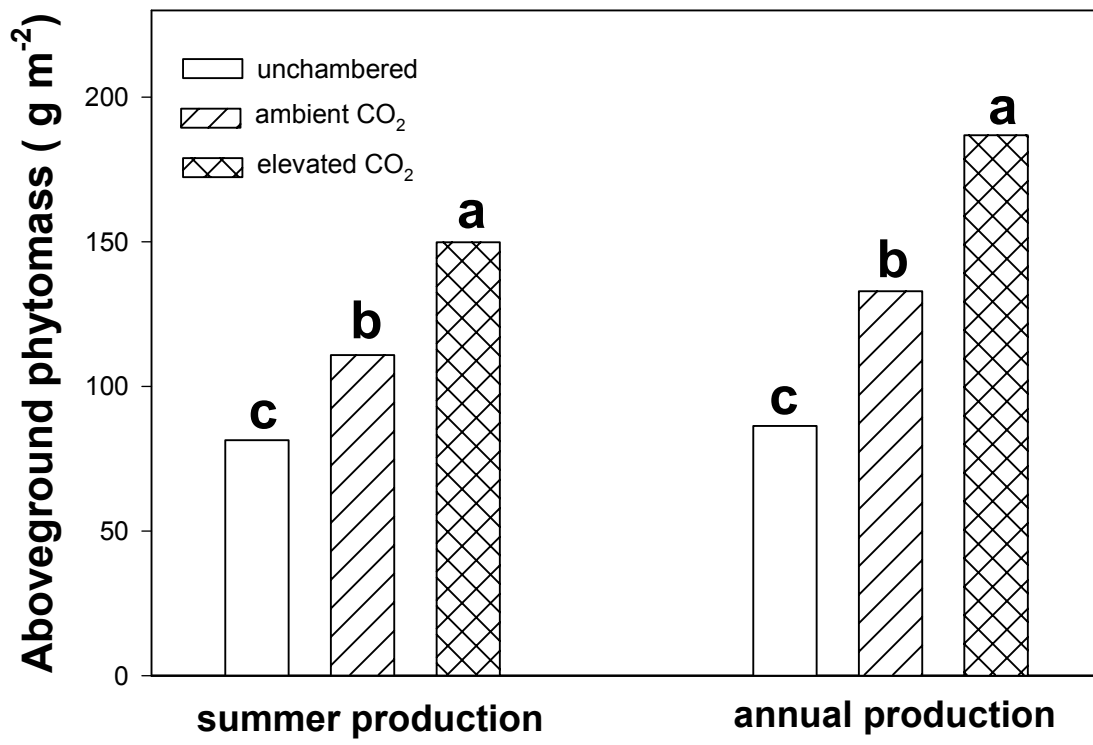


Fig. 1

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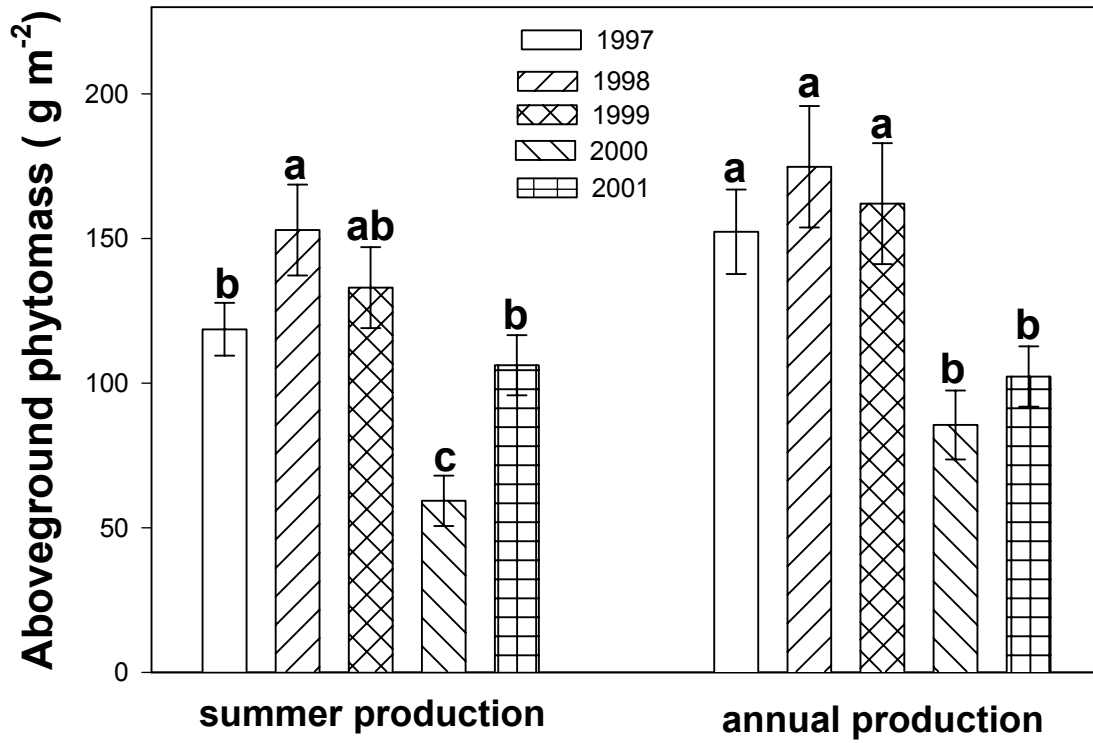
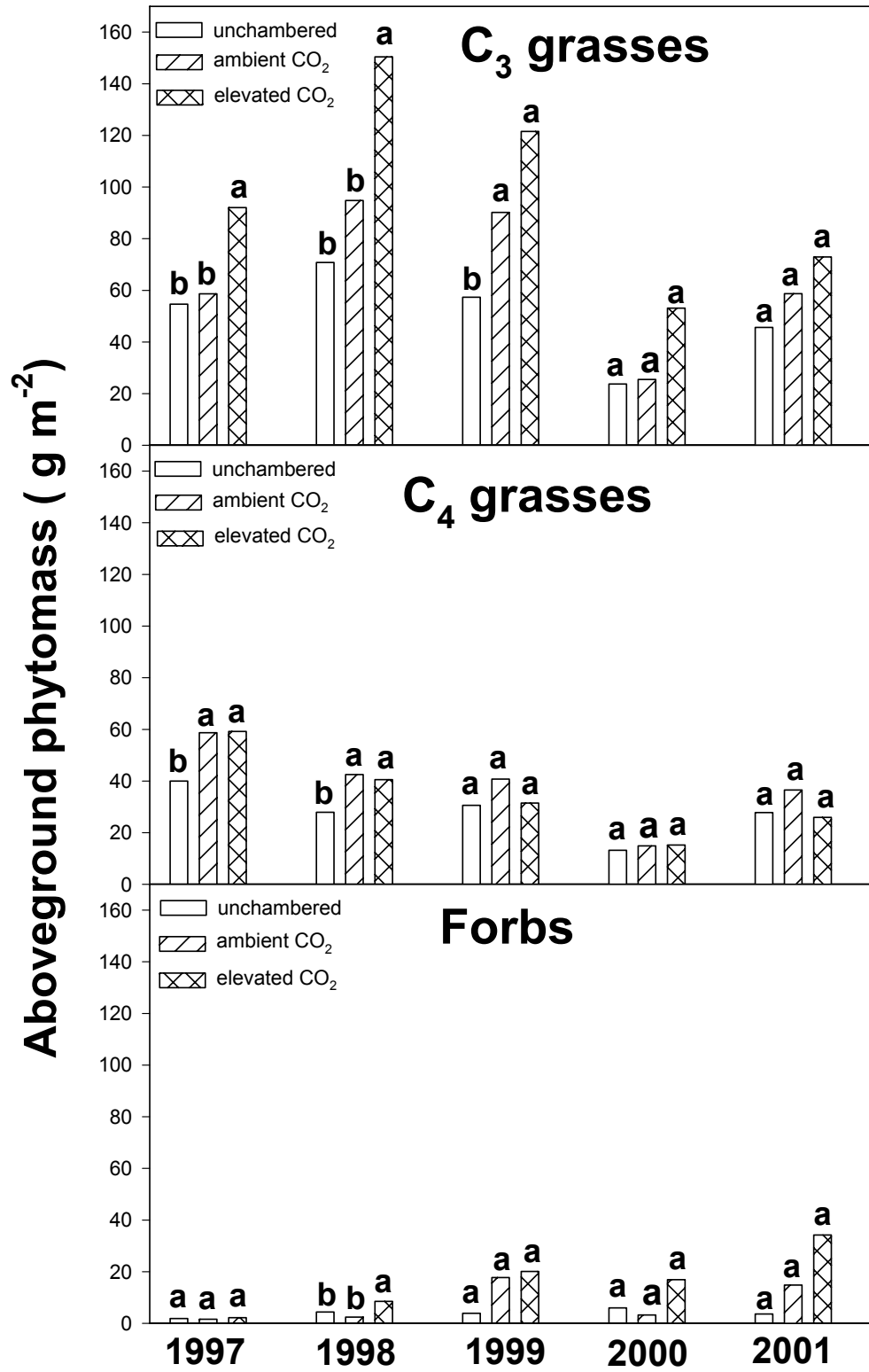


Fig. 2

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Fig. 3



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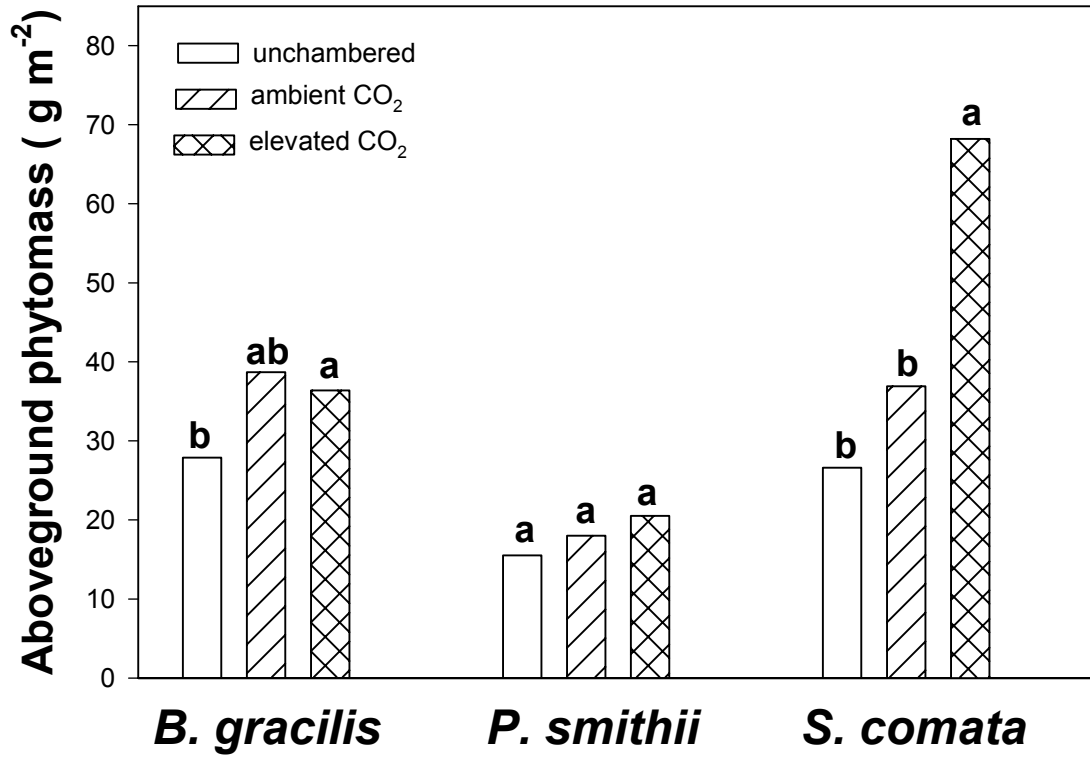


Fig. 4

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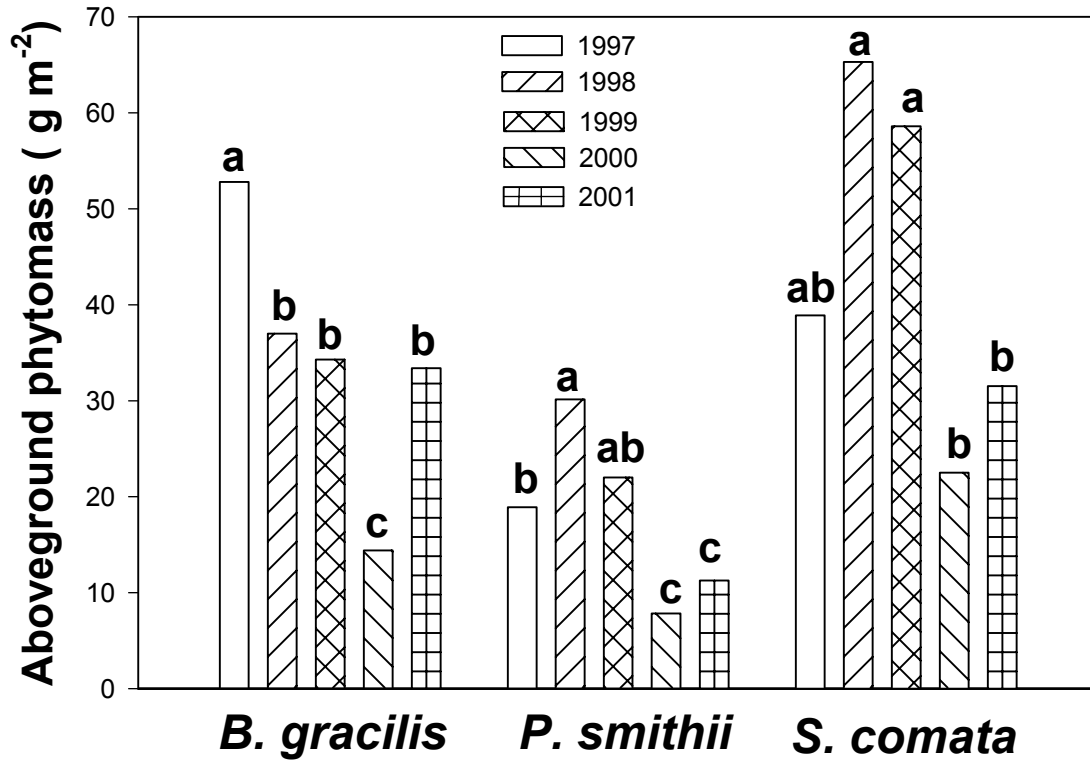


Fig. 5

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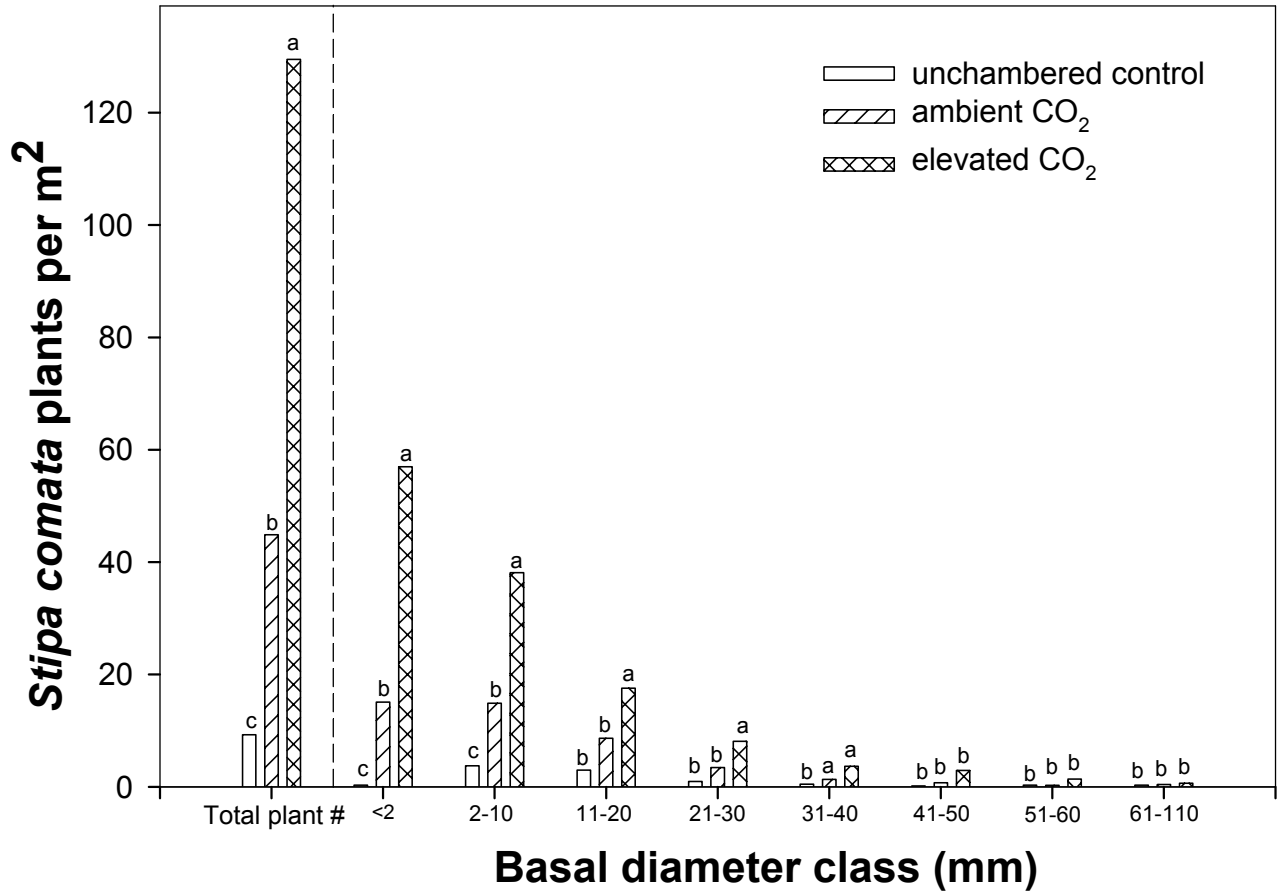


Fig. 6

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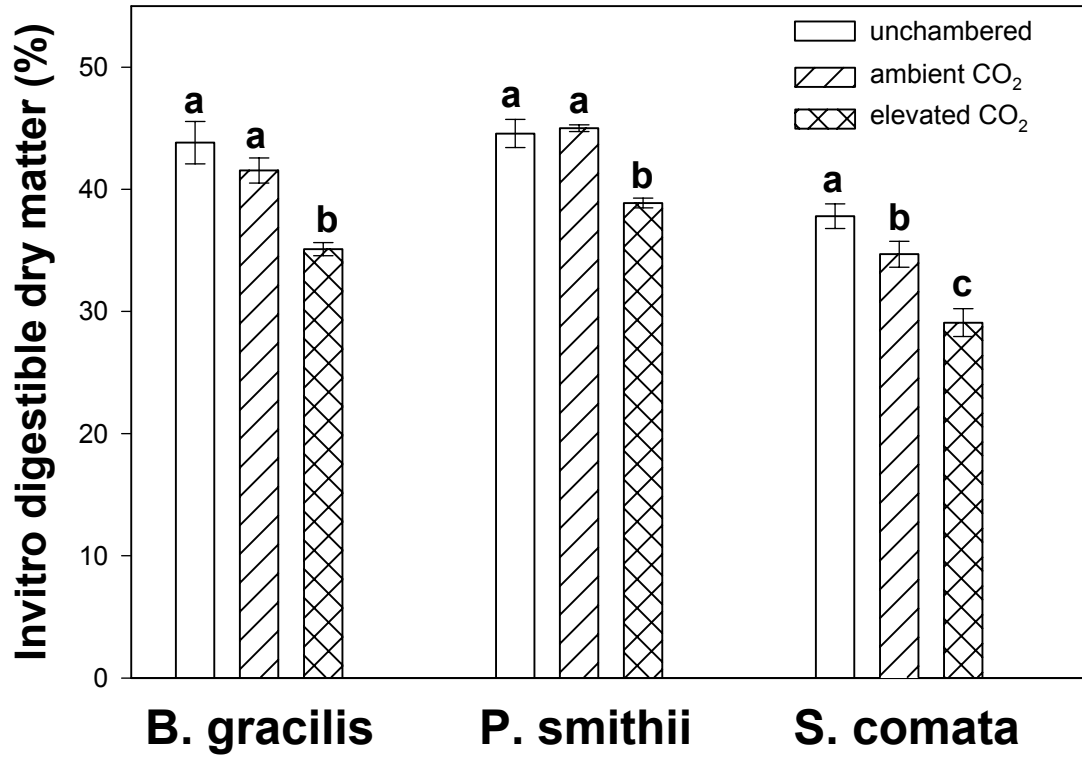


Fig. 7

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