Plant nitrogen dynamics in shortgrass steppe under elevated atmospheric CO_2

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ABSTRACT

The direct and indirect effects of increasing atmospheric carbon dioxide (CO_2) levels on plant nitrogen (N) content were studied in a shortgrass steppe ecosystem in northeastern Colorado, USA beginning in 1997. Nine experimental plots were established: three open-top chambers with ambient CO_2 levels (~365 μmol mol^{-1}), three open-top chambers with twice-ambient CO_2 levels (~720 μmol mol^{-1}), and three unchambered control plots. Following three years of growing season CO_2 treatment, the aboveground N concentration of plants grown under elevated atmospheric CO_2 decreased, and the C:N ratio increased. At the same time, increased aboveground biomass production under elevated atmospheric CO_2 conditions increased the net transfer of N out of the soil of elevated CO_2 plots. Aboveground biomass production following simulated herbivory was also greater under elevated CO_2 compared to ambient CO_2. Surprisingly, no significant changes in belowground plant tissue N content were detected in response to elevated CO_2. Measurements of individual species at peak standing phytomass showed significant CO_2 treatment effects on aboveground plant tissue N concentration and significant differences between species in N concentration, which suggest that changes in species composition under elevated CO_2 will contribute to overall changes in nutrient cycling. Changes in plant N content, driven by changes in aboveground plant N concentration, could have important consequences on biogeochemical cycling rates and the long-term productivity of the shortgrass steppe as atmospheric CO_2 concentrations increase.

Keywords: Elevated CO_2, rangelands, plant nitrogen, *Bouteloua gracilis*, *Pascopyrum smithii*, *Stipa comata*, C_3 grasses, C_4 grasses, nitrogen yield

INTRODUCTION

The effects of increasing atmospheric concentration of carbon dioxide (CO_2) on nitrogen (N) cycling will shape the way that ecosystems respond to elevated CO_2 because plant productivity in most ecosystems is limited by N availability (Hungate 1999). Specifically, ecosystem dynamics will be altered as a result of changes such as increased plant nutrient use efficiency, altered plant species composition, altered plant resource allocation, and increased plant water use efficiency (Drake and others 1997; Stitt and Krapp 1999; Polley and others 2000). Productivity, herbivory, nutrient cycling, and decomposition rates will be affected as a result. Previous studies also suggest that the physiological responses of C_3 and C_4 plant species to elevated CO_2 will differ (Bazzaz 1990; Morgan and others 1994; Wand and others 1999; Kimball and others 2002). Increased carbon to nitrogen (C:N) ratios in the aboveground plant biomass grown under elevated CO_2 conditions (Chu and others 1996; Ball and Drake 1997; Rogers and others 1999) will likely have a negative feedback on net primary production (NPP) because increased immobilization of N in the soil will limit NPP (Rice and others 1994; O'Neill and Norby 1996; Williams and others 2001; Gill and others 2002). However, the results of Hu and others (2001) suggest that reduced availability of soil nitrogen will slow microbial decomposition and will increase plant N utilization as plants and microbes compete for N (Kaye and Hart 1997). Similarly, Hungate and others (1997) found that elevated CO_2 increased plant N uptake. Although many studies have shown changes in C:N ratios of green plant biomass and of litter under elevated CO_2, few studies show evidence that decomposition rates are directly affected by elevated CO_2 (Kemp and others 1994; O'Neill and Norby 1996; Franck and others 1997; Cotrufo and others 1998; Norby and others 2001), and other mechanisms by which elevated CO_2 may affect decomposition continue to be explored (Dukes and Hungate 2002). The effects of rising atmospheric CO_2 levels on N cycling and on N distribution in ecosystems are not well understood. Predicting changes in N cycling will require an understanding of the mechanisms by which elevated CO_2 can change N cycling (Hungate 1999; Zak and others 2000).

The shortgrass steppe represents an important grassland ecosystem of the Great Plains of North America. This ecosystem is also comparable to regions which account for approximately 11% of global land area (Bailey 1979). The shortgrass steppe occupies about 280,000 km^2 in the central Great Plains and extends from western Texas to the Colorado-Wyoming border (Lauenroth and Milchunas 1991). Approximately 40% of the shortgrass steppe region remains in native grassland and is economically valuable as grazing land for livestock. Changes in plant tissue quality (e.g. N content) can affect both domestic and native herbivores, as well as overall ecosystem productivity. Reduced forage quality can result in lower animal weight gains and lower reproductive success as digestibility decreases (Owensby and others 1996).

We investigated the effects of elevated atmospheric CO_2 on changes in plant N content in shortgrass steppe following three years of experimental treatment (1997-1999). This is the first *in situ* study of the effects of elevated atmospheric CO_2 on plant N concentrations in shortgrass steppe. Based on results of studies in other ecosystems (Campbell and others 2000), we hypothesized that increased aboveground biomass production under conditions of elevated atmospheric CO_2 would decrease N concentration in shortgrass steppe vegetation. In addition we hypothesized that changes in plant N concentration would be influenced by changes in plant species composition and relative differences among species in changes in plant N concentration due to growth under elevated atmospheric CO_2. Our study addressed the following questions: (1) how does growth under elevated CO_2 affect plant N concentrations at the species level and at the plant community level?, and (2) are there interactions between elevated atmospheric CO_2 and simulated herbivory in determining aboveground plant productivity and plant tissue N concentration?

METHODS

Study site

This study was conducted at the USDA-ARS (U.S. Department of Agriculture – Agricultural Research Service) Central Plains Experimental Range (40º50'31"N, 104º42'50"W) located approximately 60 km northeast of Fort Collins, Colorado, USA at an elevation of 1650 m above sea level. The dominant plant species at the site are: *Bouteloua gracilis* (H.B.K.) Lag. (blue grama), a C_4 grass, and *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass) and *Stipa comata* Trin and Rupr. (needle-and-thread grass), two species of C_3 grasses. The soil is a Remmit fine sandy loam (Ustollic Camborthids). Meteorological data are recorded by the USDA-ARS station located ~200 m from the study site. Mean annual precipitation is 32 cm, of which approximately 70% falls between the months of April and September. Mean seasonal air temperatures range between 0.6ºC in winter and 15.6ºC in summer.

Experimental design

Nine experimental plots were established in 1996 to study the effects of elevated atmospheric CO_2 on shortgrass steppe. Baseline measurements were made in 1996. Open-top chambers were placed on plots and CO_2 treatments were started in 1997. Open-top chambers (4.5 m diameter, 3 m height) constructed of galvanized steel frame and enclosed with clear polycarbonate sheet were placed on six of the plots. Three chambered plots were maintained at ambient atmospheric CO_2 levels by circulating outside air through the chambers. The other three chambered plots were maintained at elevated CO_2 levels (~720 μmol mol^{-1}) by addition of pure CO_2 to ambient air passing through the chambers. The chambers were placed on the plots in March and were removed in October of every year. The three unchambered plots served as controls for monitoring the effects of the chambers themselves. Further details on the experimental design are described by Morgan and others (2001).

Sample collection

The south half of the ground area of each experimental plot was designated for aboveground biomass harvests. A metal wire grid was designed to exactly relocate

sampling sites at each harvest. The grid consisted of 56 quadrats each 40.5 cm by 15.3 cm (3.46 m^2 total area). To simulate herbivory, the grid was placed over the south half of each plot in late July, and aboveground vegetation in every other quadrat was clipped to crown level, separated by species, dried at 60ºC, and weighed. Species representing a small fraction (less than 5%) of the biomass in the sample area were grouped in general categories by plant growth form (C_3 grass, C_4 grass, forb). This defoliation procedure removed approximately one-half of the green vegetation in one growing season and was equivalent to a moderate to heavy level of grazing. In late October, after the chambers were removed, the grid was placed over the same area in each plot. By October, all plants were past maturity and had senesced. The quadrats that were harvested in July were harvested for re-growth following simulated herbivory, and the remaining 28 quadrats were harvested to assess end of season phytomass. This pattern was reversed each year such that quadrats that were not clipped the previous July were clipped in July at peak standing phytomass as well as at the end of the growing season, while the alternate quadrats were harvested only at the end of the season in October.

To collect soil cores for belowground biomass measurements, steel cylinders (20.3 cm diameter) were inserted to 60 cm depth in the north half of the ground area of each experimental plot. To minimize disturbance caused by inserting the cylinders, all cylinders for the study were installed before the CO_2 treatments began. Two cylinders containing soil cores were removed from each experimental plot in October each year to coincide with aboveground biomass sampling. Cores were brought back to the laboratory where roots and crowns (bases of stems) were separated from the soil, dried at 60°C, and weighed.

Dry plant biomass collected aboveground and belowground was ground through a 0.02 mm sieve (Retsch GmbH & Co. KG, Haan, Germany), and representative subsamples were analyzed for total carbon (C) and N using an automated C/N combustion analyzer (PDZ Europa Ltd., Cheshire, England). Ash content was determined by combustion at 600°C. Standing crop of N (also reported

in the literature as N uptake) was calculated by multiplying N concentration by biomass.

Data analysis

The study was a randomized block design. Data were analyzed using the SAS PROC MIXED procedure (SAS Institute Inc., Cary, NC). We performed two-way repeated-measures analyses of variance (ANOVA), with main effects of CO_2 treatment (unchambered, ambient CO_2, and elevated CO_2) and year (1997, 1998, and 1999), consistent with Morgan and others (2001). Aboveground biomass was separated by species only in July, and therefore "species" was also included as a main effect in the analysis of July measurements. Belowground biomass was not separated by species in this study. The main effects were evaluated using Tukey-Kramer *post hoc* means comparison tests to determine significant differences among means when the main effects were significant ($P \leq 0.05$). Analysis of covariance was performed to examine the effect of precipitation, calculated for annual period (January-December) or growing season (April-October) or spring season (March-June), as a covariate.

RESULTS

Comparison of monthly averages with the 80-yr mean based on measurements since 1912 shows above average precipitation in all years of this study (Figure 1). These measurements also show slightly cooler than normal maximum daily air temperatures during our study, as well as slightly warmer than normal minimum daily temperatures, especially during winter months.

Aboveground plant biomass

Aboveground plant biomass and plant N measurements were made in 1996 before CO_2 treatments began. Data collected in 1996 are presented here as a reference to background conditions. In 1996, total aboveground biomass production was not different between plots designated for ambient CO_2 and elevated CO_2 chambers. The CO_2 treatments were begun in March 1997 and were applied from March until October each year. There were highly significant treatment (unchambered, ambient

CO_2, and elevated CO_2) and year effects on July, October, and regrowth biomass (Table 1, Figure 2). In July (Figure 2a), during peak standing phytomass, aboveground biomass production was significantly higher (35%) in elevated CO_2 chambers than ambient CO_2 chambers, as reported by Morgan and others (2001). The same pattern of increased biomass production under elevated CO_2 was observed in the October harvests (Figure 2b). Aboveground biomass regrowth following simulated herbivory was significantly higher in the elevated CO_2 plots than in ambient CO_2 and unchambered plots in all three years of CO_2 treatment (Figure 2c). Biomass production varied from year to year, and total aboveground biomass was highest in 1998.

Aboveground plant N content

There were no differences in N concentration in October and regrowth biomass in 1996, before the CO_2 treatments started. Nitrogen concentration in biomass collected in July and regrowth harvests varied significantly among treatments, and there was a significant effect of year on N concentration of all three plant biomass harvests (Table 1). Nitrogen concentration of aboveground biomass in July was significantly lower (by 21%) in elevated CO_2 plots compared to ambient CO_2 plots (Table 2). The percentage decreases in July aboveground plant tissue N concentration under elevated CO_2 compared to ambient CO_2 were 22%, 17%, and 25% in 1997, 1998, and 1999, respectively. In October, N concentration of aboveground biomass did not differ significantly among CO_2 treatments (Table 2), but there was a slight trend in the N concentration consistent with July and regrowth biomass N concentration. The October results were as we expected for senesced plant biomass. Nitrogen concentrations in regrowth biomass in elevated CO_2 plots were on average 11% lower than in ambient CO_2 plots (Table 2). Across all CO_2 treatments, overall N concentrations in aboveground plant biomass were lowest in 1999, and N concentration of July and October aboveground biomass decreased in each year of the study.

The total standing crop of N in aboveground biomass in October and regrowth harvests tended to be greater (P=0.12 and 0.09, respectively; Tukey-

Kramer means comparisons) in elevated CO_2 plots compared to ambient CO_2 plots (Table 2). There was no difference between ambient and elevated CO_2 treatments in total standing crop of N in July (P=0.9). Aboveground biomass production was higher in elevated CO_2 plots compared to ambient CO_2 and unchambered plots and more than compensated for lower N concentrations. Overall, the total standing crop of N was highest in July while plants were green (1.2 ± 0.1 g N m^{-2}), whereas biomass collected in October had senesced and contained less N (0.8 ± 0.1 g N m^{-2}). The total standing crop of N in regrowth biomass was low (0.4 ± 0.1 g N m^{-2}), reflecting the low availability of N in the latter half of the growing season. There was also a significant effect of year on total N in aboveground biomass (Tables 1, 2). Total standing crop of N in regrowth biomass decreased over the course of the experiment. These changes in N concentration and standing crop of N in aboveground plant biomass resulted in a steady overall increase in the C:N ratio of plant biomass (average ± standard error) from 38.4 ± 3.4 in 1997 to 46.0 ± 3.4 in 1998 to 54.4 ± 6.9 in 1999. From the covariate analyses, there were no statistically significant relationships between precipitation (annual, growing season, or spring season precipitation amounts) and aboveground plant biomass, N concentration, or standing crop of N.

Species responses

Biomass harvests were separated by species in the July harvest only. We analyzed biomass and N content of the three dominant grass species in our study site, *B. gracilis*, *P. smithii*, and *S. comata*. These three species collectively represent approximately 85% of the total aboveground biomass at the site. The measurements of biomass of each of these individual species are presented in Figure 3. We observed a significant CO_2 treatment effect on July biomass, as described above. Our data show that species had an important effect on July biomass (P=0.02, Table 3). Species biomass varied significantly between years, and differences between years depended on species (Table 3). Biomass production was higher overall in 1998 compared to 1997 and 1999. This difference was driven by higher productivity of both *P. smithii* and *S. comata* in 1998. Overall, the productivity of *B. gracilis*

decreased slightly over the three years of CO_2 treatment. The biomass of *S. comata* was greater under elevated CO_2 compared to ambient CO_2 and was also highly variable between plots.

In general, N concentration of aboveground biomass of individual species decreased with time in all treatments (Table 3, Figure 4). Differences in N concentration among years were larger than differences among CO_2 treatments (unchambered, ambient CO_2, and elevated CO_2). The N concentrations of all three species were lower during the experiment than in 1996, before the CO_2 treatments began. In *B. gracilis*, the N concentration was 9% lower in the elevated CO_2 plots compared to ambient CO_2 plots. The N concentrations of *P. smithii* were on average 20% lower in elevated CO_2 plots compared to ambient CO_2 plots, and in *S. comata* the N concentration was 25% lower in elevated CO_2 plots compared to ambient CO_2 plots (P=0.06).

The effects of CO_2 treatment on total standing crop of N separated by individual species were not statistically significant (P=0.08, Table 3, Figure 5). Trends in the data suggest that the N content of aboveground biomass of *S. comata* increased with elevated CO_2 while the N content in *B. gracilis* decreased. Total standing crop of N was strongly affected by year. The total N in *B. gracilis* biomass steadily decreased through time and was approximately 50% lower in 1999 compared to 1997. Total N in *P. smithii* standing biomass was variable between years. Total N in *S. comata* standing biomass stayed relatively constant through time due to a decrease in N concentration and an increase in biomass production through time.

Belowground plant biomass and N content

Roots and crowns were sampled from soil cores collected in October of each year, after the plants had senesced. Measurements of root and crown biomass did not show statistically significant treatment (P=0.3 and 0.1, respectively) or year (P=0.1 for both roots and crowns) effects (Figure 6). There was a trend towards higher root and especially crown biomass in elevated CO_2 compared to ambient CO_2 chambers. There were no differences in standing crop of N in roots or crowns among

treatments (P=0.4 and 0.8, respectively) or years (P=0.1 and 0.2, respectively; Table 4). As expected for senesced plant material, standing crop of N in roots and crowns was higher than in aboveground biomass overall.

DISCUSSION

Aboveground plant biomass

Aboveground biomass production was significantly higher under elevated CO_2 compared to ambient CO_2 in the first two years of CO_2 treatment. Phytomass in elevated CO_2 plots was 30% higher than in ambient CO_2 plots in 1997 and 47% higher in 1998 (Morgan and others 2001). In the analysis of variance, "year" explained a significant amount of the variance in July and regrowth biomass without an interaction with CO_2 treatment. Although precipitation did vary from year to year, annual precipitation, growing season and spring season precipitation were not significant covariates for July, October, or regrowth biomass. While we expect precipitation to be a primary driver of biomass production in this semi-arid environment (Milchunas and others 1994), annual precipitation amounts were above average in all years from 1996 to 1999, and therefore other environmental factors varying on timescales shorter than a year or growing season may account for the interannual differences in biomass production. In a California grassland elevated CO_2 study, Hungate and others (1997) found that changes in soil moisture under elevated CO_2 best explained increased plant N uptake and plant productivity. Simulated herbivory in our study may have interacted with precipitation to determine aboveground biomass production by increasing precipitation use efficiency, as described by Varnamkhasti and others (1995).

Our results confirm previous laboratory growth chamber studies that showed enhanced productivity of the dominant grass species of shortgrass steppe under elevated CO_2 conditions (Hunt and others 1996; Morgan and others 1998). Other studies have reported similar, but generally smaller, increases in aboveground biomass following exposure to elevated CO_2 (Owensby and others 1993b; Leadley and Stöcklin 1996; Lüscher and Nösberger 1997; Dijkstra and others 1999; Kimball

and others 2002). In an open-top chamber study of tall grass prairie in Kansas, Owensby and others (1999) observed differences in aboveground biomass production only in years of below average precipitation. Their results suggest that plants exposed to elevated CO_2 have greater water use efficiency and improved soil water status which allows the plants to continue to photosynthesize longer during periods of water stress. Thus, plants exposed to elevated CO_2 are more productive during dry years than plants exposed to ambient CO_2. In our study, measurements of soil moisture indicate increased soil water content under elevated CO_2 (Morgan and others 2001), which is probably the result of increased water use efficiency in plants under elevated CO_2. Growth chamber studies of *B. gracilis* suggest that enhanced growth is also a result of a positive photosynthetic response to elevated CO_2 (Hunt and others 1996). Growth chamber studies of *P. smithii* also show a positive photosynthetic response to elevated CO_2 (Morgan and others 1998), but *in situ* measurements of photosynthesis in this study show no direct benefit for either of these species (D.R. LeCain and others unpublished data).

Aboveground plant N content

Aboveground plant tissue N concentrations decreased under elevated CO_2, though the differences present in July disappeared in senesced phytomass by October. Decreases in aboveground tissue N concentration under elevated CO_2 in this study are similar to values reported for forest ecosystems (reviewed by McGuire and others 1995) and grasslands (Owensby and others 1993a; Zanetti and others 1997; Wand and others 1999). The results from our open-top chamber study are in agreement with elevated CO_2 studies which used the FACE (Free Air CO_2 Enrichment) approach (Kimball and others 2002). Insignificant treatment differences in N concentration in senesced biomass (collected in October) compared to green biomass (collected in July) indicate that these perennial plants efficiently reallocated N resources below ground at the end of the growing season. Nitrogen leaching from herbage during plant senescence is not likely to be significant. Precipitation is a primary determinant of N loss through leaching in this ecosystem (Hook and Burke 2000), and precipitation amounts in September and October are

generally half of precipitation amounts in May through August. Similarly, the N concentration of senesced plant tissue in a Swiss grassland FACE experiment was not significantly changed under elevated CO_2 (Blum and others 1997). Significant decreases in green aboveground plant tissue N concentration under elevated CO_2 may result from a reduction in the amount of carboxylating enzymes required by the plants (Conroy 1992; Read and Morgan 1996). Growth chamber studies of *B. gracilis* and *P. smithii* also showed decreases in plant N concentration under elevated CO_2, which were described to be a result of greater plant N limitation under elevated CO_2 (Hunt and others 1996; Read and others 1997).

Higher biomass production more than compensated for lower N concentration in elevated CO_2 plots so that total N in aboveground biomass was greater on average in elevated CO_2 plots compared to unchambered and ambient CO_2 plots. The difference in total aboveground plant N may be linked to increased root biomass production and therefore greater N acquisition under elevated CO_2 or may be linked to increased N use or uptake efficiency. Increased N mobilization and N mineralization rates, as suggested by Carnol and others (2002), may account for the higher N content in plant biomass under elevated CO_2. Increased soil organic matter decomposition under elevated CO_2 may also have enhanced N mineralization. A laboratory growth chamber study of *B. gracilis* showed decreases in overall plant N content but also a significant increase in total root N content under elevated CO_2 (Morgan and others 1994). In an open-top chamber study in Kansas tallgrass prairie, Owensby and others (1993a) observed lower N concentrations in plants grown under elevated CO_2 in some years of their study, but total standing crop of N was also higher under elevated CO_2. This result was attributed to increased N acquisition and increased N retention by the plants.

We observed significant differences in aboveground plant N concentration and standing crop of N by year. These differences could not be explained by annual or growing season or spring season precipitation as a covariate. The differences are probably driven by a more complex combination of precipitation and other environmental factors. Soil available N in the experimental plots did not change

detectably over the course of this study (A.R. Mosier unpublished data). In addition, N cycling rates, as reflected by N trace gas fluxes, did not change significantly under CO_2 treatment (Mosier and others 2002). However, the amount of N in plant litter is small (1.2 g N m^{-2}) compared to the total soil N pool (230 g N m^{-2} in 0-20 cm depth), and it is possible that small changes in soil N that would account for differences in plant N could not be detected. Biomass clipped from the experimental plots in all harvests was not returned to the plots in this study. Although plant biomass had senesced at the time of October harvests, removal of all aboveground biomass represents relatively severe grazing or disturbance. The harvest technique could explain some of the "year" effects observed in the analysis of variance. The net removal of biomass (and therefore N) from the system may have contributed to the decreases in total N in plant biomass over the years.

Defoliation effects

Recovery from simulated herbivory (defoliation) was significantly higher under elevated CO_2 compared to ambient CO_2. Aboveground biomass production following simulated herbivory was higher in elevated CO_2 plots compared to ambient CO_2 plots by 26%, 47%, and 65% in 1997, 1998, and 1999, respectively. At the same time, the N concentration of aboveground regrowth following simulated herbivory was only slightly lower in elevated CO_2 plots compared to ambient CO_2 or unchambered plots, suggesting that N mineralization or remobilization from belowground organs may have been greater in elevated CO_2 plots. Past studies of long-term grazing of shortgrass steppe show negative effects of increased soil water availability on grass N concentration, but the effects depended on grazing intensity (Milchunas and others 1995). Aboveground productivity in historically heavily grazed systems was not significantly affected by simulated herbivory or water supplementation treatments (Varnamkhasti et al 1995), but plant N concentrations tended to be lower under these treatments (Milchunas and others 1995). These results suggest that plants subjected to herbivory may respond positively to the direct effects of elevated CO_2 rather than the indirect effects of improved soil water status or other CO_2-induced factors. Greenhouse studies have shown that regrowth

following simulated herbivory is increased under elevated CO_2 for *P. smithii* but decreased for *B. gracilis* (Skinner and others 1999). In that study, remobilization of total nonstructural carbohydrates and soluble N from root and crown tissues following simulated herbivory was lower under elevated CO_2 in *B. gracilis*, but higher under elevated CO_2 in *P. smithii*. The differences in contribution of remobilized N for shoot growth following simulated herbivory between *B. gracilis* and *P. smithii* may account for some of the decrease in biomass of *B. gracilis* relative to *P. smithii*.

Plant N allocation and plant tissue quality

Changes in aboveground plant N concentration may be driven by changes in the production of different structural tissues (lignin, cellulose). Laboratory analyses of *B. gracilis* and *P. smithii* indicate higher total nonstructural carbohydrate concentrations in response to elevated CO_2 (Read and Morgan 1996; Morgan and others 1998). Increased production of nonstructural carbohydrates would tend to decrease plant N concentration (Conroy 1992; LeCain and Morgan 1998; Stitt and Krapp 1999). Studies of CO_2 effects on forage chemical composition have reported changes in lignin content (Fritschi and others 1999). Since lignin has a relatively high C:N, increased lignin could account for some of the differences in C:N between CO_2 treatments. The increase in tissue lignin concentrations we measured in October in elevated CO_2 plots (D.G. Milchunas and others unpublished data) is similar to results in some studies (Cotrufo and others 1994), but contrasts with other studies in which no changes or decreases in lignin concentration with elevated CO_2 were observed (Chu and others 1996; Ball and Drake 1997). Reduced tissue N concentrations and tissue quality could have significant impacts on microbial decomposition rates as well as herbivore nutrition. Although aboveground biomass production may increase under elevated CO_2 conditions, grazing ruminants in this system will need to increase intake rates to meet their N demands.

Species responses

We observed a significant effect of year on species biomass and a significant year by species interaction. The data show an overall increase in *S. comata* and decrease

in *B. gracilis* through time. Biomass production of the three dominant grasses was significantly affected by CO_2 treatments (unchambered, ambient CO_2, and elevated CO_2). Though differences between ambient and elevated CO_2 could not be detected within individual species, the trend in the data suggests increasing productivity of *S. comata* under elevated CO_2. The three dominant grasses differed in N concentration at peak growing season, perhaps reflecting differences in N acquisition or N use efficiency. Variation in plant structural composition, such as lower ratios of leaves to stems, which are generally lower in N concentration, in *S. comata*, may also account for differences in N concentration. Contrasting species responses to elevated CO_2 have important implications for the shortgrass steppe. Changes in the relative abundance of the three dominant grass species in this ecosystem, as shown by data after five years of elevated CO_2 treatment (J.A. Morgan and other unpublished data), suggest that species composition may change under long-term elevated CO_2. A shift from a community dominated by the C_4 shortgrass *B. gracilis* to a community dominated by the C_3 mid-grass *S. comata* would change ecosystem N cycling dynamics.

The data on total standing crop of N by species reflect the shift in species composition mentioned above. Both N concentration and biomass production of *B. gracilis* decreased between 1997 and 1999, which is reflected in the downward trend in total N content in *B. gracilis*. On the other hand, the N concentration of *S. comata* also decreased significantly between 1997 and 1999, but increased biomass production of *S. comata* (Figure 3) compensated for the N concentration decrease so that the total standing crop of N in *S. comata* remained unchanged. In this way, *S. comata* outpaced *B. gracilis* in acquisition of ecosystem N resources, regardless of CO_2 treatment.

Our results are similar to those from a Swiss grassland study in which significant interspecific differences in response to elevated CO_2 were observed while intraspecific differences in response to elevated CO_2 could not be detected (Lüscher and Nösberger 1997). Leadley and Stöcklin (1996) also reported important species-specific responses of calcareous grassland species to elevated CO_2 that are

predicted to cause changes in community structure under increasing atmospheric CO_2 concentrations. Important species effects have also been observed in other studies (Garbutt and others 1990; Diaz and others 1993; Owensby and others 1993a, b; Chu and others 1996; Hungate and others 1996; Grünzweig and Körner 2001; Joel and others 2001; Reich and others 2001). Berntson and others (1998) suggest that plants' abilities to take up N may drive community composition changes under elevated CO_2. The variation in responses of individual species to elevated CO_2 in terms of multiple characteristics such as leaf area, leaf biomass, plant phenology, biomass of reproductive parts, and seed N content complicates estimates of ecosystem responses to elevated CO_2. Understanding ecosystem responses to elevated CO_2 will require understanding many of these responses at the species level.

Belowground plant biomass and N content

Although aboveground plant biomass production increased significantly under elevated CO_2, similar responses were not observed in belowground biomass. Many studies have shown increases in root growth and root to shoot ratios under elevated CO_2 (Rogers and others 1999), but relatively few of those studies focused on natural ecosystems or grasslands in particular. One study in annual grasslands showed a slight trend of lower root to shoot ratios with increasing atmospheric CO_2 (Berntson and others 1998). We did not calculate root to shoot ratios because the aboveground and belowground biomass data reported here were collected from separate areas. However, our measurements of overall increases in aboveground biomass and no change in belowground biomass suggest lowered root to shoot ratios under elevated CO_2. The wide range of root to shoot ratio responses to elevated CO_2 in the literature suggests that analysis of root to shoot ratio alone may be inadequate for describing the effects of elevated CO_2 on plants (Norby 1994). Changes in root to shoot ratios under elevated CO_2 might depend strongly on other conditions, including nutrient and water availability (e.g. Sindhøj and others 2000). Results from the tallgrass prairie study conducted in Kansas suggested that changes in root biomass were related to changes in species composition (Owensby and others

1993b). In our study, shifts in plant species composition combined with long average root turnover times of 5 to 7 years (Milchunas and Lauenroth, 1992; 2001) might explain why changes in root biomass may be too small to be detected. In addition, Kimball and others (2002) indicate that open-top chambers may have a negative effect on root growth when compared to FACE studies. Similar to root biomass, the biomass of plant crowns did not change significantly under elevated CO_2 compared to ambient CO_2. These data suggest that greater aboveground biomass production under elevated CO_2 resulted primarily from increased leaf area of individual plants rather than an increase in the number of plants.

Changes in N concentration in aboveground biomass may be the result of changes in plant allocation of N between aboveground and belowground tissues (Gorissen and Cotrufo, 1999); however, root and crown N contents were not significantly altered under elevated CO_2 compared to ambient CO_2. At the end of the growing season, we expect that senesced plants have reallocated N to their roots and crowns. The increase in aboveground productivity under elevated CO_2 may mask any impacts on root and crown standing crop of N despite changes in aboveground plant N concentration (Table 2). Shifts in species composition may also make it difficult to detect changes in root and crown N in response to elevated CO_2. Important species differences in response to elevated CO_2 have been reported for root biomass and nitrogen content. Curtis and others (1990) found that while a C_3 species showed increased root biomass and decreased N concentration in root biomass, a C_4 species in a similar environment showed no root growth or N content response to elevated CO_2.

CONCLUSIONS

Nitrogen concentrations of aboveground plant biomass decreased under elevated CO_2, but increased aboveground biomass production under elevated CO_2 resulted in higher total standing crop of N in elevated CO_2 plots. Aboveground biomass production following simulated herbivory was also greater under elevated CO_2 compared to ambient CO_2 conditions. At the same time, elevated CO_2 did not result in significant changes in belowground biomass production or belowground plant N

content after 3 years of CO_2 treatment. Despite obvious aboveground plant responses to elevated CO_2, our results indicate that overall changes in ecosystem N cycling in response to elevated CO_2 will occur slowly and will be difficult to detect in grasslands in which the majority of plant biomass is located belowground and turns over on 5- to 7-year timescales. Differences in aboveground tissue N concentration between species and changes in the proportion of total biomass represented by each species under elevated CO_2 suggest changes in ecosystem N cycling as a result of community composition changes under elevated CO_2. These indirect effects of elevated CO_2 on ecosystem N cycling through long-term changes in community composition may be more important than the direct effects of elevated CO_2 on biomass production and plant nutrient content.

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Figure Captions

Figure 1. Meteorological data are recorded daily at a station located ~200 m from the study site. Monthly values presented for: a) average daily maximum air temperature, b) average daily minimum air temperature, and c) precipitation. The thick solid line represents measurements and the lighter line represents an 80-year average for the site, taken as "normal."

Figure 2. Aboveground biomass shown for main effects of year and CO_2 treatment a) in July at peak standing crop, b) in October at the end of the growing season, and c) regrowth in October (*following simulated herbivory in July). Measurements in 1996 were made before experimental treatments began. They are provided here for reference but were not included in the statistical analyses. Error bars represent standard errors (n = 3). When the main effect was significant, treatment differences were determined at $P \leq 0.05$. Bars within each main effect marked with the same letter are not different.

Figure 3. Species biomass of the three dominant grasses, a) *B. gracilis*, b) *P. smithii*, and c) *S. comata*, collected in July of each year. Measurements in 1996 were made before experimental treatments began. They are provided here for reference but were not included in the statistical analyses. Error bars represent standard errors (n = 3). When the main effect was significant, treatment differences were determined at $P \leq 0.05$. Bars within each main effect marked with the same letter are not different.

Figure 4. Aboveground plant biomass nitrogen concentration of three dominant grasses, a) *B. gracilis*, b) *P. smithii*, and c) *S. comata*, collected in July of each year. Data are presented as in Figure 3.

Figure 5. Total standing crop of nitrogen in aboveground biomass of three dominant grasses, a) *B. gracilis*, b) *P. smithii*, and c) *S. comata*, collected in July of each year. Data are presented as in Figure 3.

Figure 6. Biomass of a) roots and b) crowns collected from soil cores taken from 0-60 cm depth. Error bars represent standard errors (n=3).

Figure 1

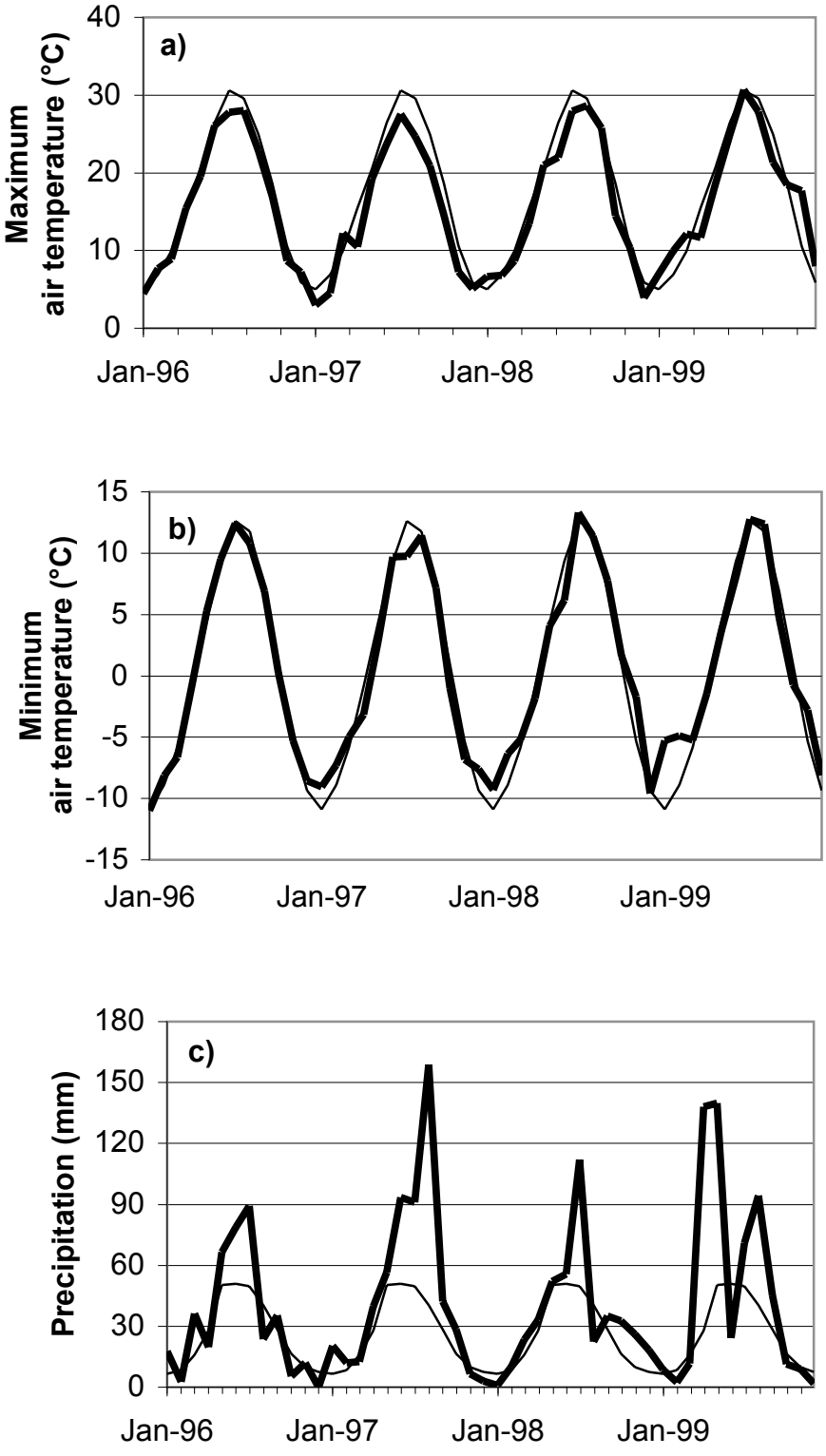
a)
Maximum air temperature (°C)
40
30
20
10
0
Jan-96
Jan-97
Jan-98
Jan-99
b)
Minimum air temperature (°C)
15
10
5
0
-5
-10
-15
Jan-96
Jan-97
Jan-98
Jan-99
c)
Precipitation (mm)
180
150
120
90
60
30
0
Jan-96
Jan-97
Jan-98
Jan-99

Figure 2

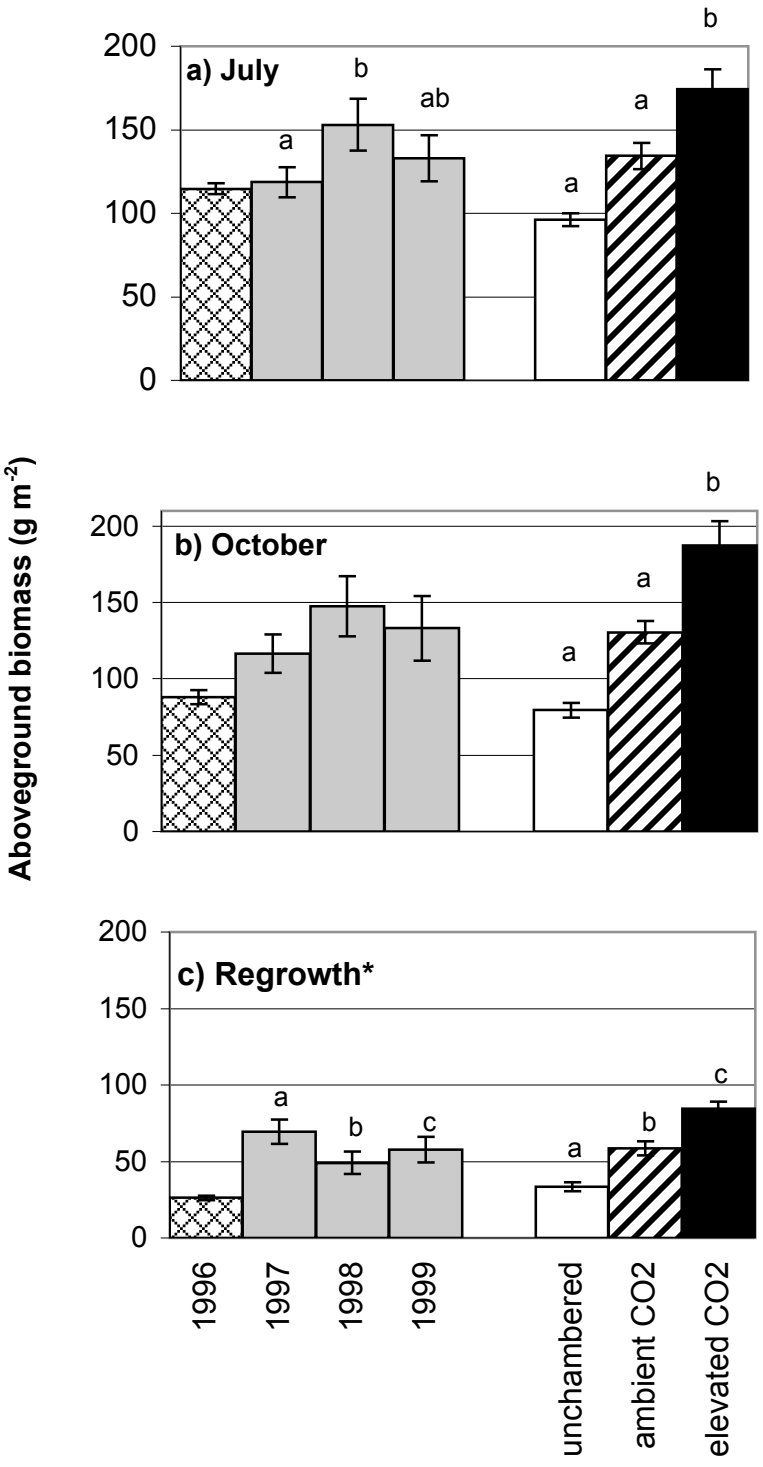
a) July
b) October
c) Regrowth*
Aboveground biomass (g m-2)
200
150
100
50
0
a
b
ab
a
a
b
a
b
a
b
a
b
c
a
b
c
1996
1997
1998
1999
unchambered
ambient CO2
elevated CO2

Figure 3

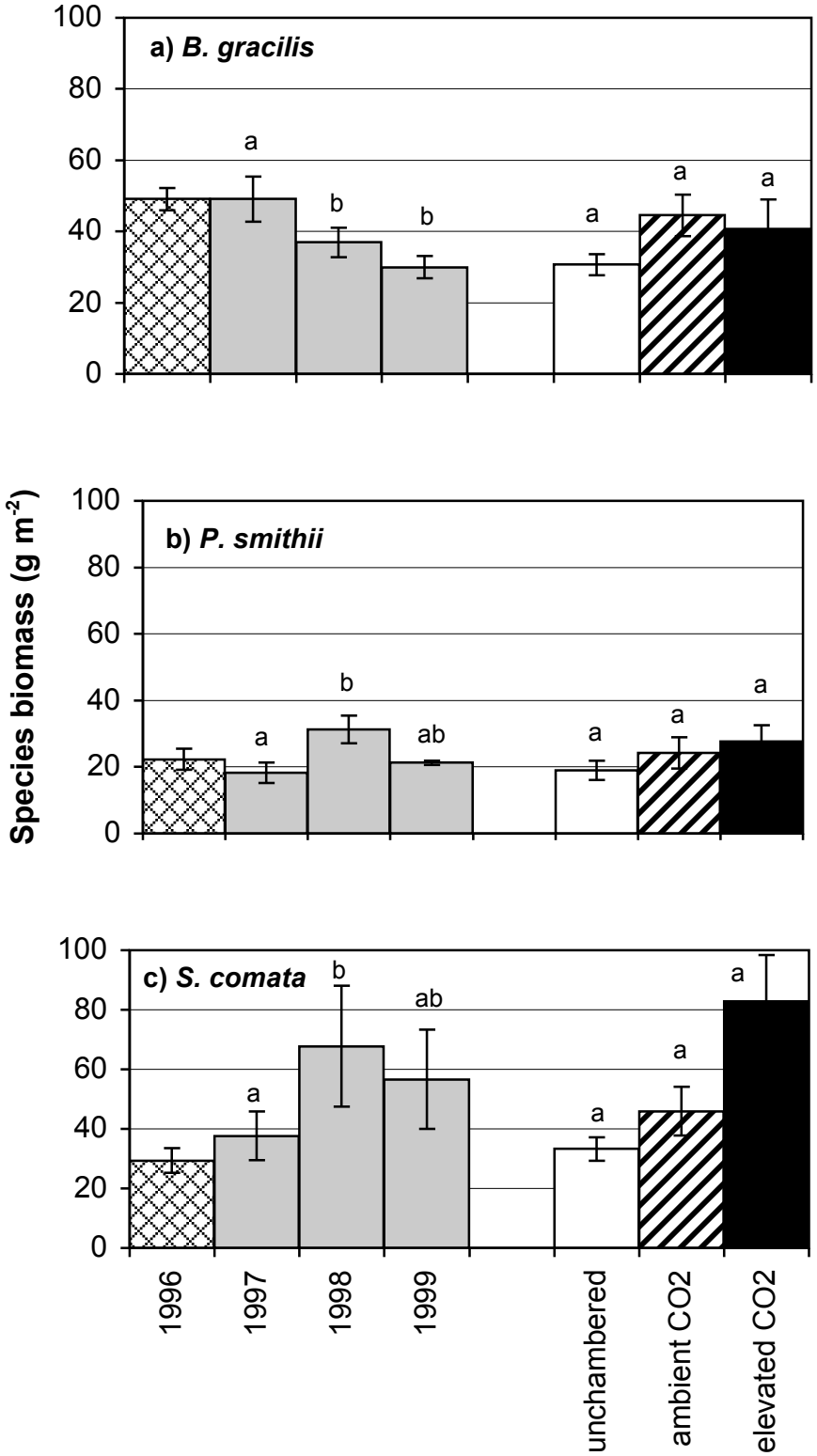
a) B. gracilis
b) P. smithii
c) S. comata
Species biomass (g m-2)
100
80
60
40
20
0
a
b
b
a
a
a
a
b
ab
a
a
a
a
b
ab
a
a
a
1996
1997
1998
1999
unchambered
ambient CO2
elevated CO2

Figure 4

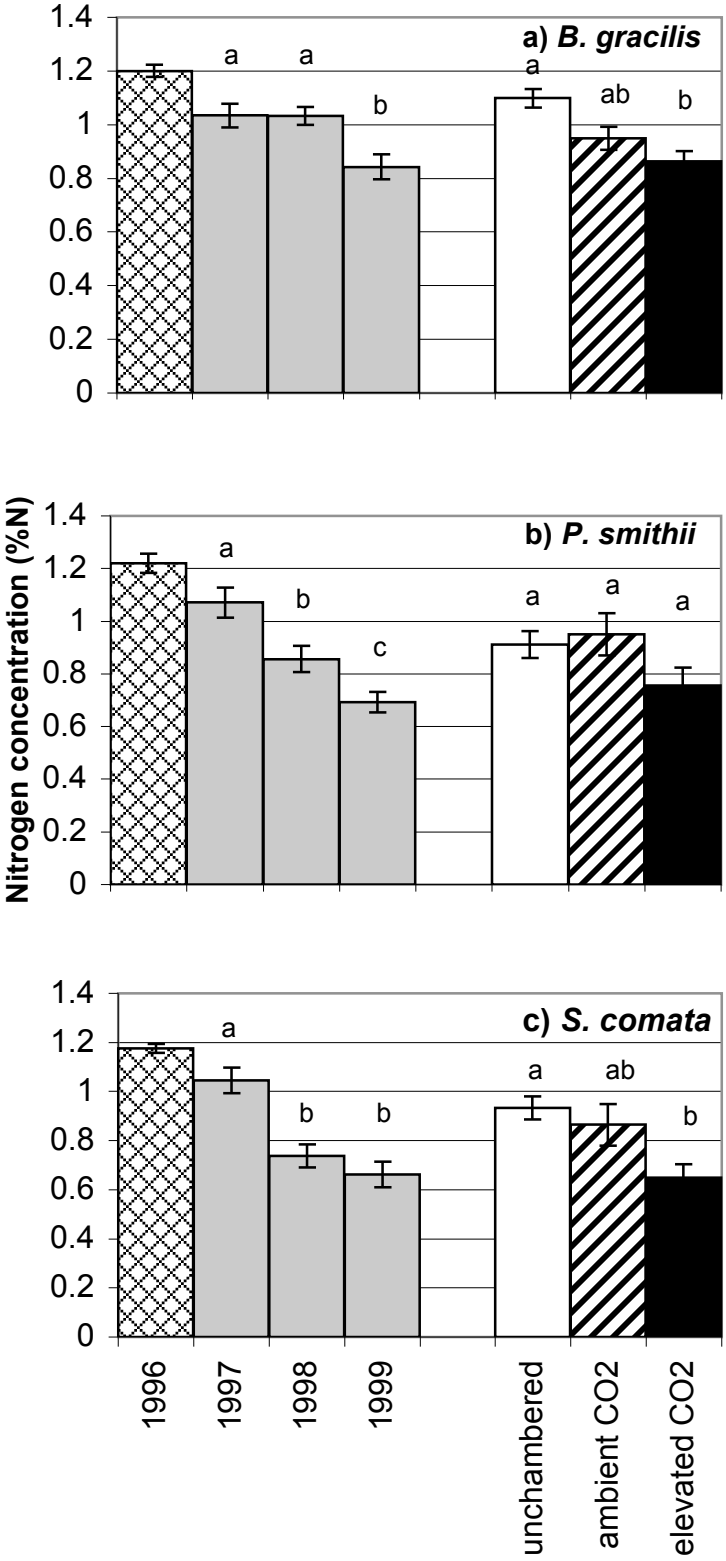
a) B. gracilis
b) P. smithii
c) S. comata
Nitrogen concentration (%N)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
a
ab
b
a
b
c
a
a
a
a
b
b
a
ab
b
1996
1997
1998
1999
unchambered
ambient CO2
elevated CO2

Figure 5

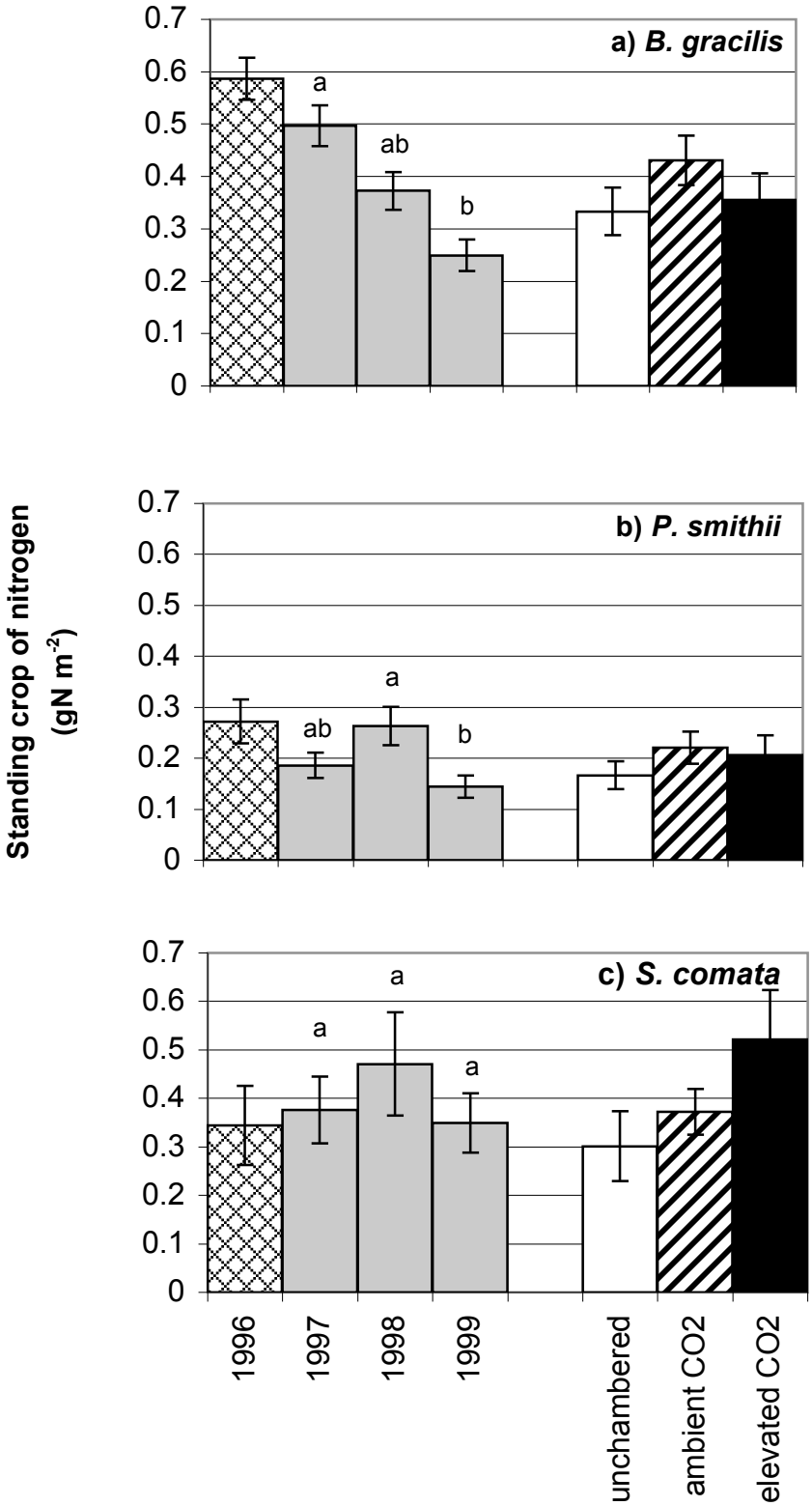
a) B. gracilis
b) P. smithii
c) S. comata
Standing crop of nitrogen (gN m-2)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
ab
b
a
ab
b
a
a
a
1996
1997
1998
1999
unchambered
ambient CO2
elevated CO2

Figure 6

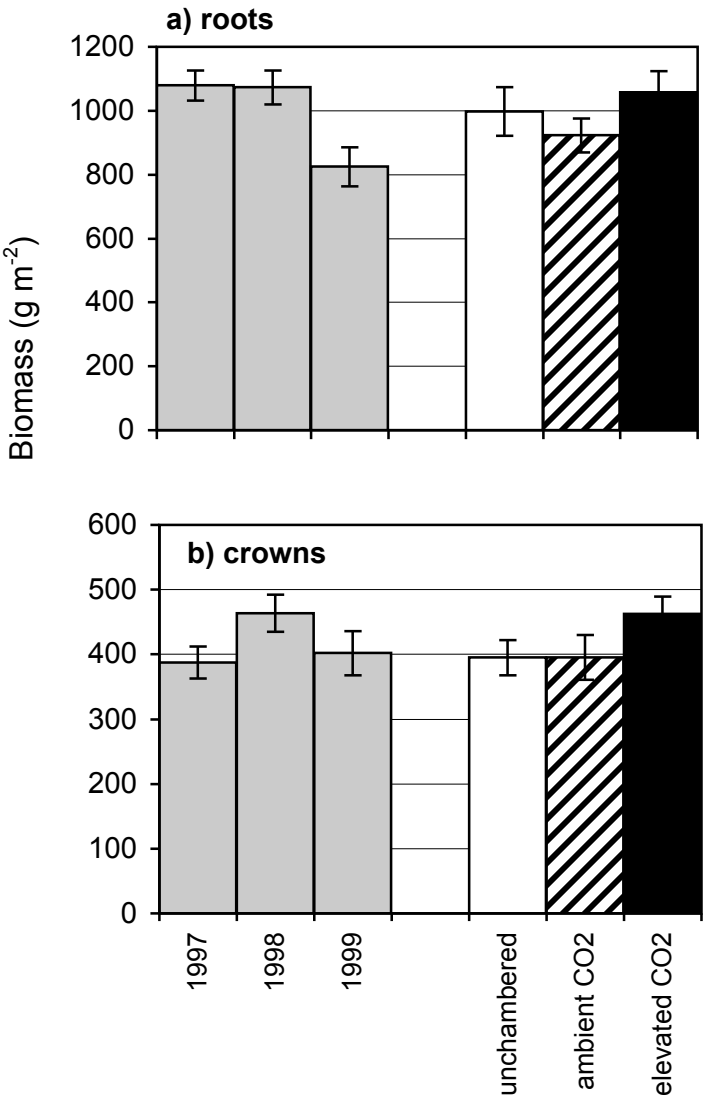
a) roots
1200
1000
800
600
400
200
0
Biomass (g m-2)
b) crowns
600
500
400
300
200
100
0
1997
1998
1999
unchambered
ambient CO2
elevated CO2

Table 1. Results of repeated measures analysis of variance (ANOVA) showing main effects and interactions for biomass, tissue nitrogen concentration, and standing crop of nitrogen for three years of experimental treatment (1997-1999).

	Biomass (g m^{-2})			Nitrogen Concentration (%N)			Standing Crop of Nitrogen (gN m^{-2})		
	July	October	Regrowth[2]	July	October	Regrowth[2]	July	October	Regrowth[2]
CO_2 treatment[1]	□□	□□	□□□	□□□	NS	□□	□	□□	□□
year	□□	NS	□	□□□	□□□	□□□	□□	□	□□□
CO_2 trt x year	NS	NS	NS	NS	□□	NS	NS	NS	NS

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
NS, not significant

[1] CO_2 treatment includes unchambered, ambient CO_2, and elevated CO_2 plots
[2] Regrowth following simulated herbivory

Table 2. Aboveground plant nitrogen in July, October, and regrowth biomass, standard error in parentheses.
Values in each group followed by different letters indicate significant difference ($P \leq 0.05$). Data from 1996 included for reference.

CO_2 treatment or year	Nitrogen concentration (%N)			Standing crop of nitrogen (gN m^{-2})		
	July	October	Regrowth*	July	October	Regrowth*
unchambered	0.98 (0.04) a	0.68 (0.05) a	0.82 (0.07) a	0.94 (0.05) a	0.54 (0.06) a	0.28 (0.04) a
ambient CO_2	0.94 (0.05) a	0.65 (0.05) a	0.79 (0.05) a	1.24 (0.06) ab	0.85 (0.07) ab	0.47 (0.05) b
elevated CO_2	0.74 (0.04) b	0.60 (0.03) a	0.70 (0.05) b	1.28 (0.09) b	1.12 (0.09) b	0.59 (0.05) b
1996	1.21 (0.02)	0.76 (0.02)	0.92 (0.02)	1.39 (0.05)	0.68 (0.05)	0.24 (0.01)
1997	1.04 (0.04) a	0.75 (0.02) a	0.88 (0.02) a	1.21 (0.05) a	0.86 (0.08) ab	0.60 (0.06) a
1998	0.86 (0.03) b	0.69 (0.03) b	0.88 (0.04) a	1.28 (0.09) a	0.98 (0.11) a	0.41 (0.05) b
1999	0.76 (0.04) c	0.49 (0.01) c	0.55 (0.01) b	0.98 (0.07) b	0.67 (0.12) b	0.32 (0.04) c

* Regrowth following simulated herbivory

Table 3. Results of repeated measures analysis of variance (ANOVA) showing main effects and interactions for species biomass, tissue nitrogen concentration, and standing crop of nitrogen for three years of experimental treatment (1997-1999).

	Biomass (g m^{-2})	Nitrogen Concentration (%N)	Standing Crop of Nitrogen (gN m^{-2})
CO_2 treatment[1]	□□	□□	NS
year	□□□	□□□	□□□
species	□	□□□	□
CO_2 trt x year	NS	NS	□
CO_2 trt x species	NS	NS	NS
year x species	□□□	□□□	□□
CO_2 trt x year x species	NS	NS	NS

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
NS, not significant

[1] CO_2 treatment includes unchambered, ambient CO_2, and elevated CO_2 plots

Table 4. Belowground standing crop of nitrogen in October. Standard error in parentheses.

CO_2 treatment or year	Standing crop of nitrogen ($gN\ m^{-2}$)	
	Roots	Crowns
unchambered	11.02 (0.90)	4.65 (0.46)
ambient CO_2	9.77 (0.67)	4.54 (0.45)
elevated CO_2	11.12 (0.70)	4.96 (0.21)
1997	11.63 (0.64)	4.44 (0.40)
1998	11.14 (0.83)	5.40 (0.39)
1999	9.15 (0.60)	4.32 (0.27)