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Elevated CO_2 and defoliation effects on a shortgrass steppe: Forage quality versus quantity for ruminants

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Abstract

We assessed the effects of elevated atmospheric CO_2 on ruminant forage quality and nutrient yields during 4 years in semiarid shortgrass steppe where grazing by domestic livestock is the primary land-use. A defoliation and a nitrogen fertilization treatment were superimposed on CO_2 treatments in large open-top chambers. CO_2 effects on forage soluble and fiber (celluloses, lignin) constituents were small, even though mid-growing season yield and end of season production increased. However, large negative effects of elevated CO_2 were evident in crude protein concentrations and digestibility of forages. While the effects were more negative mid-growing season than autumn, a reduction in already poor quality autumn forage may be more critical to animals. Crude protein concentrations of autumn forage on the elevated CO_2 treatment fell below critical maintenance requirements 3 out of 4 years, compared to 1 of 4 for ambient and control treatments. Forage digestibility declined 14% mid-season and 10% in autumn with elevated CO_2. Negative effects of elevated CO_2 on animal performance mediated through forage quality are likely to be greater than the positive effects of increased quantity, because quality drops to critically low levels that can inhibit utilization. Further, elevated CO_2 shifted the proportional availability of protein and energy to a species of lower overall quality and the species most negatively affected by drought. Current-year defoliation increased both quality and production of protein and energy compared to non-defoliated plots, but no CO_2 by defoliation treatment interactions were observed. Nitrogen fertilization increased crude protein concentrations and digestibilities, but not in the least nutritious species that increased with elevated CO_2 or in autumn when quality was lowest.

Keywords: Compensatory regrowth; Digestibility; Nitrogen fertilization; Grassland; Herbivory; Lignin

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1. Introduction

There are relatively few assessments of the effects of elevated atmospheric CO_2 on large herbivores (Owensby et al., 1996; Frehner et al., 1997; Fritschi et al., 1999). Even for studies that assess impacts, the amount of consumption relative to size of treatment plots necessitates extrapolation from indices of forage quality. Direct estimates of performance in response to CO_2 can be obtained for smaller arthropod herbivores (Lindroth, 1996). However, ruminant response would not be expected to be similar to arthropod response because of the microbial processing of food in the rumen, and because ruminants cannot increase intake in response to low quality forage (Owensby et al., 1996). In temperate regions, unsupplemented native ruminants are often subject to periods of bulk limitation (further intake is not possible) and periods when crude protein levels fall below maintenance requirements. Overwinter periods of body-protein catabolism and starvation death of wild herbivores with full rumens are not uncommon. Supplementation of livestock is an expense, and the balance between maintaining land in native range or converting it to crop agriculture can depend on economics (Joyce, 1989).

Grazing by domestic livestock is the primary economic land-use in shortgrass steppe in the Great Plains of North America, where 40% remains in native vegetation due to the semiarid, marginal conditions for crop production (Lauenroth et al., 1994). Smaller native ruminants, such as pronghorn antelope, are important faunal components of the system. Elevated levels of atmospheric CO_2 can potentially affect forage quality for both native and domestic consumers, and there are potential broad implications for biodiversity and land-use practices. Defoliation can feed back on forage quality, because tissue regrown is different in quality from non-defoliated tissue in the shortgrass steppe (Milchunas et al., 1995).

Protein, digestible energy, and rate of passage are three basic, important, interrelated components of ruminant nutrition for which inferences can be made based on indices of forage quality. Forage nitrogen concentration is an index of crude protein for ruminants, because sources of non-protein-N in plants are converted to protein by rumen microbes. A large proportion of the nitrogen in plants is in cell solubles, which are highly digestible. Digestible energy from soluble carbon compounds is also readily assimilated from cell solubles. Hemicellulose and cellulose of the fibrous fraction are potentially digestible but rate limiting, the degree of which depends on encrustation by lignin, the availability of nitrogen in the rumen, and the rate of particle-size breakdown (VanSoest, 1982). Lignin is virtually indigestible by the ruminant. Rate of passage in the ruminant is complex, but in general, the greater the digestibility and the lower the lignin, the higher the rate of passage. Therefore, reasonably good estimates of the protein and energy value of a forage, and relative passage rate limitations, may be obtained from nitrogen, soluble/fiber fractions, and in vitro digestible dry matter (IVDDM) analyses. IVDDM integrates over other mineral nutrient compositions/limitations as well as over concentrations, ratios, and structure of carbon and nitrogen in an index of microbial digestion, but does not account for physical breakdown processes such as mastication. There are established, good relationships between IVDDM and in vivo digestible energy for cattle, sheep, and deer (Milchunas et al., 1978).

C:N ratios are commonly higher in growing plant tissue exposed to elevated compared to ambient CO_2 (reviewed by Rogers et al., 1999; Körner, 2002). However, this may not persist after plant senescence, as cell solubles are translocated to roots and crowns or leached from structural components during freeze–thaw and precipitation events. This has been proposed as a factor explaining the somewhat common observations of no or small effects of CO_2 on field-grown, senescent litter decomposition on the soil surface (O'Neill and Norby, 1996; Owensby et al., 1996). CO_2 may also affect cell soluble/fiber composition of plant tissue. Increased plant growth would increase fiber fractions, whereas increased carbon assimilation may result in greater cell soluble content due to storage of excess carbohydrates. Very few forages grown under ambient and elevated CO_2 have been assessed for IVDDM and mixed results have been reported (Akin et al., 1994, 1995; Owensby et al., 1996; Frehner et al., 1997; Carter et al., 1999; Fritschi et al., 1999).

Another aspect of forage quality in response to CO_2 is the feedback between plant and animal due to defoliation effects on plant tissue quality. Regrowth tissue of defoliated plants can be of different quality than undefoliated plant tissue (Polley and Detling,

1989; Coppock et al., 1983), and this has been observed in shortgrass steppe vegetation where this study was conducted (Milchunas et al., 1995). There is the potential for CO_2 by defoliation interactions on forage quality (Soussana et al., 1996). Catovsky and Bazzaz (1999) observed that one species increased growth at the dry end of a soil water gradient in response to elevated CO_2 while another species responded more at the wet end, and attributed the differences in sensitivity to drought. Forage quality also varies with precipitation from year to year, with greater growth often diluting nutrient concentrations and resulting in greater stem to leaf ratios and greater fiber concentrations. Shortage of available soil nitrogen may limit the ability of plants to respond to elevated CO_2 (Curtis et al., 1994; Daepp et al., 2000). Alternatively, plants may respond to elevated CO_2 even in N-poor soil (Norby et al., 1992) if increased exudation stimulates microbial mineralization of nitrogen (Zak et al., 1993). CO_2 studies with N-additions have most often been conducted in forest or subhumid to mesic grasslands. Water is often the most limiting factor in the semiarid shortgrass steppe, and interactions between CO_2 and nitrogen may depend on precipitation during a particular year.

Our broad objectives in this study were to assess forage quality of native shortgrass steppe vegetation exposed to ambient and elevated CO_2 in the field. The study ran for 5 years with forage quality data collected for the last 4 years, thereby allowing for comparisons under different seasonal annual weather conditions. A recent report from this study site indicated increased production, a shift in species composition, and lower forage quality based on 2 years of digestion data (Morgan et al., 2004). In this paper, we focus in greater detail on tradeoffs between the potential for elevated CO_2 to decrease quality while increasing quantity, and how this may be accentuated by shifts in species contributing to nutrient and energy yields. We here also specifically assess how defoliation and N-fertilization treatments, superimposed within the large open-top chambers, may interact with CO_2 treatment in affecting forage quality. A previous study showed that the effects of defoliation on forage quality in this plant community can differ among long-term grazing treatments (Milchunas et al., 1995). We hypothesized that defoliation and N-fertilization would both result in greater increases in forage quality under elevated than ambient CO_2, because the effects of defoliation are often greater in more productive systems (Milchunas and Lauenroth, 1993) and nitrogen can become limiting under elevated CO_2 (Mosier et al., 2003). We further hypothesize that the preliminary data indicating a decline in forage digestibility: (1) is primarily due to lower crude protein rather than changes in fiber fractions, because of possible counteracting influences of increasing solubles and fibers with elevated CO_2, (2) will not be as large during later dry years, because nitrogen may not be as limiting as in wet years, and (3) the largest differences in forage quality will be during the green-season rather than after plant senescence, because of the possibility for translocation of nutrients out of leaves during senescence.

2. Methods

The study was conducted at the Central Plains Experimental Range (CPER) (lat. 40°49′N, long. 104°46′W) in north central Colorado. Mean annual precipitation is 321 mm, with 71% occurring during the May through September growing season (Lauenroth and Milchunas, 1991). Mean monthly air temperatures range from 22 °C in July to below 0 °C in January. During the 5 years of study, the first 3 years had much higher precipitation than the long-term mean (Fig. 1). Years 2 and 3 had a normal seasonal distribution, but year 1 had an unusually wet late season. Years 4 and 5 were slightly below and slightly above the long-term mean, respectively. Both years 4 and 5 had long periods of drought; year 4 was very dry until after the mid-season sampling of vegetation and year 5 was very dry after the mid-season sampling. Years 1 through 5 were 1997–2001.

Total vegetative basal cover at the CPER is typically 30–40% (Milchunas et al., 1989). Prior to starting CO_2 fumigation, *Bouteloua gracilis* (H.B.K.) Lag. comprised 45%, *Stipa comata* (Trin and Rupr.) 25%, and *Pascopyrum smithii* (Rydb.) A. Löve 18% of plant biomass in 1996 (Morgan et al., 2004). *B. gracilis* is a caespitose, C_4 warm-season short-grass that is drought and grazing tolerant. *S. comata* is a mid-height, C_3 cool-season, bunchgrass. *P. smithii* is a mid-height, rhizomatous, C_3 cool-season grass that usually occurs in groups of one to four tillers, is highly preferred by small and large herbivores and decreases

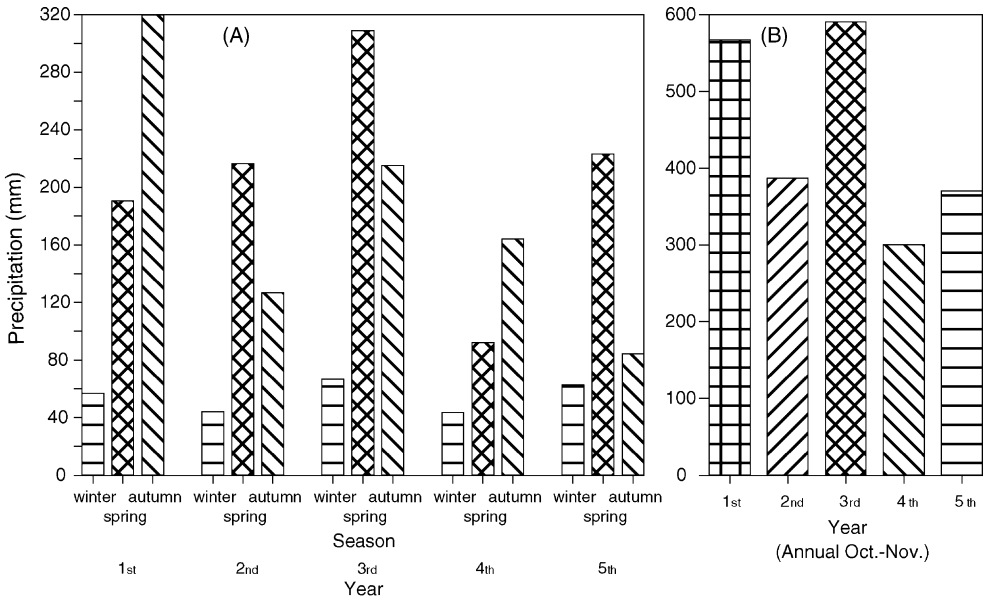

Fig. 1. Seasonal and annual precipitation during the 5 years (1997–2001) of CO_2 treatments at a shortgrass steppe site. Long-term average precipitation is 321 mm year^{-1}. Annual precipitation was calculated from November through October, since plots were clipped in October after senescence. Winter was considered November–March, early growing season was April–July 15, and late-growing season was July 15–October. July 15 is the approximate time of the mid-season sampling, and is also the average-year approximate time of peak-green-standing crop.

with grazing (Milchunas et al., 1989). Soil at the site is a Remmit fine sandy loam (Ustollic camborthids).

The experimental design included three blocks with each of an ambient (360 ± 20 μmol mol^{-1}) and an elevated (720 ± 20 μmol mol^{-1}) CO_2 large open-top chambers (OTCs) (4.5 m diameter by 3.8 m high, 15.5 m^2 ground area) and a non-chambered control of equal area. Each year, OTCs were placed on plots in early spring just before first vegetation green-up, and removed in the autumn after senescence. Precipitation was returned to the chambers by an automated system, with inefficiencies in capture supplemented back to the plots. See Morgan et al. (2001) for details of design and operation of the OTCs. Comparisons of micro-climatic and plant responses indicated significant chamber effects (Morgan et al., 2001, 2004). Air temperatures at canopy height and soil temperatures averaged 2.6 and 1.25 °C warmer inside than outside chambers throughout the experiment. Periodic measurements of PAR indicated the chambers reduced PAR by 28%.

The shortgrass steppe has a long evolutionary history of grazing by large herds of bison, and is among the most resistant to grazing by domestic livestock of all grasslands in the world (Milchunas et al., 1988a,b; Milchunas and Lauenroth, 1993). As a result, current-day grazing intensities are also high relative to many other systems. Moderate and heavy grazing at the CPER is considered 40 and 60% utilization of aboveground primary production, respectively, and private lands at 50–70% utilization (Bement, 1969; Milchunas et al., 1995). For this experiment, large wire grids made from cattle panels that contained fifty-six 40.5 cm × 15.3 cm contiguous quadrats (3.46 m^2 total area) were placed in a fixed location in plots. Every other quadrat (painted either red or green) was clipped by species to crown level at mid-growing season (mid-July), with the other colored alternating quadrats clipped the following year. All quadrats were clipped in late October after plant senescence, by grid color with all quadrats combined within a color for each plot. These two sampling dates represented green forage during the growing season and standing dead peak crop at the beginning of the winter dormant period. The July clip represented a defoliation treatment, whereby regrowth was sampled from the same quadrats in October. These quadrats were compared to the non-defoliated

quadrats that were clipped only in October when clipping of senescent material would not elicit a physiological response. Annual alternation of the color of the quadrats clipped each July allowed for a rest from mid-season defoliation, and resulted in a treatment where 50% of individuals were defoliated in a patchy pattern that is similar to the small-scale defoliated–undefoliated pattern created naturally by the grazing animals (Varnamkhasti et al., 1995; Milchunas et al., 1995). A disadvantage of this approach is that defoliations occurred at one time in the season rather than spread throughout, and selectivity for species by the animal is not accounted for. Average removal intensity for the defoliated grids was 67% of above-ground production, excluding the year with the late-season drought (2001, 5th year of treatment). October clippings were for total biomass, not by species, except in 2000. Samples of the three dominant species were obtained in 2001, but the drought after the July clip that year limited the quantity of sample from the defoliated quadrats. Vegetation samples were dried at 55 °C, weighed, and milled.

In April 2000, a nitrogen addition was applied to each plot in a 1.115 m^2 area on the east end of the clip grid described above. Nitrogen was applied as NH_4NO_3 in an amount equivalent to 2 g N m^{-2} in 1 cm water by hand sprinkler cans. An equivalent amount of water was applied to the non-fertilized part of the grid.

Plant tissue samples were analyzed for cell solubles, hemicellulose, cellulose, and lignin by the NDF, ADF, and sulphuric acid lignin fractionation method (VanSoest, 1963, 1967, 1975), modified for block refluxing and without sodium sulphite. Values were expressed on an ash-free basis (sub-samples in muffel furnace at 550 °C) in order to compare with root samples reported elsewhere. Hemicellulose and cellulose values were added together for statistical analyses and presentation, because of their nutritional similarity (VanSoest, 1975). Nitrogen and carbon were assessed using an automated C/N combustion analyzer (PDZ Europa). Nitrogen was converted to crude protein by multiplying values by 6.25 (Maynard and Loosli, 1969).

Inoculum for IVDDM was collected from a fistulated cow maintained on an exclusively grass-hay diet for 1 week prior to collection, with feed removed 12 h and water removed 3 h 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 Milchunas and Baker (1982, strain-layer method). IVDDM was run according to the two-stage, 96 h, Tilley and Terry (1963) method with modifications and quality controls described in Milchunas and Baker (1982). The first stage is a 48 h microbial digestion, that simulates rumen processing of the forage, followed by a second 48 h acid pepsin stage that simulates lower gut digestion.

CO_2 and defoliation treatments, or CO_2 and nitrogen fertilization treatments, were analyzed for their effects on cell solubles, hemicellulose plus cellulose, lignin, crude protein (N times 6.25), and IVDDM using the SAS PROC MIXED analysis (SAS Institute Inc., Cary, NC, USA). ''Year'' was used as a repeated measure variable; ''block'' was specified as a random effect (thereby removing the variability due to blocking); and block × CO_2 treatment was used as the error term for CO_2 treatment comparisons. Species was a factor only for mid-season analyses, while defoliation or fertilization were factors in autumn data analyses. 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.

3. Results

3.1. Soluble and fiber constituents

Soluble and fiber concentrations of vegetation harvested at mid-growing season showed significant CO_2 treatment main effects, and plant species by year interactions (Fig. 2A–C). CO_2 treatment effects were small, however. Cell solubles and lignin concentrations were slightly lower in elevated compared to ambient CO_2 treatment, and celluloses were higher. Control and ambient CO_2 treatments did not significantly differ in any of the fiber constituents. Differences among species were generally larger than among CO_2 treatment or year. *P. smithii* had higher concentrations of cell solubles than either *B. gracilis* or *S. comata*, and *S. comata* had higher concentrations of lignin than the other two species.

In contrast to concentrations, large differences among CO_2 treatments were observed for yield (or

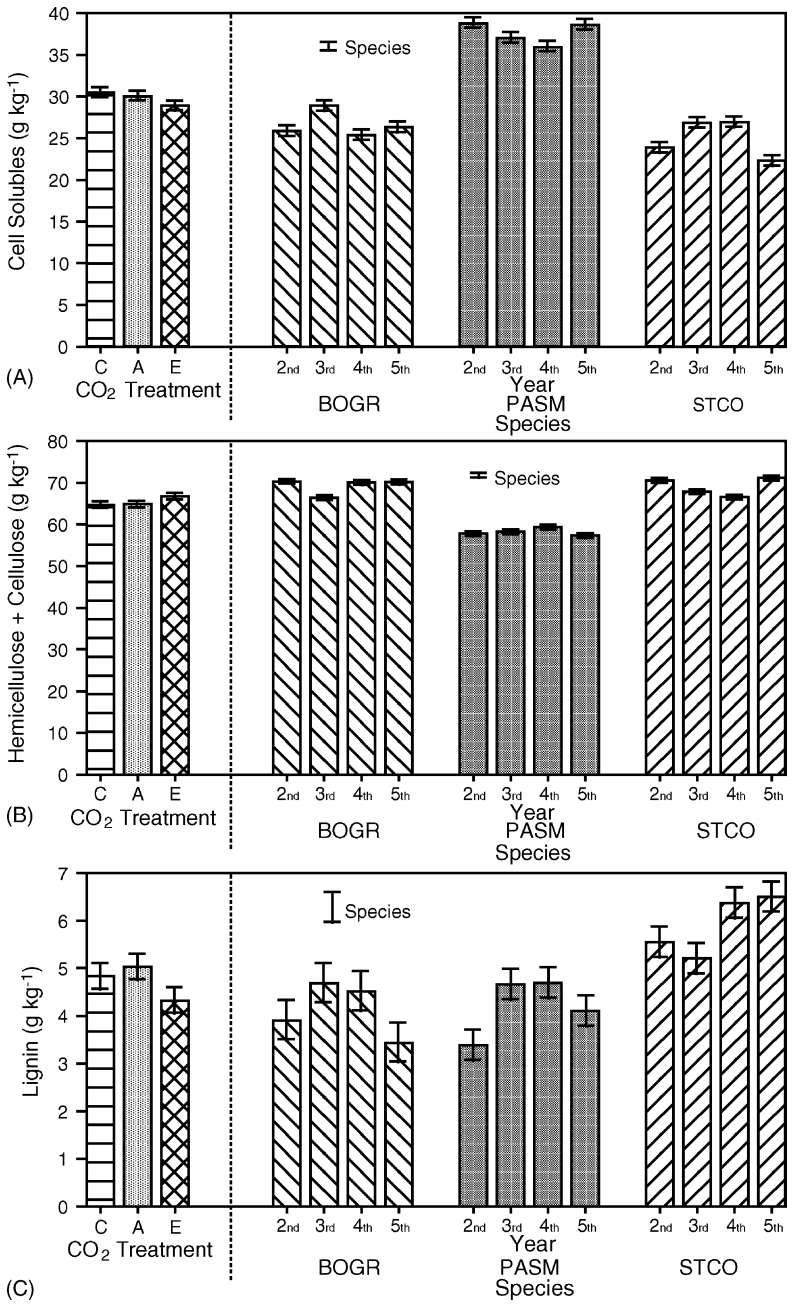

Fig. 2. (A) Cell solubles, (B) hemicellulose plus cellulose, and (C) lignin concentrations (g kg^{-1}) of mid-growing season *B. gracilis* (BOGR), *P. smithii* (PASM), and *S. comata* (STCO) vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2 in the 2nd through 5th year of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

standing crop) of the fiber constituents at mid-season, but this often depended on the plant species. Yield of soluble components of forage increased from control, to ambient, to high CO_2 treatment (Fig. 3A). The spring drought during the 4th year resulted in lower yields of soluble constituents in all species, but this was particularly true for *S. comata*. A mid-season through autumn drought in the 5th year also resulted in generally lower yields of solubles, but not for *B. gracilis*. Elevated CO_2 treatment resulted in much greater yield of celluloses only for *S. comata*, and again *S. comata* was the species most affected by the drought years (Fig. 3B). The CO_2 treatment by species interaction for lignin yield displayed similar response patterns to that for celluloses, with effects of elevated CO_2 manifesting through *S. comata* yields (Fig. 3C). For yields in general, *B. gracilis* was least affected by drought and *S. comata* displayed the greatest yield fluctuation.

Autumn harvests are presented by total biomass to simplify data presentation, because species data generally followed the same pattern as for mid-season, with *P. smithii* highest in soluble concentration and *S. comata* highest in lignin. Response to CO_2 treatments in concentrations of fiber fractions of total vegetation after senescence in autumn was even less than that observed in green, mid-season tissue. Slightly lower solubles and slightly higher celluloses were observed in elevated compared to ambient CO_2 treatments, with no differences for lignin concentrations (Fig. 4). Defoliation treatment was not a significant factor in any analyses of soluble and celluloses data. The significant defoliation treatment by CO_2 treatment interaction for lignin concentration was due only to differences between defoliation of control CO_2 treatment (Fig. 4C). Differences among years in the three constituents were generally small, but greatest for lignin.

Production of the soluble and fiber fractions of the vegetation represents the addition of the July plus the October yield on the defoliated treatment compared to the October yield on the undefoliated treatment. Elevated compared to ambient CO_2 treatment resulted in greater production of the three components, but this occurred only in the 2 years that did not have a drought period, although a similar but non-significant trend occurred in the other 2 years (Fig. 5). A significant main effect of defoliation treatment was observed for solubles and celluloses, with defoliation stimulating greater production. Lignin production displayed both increases and decreases in response to defoliation, depending on year. No CO_2 treatment by defoliation treatment interactions were observed.

3.2. Crude protein

Mid-growing season responses to CO_2 treatment of plant tissue crude protein concentrations were generally greater than for fiber constituents, but depended on species and year (Fig. 6A). *B. gracilis* crude protein concentrations were the most variable with time and treatment. Mid-season crude protein concentrations of *B. gracilis* were not significantly different between ambient and elevated CO_2 in years 2 and 3 of treatment, but then displayed among the largest reductions of the three species in the 2 years with drought (4th: 35% reduction, 5th: 34% reduction). Both *P. smithii* and *S. comata* had lower mid-season crude protein in elevated compared to ambient CO_2 treatments in all 4 years, and differences were large compared to fiber fractions. Averaged overall CO_2 treatments, *B. gracilis* had highest crude protein concentrations and *P. smithii* and *S. comata* similarly lower values (6.5, 5.8, 5.8%, respectively). However, crude protein values for the elevated CO_2 treatment showed even greater differences among species, with *S. comata* lowest at 4.9%, *P. smithii* intermediate at 5.2%, and *B. gracilis* highest at 5.7%.

B. gracilis crude protein yield (standing crop) at mid-season was lower in elevated compared to ambient CO_2 treatments, but the opposite was true for *S. comata* (Fig. 6B). The increase in *S. comata* growth overcame the decrease in crude protein concentrations. No significant difference was observed between ambient and elevated CO_2 treatments in crude protein yield for *P. smithii*. No differences were observed between control and ambient CO_2 treatments. *S. comata* yield was most affected by the 4th year drought and *B. gracilis* the least.

Crude protein concentrations in total vegetation by autumn were negatively affected by elevated compared to ambient CO_2 treatment, but only during 2 of the 4 years of treatment and generally less than differences at mid-season (Fig. 7A). The years without declines with elevated CO_2 treatment were the two with wet late-growing seasons (3rd and 4th years) (Fig. 1). The largest negative effect of elevated CO_2 on

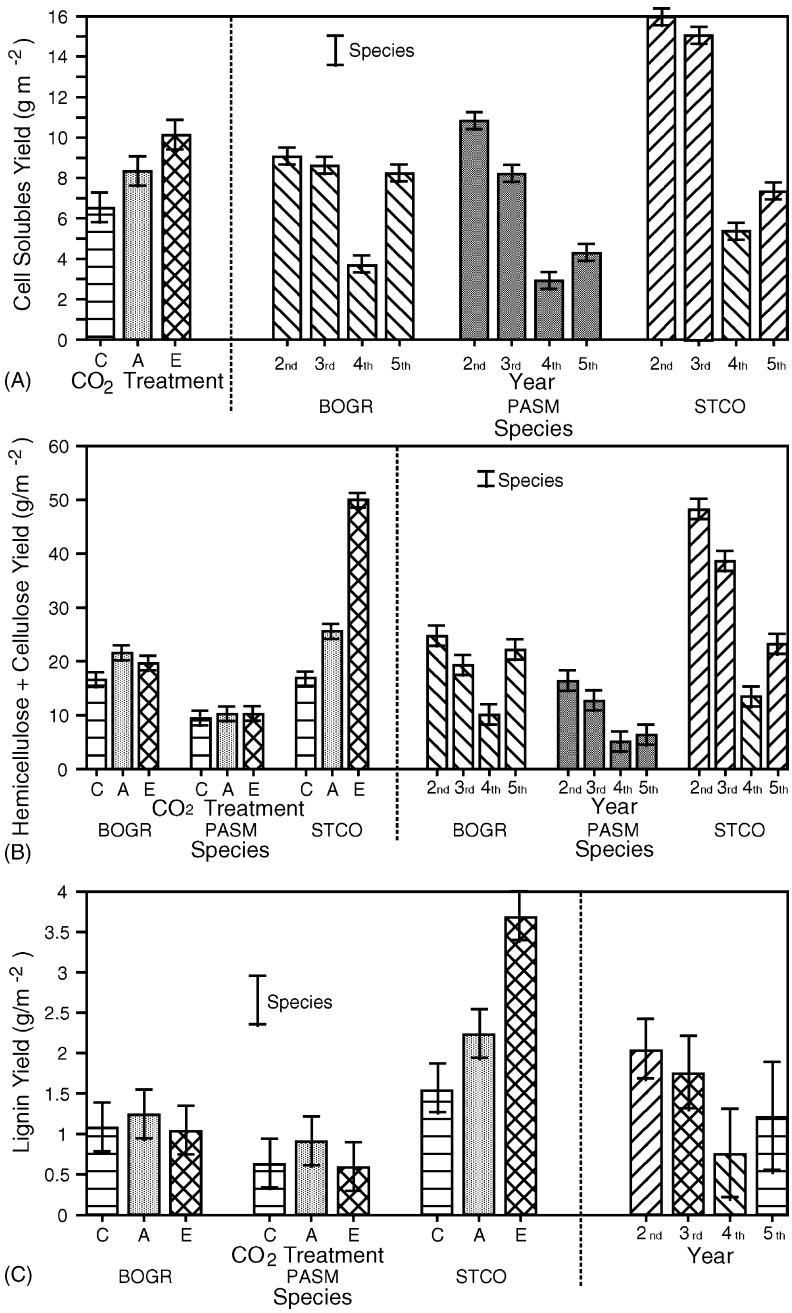

Fig. 3. (A) Cell solubles, (B) hemicellulose plus cellulose, and (C) lignin yield (g m^{-2} standing crop) of mid-growing season *B. gracilis* (BOGR), *P. smithii* (PASM), and *S. comata* (STCO) vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2 in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

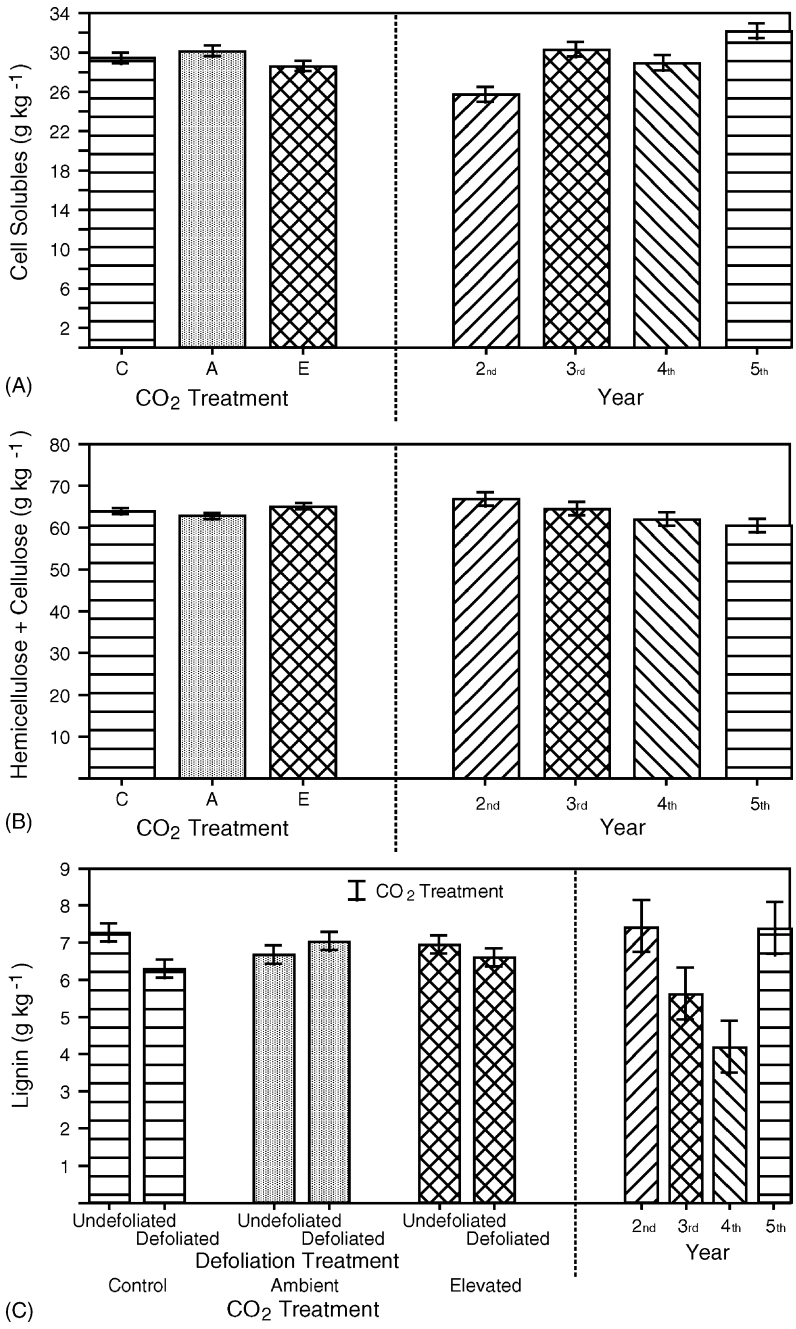

Fig. 4. (A) Cell solubles, (B) hemicellulose plus cellulose, and (C) lignin concentrations (g kg^{-1}) of autumn total vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2, and previously undefoliated or defoliated during mid-season, in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

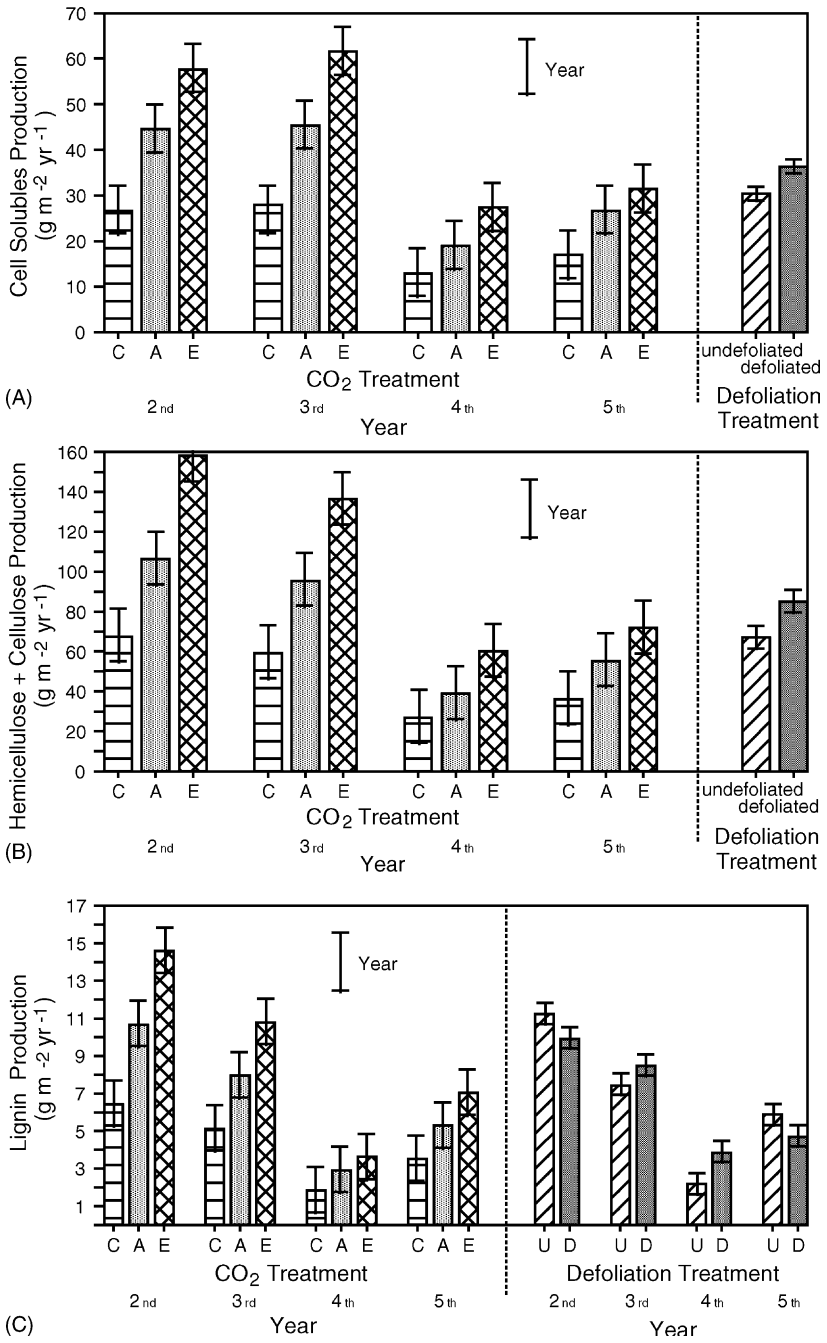

Fig. 5. (A) Cell solubles, (B) hemicellulose plus cellulose, and (C) lignin production (g m^{-2} $year^{-1}$) of autumn total vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2, and previously undefoliated (U) or defoliated (D) during mid-season, in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

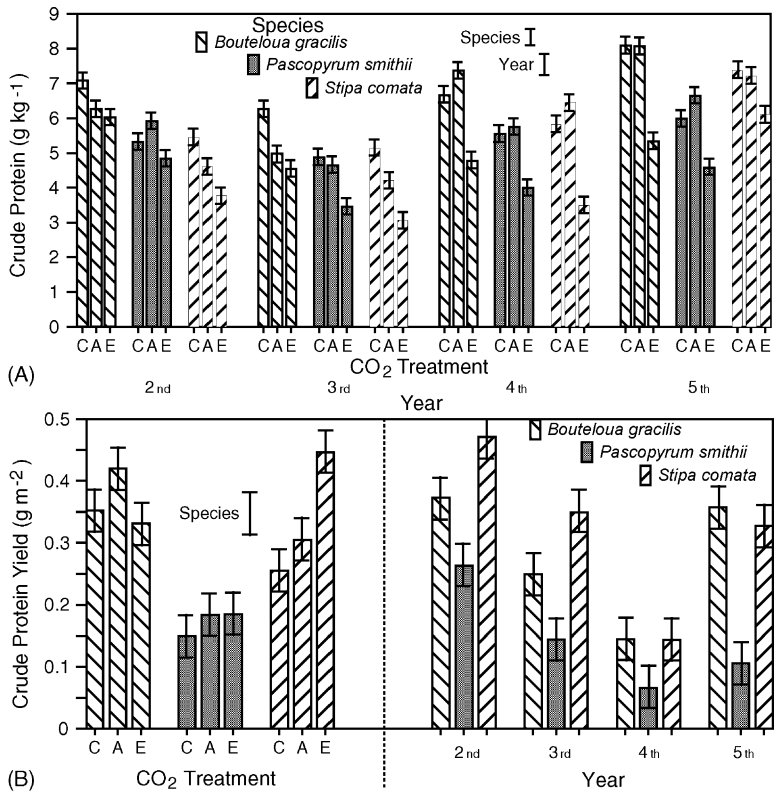

Fig. 6. (A) Crude protein concentration (g kg^{-1}), or (B) yield (g m^{-2} standing crop) of mid-growing season *B. gracilis*, *P. smithii*, and *S. comata* vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2 in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

late-season forage crude protein concentrations was the 5th year of treatment (Fig. 7A). In general, lowest nitrogen concentrations occurred during the wettest year (3rd year). Defoliation resulted in forage regrowth with higher crude protein concentrations, except in the wettest year. Annual crude protein production was greater in elevated compared to ambient CO_2 treatment in all but the 5th year of treatment (Fig. 7B). For elevated compared to ambient CO_2 treatment, crude protein production increased in 1 year when concentrations were lower, increased in 2 years when concentrations were not different, and remained the same in 1 year even though concentrations were lower. Defoliation increased crude protein production in all but the 4th year of treatment. The non-significant defoliation response was in the year with the spring drought, but there also appeared to be a general decline through time in the stimulation in production from defoliation.

3.3. Digestibility

A significant CO_2 treatment by year interaction for forage digestibility at mid-season was due to control and ambient treatments. There was a consistent and rather uniform among years reduction in digestibility with elevated compared to ambient CO_2 treatment, averaging 14% over years (Fig. 8A). *P. smithii* was the most digestible species and *S. comata* the least (19% less). Species differences in digestibility were greater than CO_2 treatment differences. Digestible forage yield at mid-season was not significantly affected by CO_2 treatment, although it averaged 11% greater under elevated compared to ambient CO_2 (data not

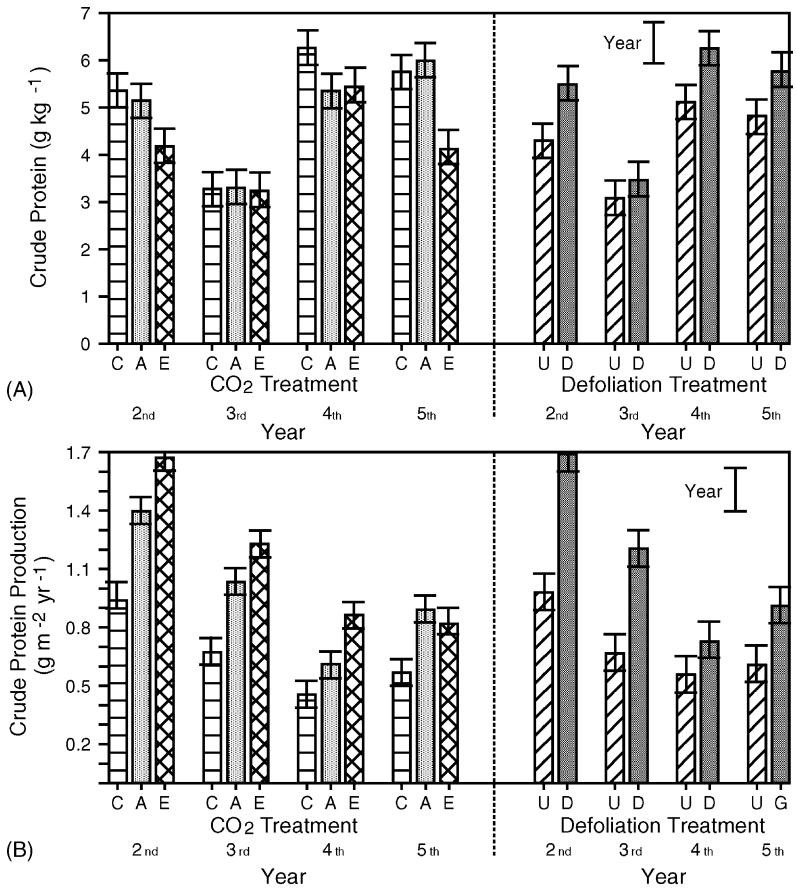

Fig. 7. (A) Crude protein concentration (g kg^{-1}), or (B) production (g m^{-2} $year^{-1}$) of autumn total vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2, and previously undefoliated (U) or defoliated (D) during mid-season, in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

shown). The two drought periods most affected the digestible forage yield of *S. comata* and least affected *B. gracilis* (Fig. 8B).

Senescent vegetation in autumn showed significantly lower digestibility in elevated compared to ambient CO_2 treatment, except for the 4th year with the spring drought where no CO_2 treatment effects were observed (Fig. 9A). Elevated CO_2 treatment resulted in a 10% reduction in digestibility of autumn forage when averaged over all years, compared to the 14% reduction for green, mid-season forage (Fig. 8A). Quadrats previously defoliated in July had vegetation of higher digestibility than the undefoliated treatment in 2 out of the 4 years (Fig. 9A). Data by species was obtained in autumn of the 4th and 5th years of CO_2 treatment. Both *B. gracilis* and *S. comata* had lower digestibilities under elevated compared to ambient CO_2 treatment, while there was only a trend of lower digestibility for *P. smithii* (Fig. 9B). Defoliation treatment effects were also species dependent. Defoliation increased digestibility of *P. smithii* and *S. comata*, but not the shorter stature *B. gracilis*.

Even though elevated CO_2 treatment reduced digestibility, digestible forage production was greater in elevated compared to ambient CO_2 treatment (Fig. 9C). Defoliated treatment produced more digestible forage than undefoliated, and years with a period of drought produced about half the digestible

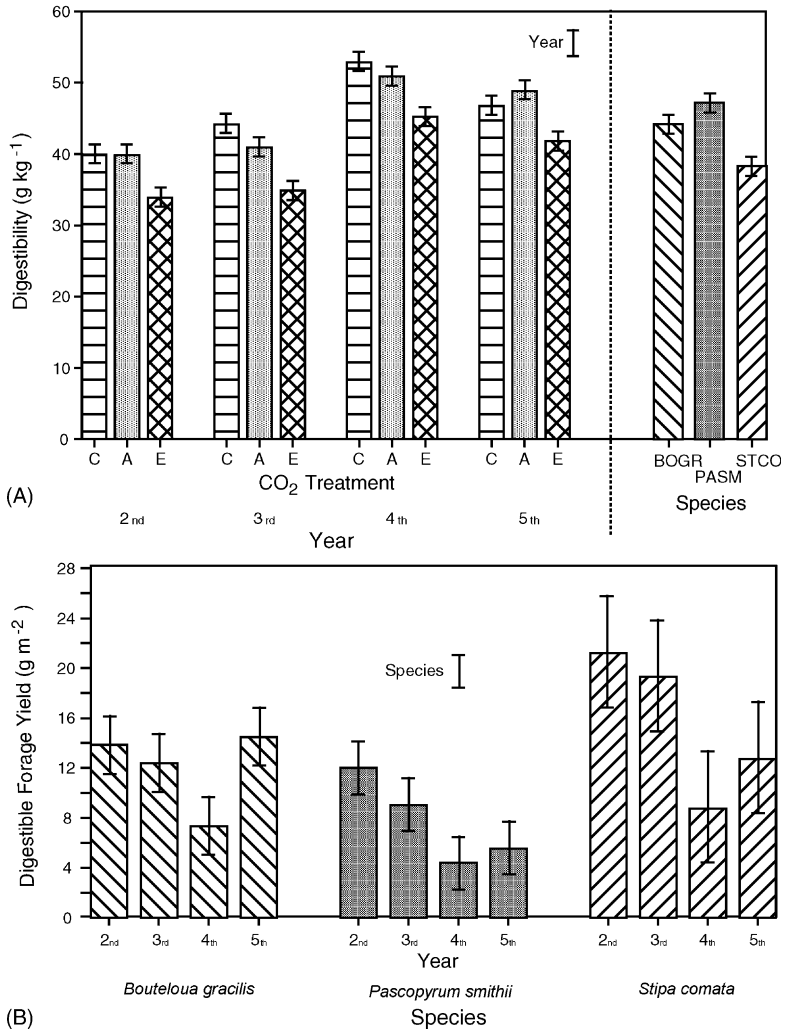

Fig. 8. (A) Digestibility (g kg^{-1} in vitro digestible dry matter), or (B) digestible forage yield (g m^{-2} standing crop) of mid-growing season *B. gracilis* (BOGR), *P. smithii* (PASM), and *S. comata* (STCO) vegetation exposed to control (C), ambient (A), and elevated (E) levels of CO_2 in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

forage yield of good years of precipitation. The year with the spring drought (4th) produced less digestible forage than the year with the late-season drought (5th).

3.4. Nitrogen fertilization

Nitrogen fertilized subplots were present only for the last 2 years of the study, and both years had a period of drought (Fig. 1). No significant nitrogen fertilization or CO_2 by nitrogen fertilization treatment interactions were observed for cell soluble, celluloses, or lignin fractions of vegetation. Nitrogen fertilization resulted in higher mid-season crude protein concentrations in *B. gracilis* in elevated but not ambient CO_2 treatment (data not shown). Nitrogen fertilization increased mid-season crude protein concentrations of *B. gracilis* and *P. smithii* in the 5th year of study with the normal spring followed by drought, but only increased concentrations in *B. gracilis* (the C_4 of the three species) in the year with the spring drought (data

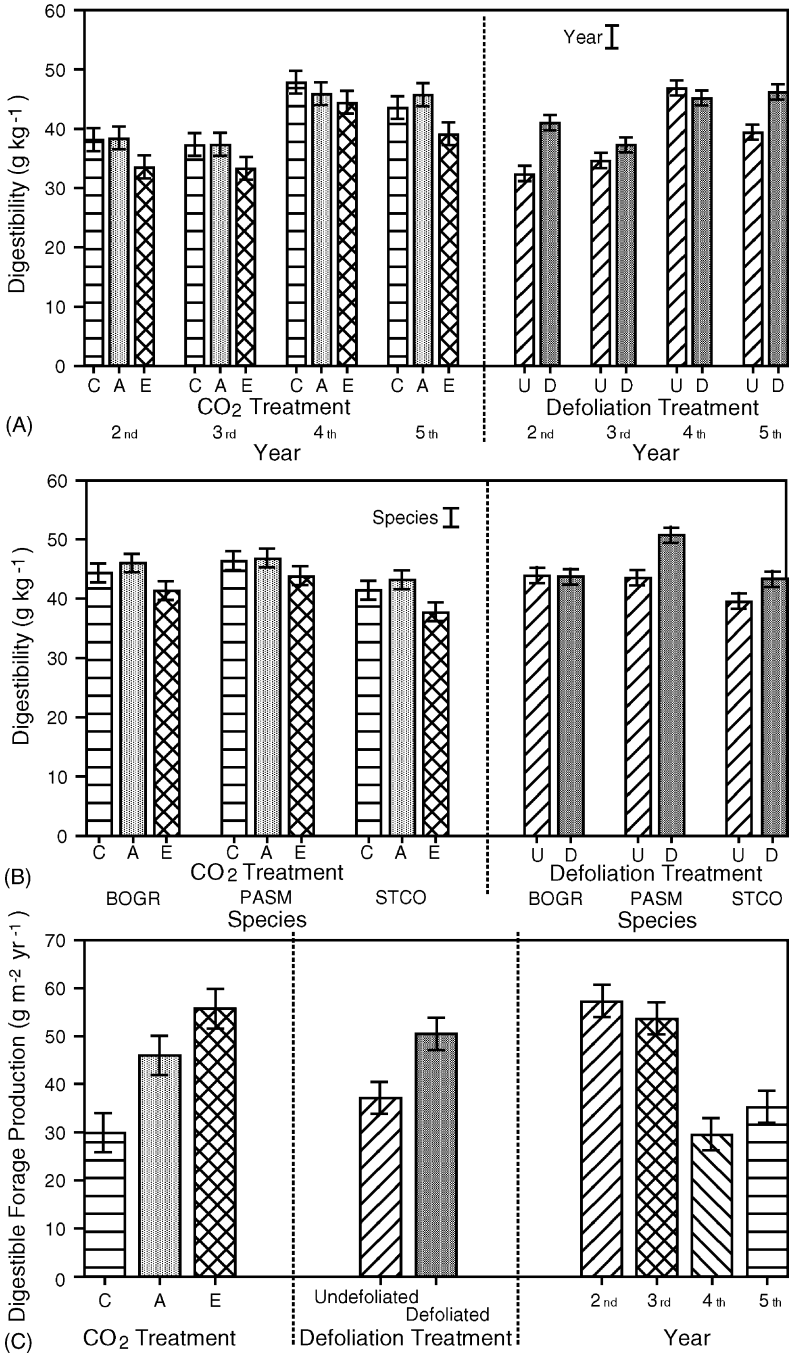

Fig. 9. (A) Digestibility (g kg^{-1} in vitro digestible dry matter) of total vegetation in autumn, (B) digestibility of *B. gracilis* (BOGR), *P. smithii* (PASM), and *S. comata* (STCO) vegetation in autumn, or (C) digestible forage production (g m^{-2} $year^{-1}$) of total vegetation, exposed to control (C), ambient (A), and elevated (E) levels of CO_2, and previously undefoliated (U) or defoliated (D) during mid-season, in the 2nd through 5th years of treatment in shortgrass steppe. Confidence intervals on bars are for within that group, and confidence interval not on bars are for the indicated variable within any other group. Dashed lines separate different significant terms within the same ANOVA.

not shown). Increases averaged about 11% for both species. *S. comata* crude protein concentrations were not significantly affected by nitrogen fertilization. No significant nitrogen fertilization treatment effects on crude protein concentrations or production were significant by autumn. Digestibility followed similar patterns, but increases with nitrogen fertilization were less than for crude protein concentrations.

4. Discussion

The effects of elevated CO_2 on forage fiber constituent concentrations were small at mid-growing season, and even smaller by autumn senescence. The large increases in yields indicated greater plant growth with elevated CO_2 (see Morgan et al., 2004 for productivity data). Increased plant growth can result in a higher lignin component of forages. The relatively short stature, low stem proportions, of vegetation in this system would tend to minimize this mechanism of change in fiber fractions, but studies in other systems also indicate only small effects of CO_2 on cell solubles, hemicellulose–cellulose, lignin fiber fractions of forages (Akin et al., 1994, 1995; Soussana and Loiseau, 1997; Booker, 2000; Fritschi et al., 1999). The effects of CO_2 on forage crude protein concentrations were greater than for fiber fractions, and this resulted in lower digestibility under elevated CO_2. This supports our hypothesis that nitrogen rather than carbon soluble and fiber fractions would mediate forage quality responses to elevated CO_2. The small effects of CO_2 on fiber constituent concentrations that were observed somewhat offset each other in terms of quality, since both soluble (readily assimilated) and lignin (indigestible and passage limiting) fractions declined with elevated CO_2, and celluloses increased. The potential for CO_2 treatment effects on rate of passage and voluntary intake in ruminants of the shortgrass steppe is therefore restricted primarily to digestibility and crude protein. The effects of CO_2 on forage fiber were primarily through quantities, not through quality, but were mediated through increases in constituent yields of *S. comata*, which was the species of relatively lowest quality.

The effects of elevated CO_2 on crude protein were large decreases in mid-season concentrations and increasing differences among species. The species of lowest crude protein concentration (*S. comata*) became increasingly important in terms of crude protein availability (yield) to herbivores. The overall effect in terms of crude protein was that quality was more affected than quantity. The decrease in quality was to some extent offset by increased plant growth (Morgan et al., 2004) and nutrient yields. However, crude protein concentration at mid-growing season in elevated compared to ambient treatments declined 18%, compared to an increase in protein yield of 14%. This raises the question as to whether the decline in quality is more important than the increase in quantity.

Nitrogen concentrations of forage in native grassland as opposed to intensively managed pastures are characteristically low, ranging from 6 to 13% crude protein, whereas the optimum diet would be 15–20% crude protein for high yielding dairy cattle or actively growing young beef cattle (Thompson and Poppi, 1990; Whitehead, 1995). Native semiarid grassland forage is often even lower in crude protein, as indicated by the values in this study. A maintenance diet requires about 7% crude protein. Crude protein requirements of rumen microbial populations are approximately 7% (VanSoest, 1982), and forage intake by sheep declines rapidly when levels fall below 7% (Milford and Minson, 1965). Similar values are applicable to deer. Productivity, survival, and condition of breeding does were adversely affected by reductions in forage crude protein concentrations from 13, 11, to 7%, and body weights and antler development of yearling and adult males were drastically retarded by a forage diet of 7% (Murphy and Coates, 1966). A 6–7% crude protein diet is generally accepted as maintenance level, and the 5% level is generally considered the ‘‘critical point’’ where muscle catabolism and negative apparent protein digestibility may begin (Milchunas et al., 1978). Total vegetation clipped from quadrats is of lower quality than that selected by foraging animals, but does illustrate relative conditions of total availability that the animal must discriminate from. Using the 5% critical point for dietary crude protein, a random feeder of mid-growing season forage on control plots would be subject to 1 of 12 possible species-year instances of below critical crude protein concentrations (from Fig. 6A). For the same random feeder, the number of instances where dietary levels of crude protein fall below critical levels increases to 4 of 12 under the ambient CO_2 treatment, and to 9 of 12 under the

elevated CO_2 treatment. Increases in quantity do of course have some positive impacts on animal performance, especially in low productive systems. Intake by foraging cattle can decline when average height of vegetation is less than 7–10 cm (Minson, 1990), and for sheep when less than 4–6 cm (Parsons et al., 1991). Bite size on shortgrass steppe is small. However, animals can compensate up to some extent by increasing foraging time or bite rate. Low dietary crude protein concentrations limits fiber digestion, lowering rate of passage, which limits intake. Increases in quantity may become of secondary concern when quality greatly reduces weight gain or potentially becomes critical where weight losses occur in wildlife and additional supplementation is required for domestic animals.

Crude protein concentrations generally become more limiting in winter than during the growing season. Mid-growing season overall average crude protein concentrations were 6.44, 6.40, and 5.26 g/kg DM for the control, ambient, and elevated CO_2 treatments, respectively, compared to 5.17, 4.95, and 4.25 g/kg DM for autumn forage. Based on values in Fig. 7A, crude protein concentrations of total forage fell below the critical 5% level in 1 of 4 years for the control and ambient CO_2 treatment compared to 3 of 4 years for the elevated CO_2 treatment. Years with higher precipitation and plant growth generally had lower crude protein concentrations of forage. However, further reductions in autumn forage crude protein concentrations due to elevated CO_2 did not occur in years with wet late-growing seasons (3rd and 4th years) or the wettest overall year (3rd year). An overall dry year produced the same lack of elevated CO_2 effect as a wet year, when the dry year had a very wet autumn. In that dry year (4th), plants were dormant for much of the growing season until late-season's rains occurred. The short growing season and a probable late flush of nitrogen mineralization and rapid growth following the long dry period may have eliminated the potential for CO_2 treatment effects to manifest. The very wet 3rd year may have also allowed for periods of rapid plant growth, thereby eliminating CO_2 treatment effects.

Mid-growing season digestibility declined consistently with elevated CO_2 in all years, unlike crude protein that depended on year and/or species. However, the average reduction in elevated compared to ambient CO_2 treatment was 14% for digestibility and 18% for crude protein. In contrast, differences among species were greater for digestibility (*S. comata* lowest at −19% that of *P. smithii*) than for crude protein concentrations (*S. comata* lowest at −11.5% that of *B. gracilis*), and species differences were greater than CO_2 treatment effects. The increase in *S. comata* aboveground net primary production and recruitment of individuals with elevated CO_2 (Morgan et al., 2004), i.e., change in species composition, would have a greater negative effect on digestibility than on crude protein concentration, but the direct effect of elevated CO_2 is greater on crude protein than on digestibility.

A main effect of CO_2 treatment on mid-season yield of either crude protein or digestible forage was not significant, although *B. gracilis* yields of crude protein decreased and *S. comata* yields increased. The lack of significant effects was probably due to low statistical power of three replicates for treatments, but trends were for an average increase of 14% for crude protein yield and 11% increase for digestible forage yield. However, the yields were associated with decreased quality and increased yield of the species of lowest quality, *S. comata*. Further, drought affected *S. comata* and *P. smithii* more than *B. gracilis* based on fiber constituent yields in two wet versus two dry years, indicating that a greater instability of forage supply would occur with elevated CO_2 in addition to a less nutritious community species composition. *B. gracilis* is known to be a drought tolerant species (Lauenroth et al., 1987). Increases in a less palatable species would place greater selection pressure on the more palatable species, potentially shifting species composition further.

Effects of elevated CO_2 on digestibility were less in autumn than in mid-growing season, supporting our hypothesis of less effect after senescence. However, of the 10% reduction in digestibility in autumn versus the 14% reduction in mid-season, the effect of CO_2 in autumn may be more critical, for the same reasons as described above for crude protein. Forage quality in autumn is poor compared with that during the growing season, and lower digestibility at that time can affect rate of passage and reduce intake to a point where muscle and fat catabolism, and even death, can occur in animals with a full rumen. This is more likely to affect small than large ruminants. The weight of deer rumen contents is about 5% of body weight (Short, 1963) compared to about 13% for cattle (Thomas et al., 1961),

and smaller animals have proportionately greater metabolic rates. Smaller ruminants may better compensate for low quality through a greater capacity to be selective, but are also more susceptible to bulk limitation and require forages that allow a faster rate of passage. Increases in quantity and lower quality generally would be relatively less favorable for smaller ruminants, but more detailed species and plant-part studies and/or foraging animal studies would be necessary to establish how tradeoffs between the capacity to select and the necessity to be selective would manifest. Domestic animals on poor range can be supplemented, but this adds substantially to operating costs.

Nitrogen fertilization increased crude protein concentrations in mid-growing season *B. gracilis* and *P. smithii*, but not in the less nutritious *S. comata*, and not in autumn forage when crude protein became most limiting for consumers. Therefore, nitrogen fertilization did not ameliorate the negative effects of elevated CO_2 on change in species to a less nutritious composition or ameliorate over-winter nutritional constraints. However, our nitrogen fertilization treatment did not span years that could be considered average or above average precipitation, when greater effects would be expected. Economics currently constrains fertilization of low productivity native grassland.

Defoliation generally increased forage quality and yields compared to undefoliated treatment. Vegetation regrowing following defoliation is younger tissue, and is commonly of greater quality (Leriche et al., 2003). However, no defoliation by CO_2 treatment interactions were observed, which appears to be the most common response (Wilsey et al., 1994, 1997; Fischer et al., 1997). This may be a function of the time after defoliation that the analyses are performed. Ryle et al. (1992) and Soussana et al. (1996) indicate that elevated CO_2 can dilute nitrogen concentrations in fully expanded leaves, but not in unexpanded leaves. Increased growth rates following defoliation may have declined to undefoliated growth rates over the mid-July to the late October sampling date. Differences in quality with defoliation could still manifest through greater numbers of older senescent leaves with leached cell contents and/or lower leaf to stem ratios of undefoliated plants.

Nitrogen fertilization and defoliation generally did not ameliorate the negative impacts elevated CO_2 had on forage quality. Negative effects on quality were most evident in crude protein, to a somewhat lesser degree on digestibility, with only small, mixed effects on quality through altered fiber fractions. The negative effects of elevated CO_2 on forage quality are likely to be greater than the positive effects on quantity, because quality drops to critically low levels that can inhibit utilization of the quantity that is available. In this particular system, the direct negative effect of elevated CO_2 on decreased forage quality is accentuated by a shift to availability of the protein and energy in a species (*S. comata*) that is of relatively low quality, and that is more negatively affected by the periods of drought experienced during the 4 years of this study than was *B. gracilis*.

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