



## Root production and tissue quality in a shortgrass steppe exposed to elevated CO<sub>2</sub>: Using a new ingrowth method

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### Abstract

A modified root ingrowth method was developed to minimize destructive sampling in experiments with limited space, and used to estimate belowground net primary production and root tissue quality in a native semiarid grassland exposed to elevated CO<sub>2</sub> for five years. Increases in root production of over 60% were observed with elevated CO<sub>2</sub> during years of intermediate levels of precipitation, with smaller effects in a very wet year and no effects in a very dry year. Aboveground to belowground production ratios, and the depth distribution of root production, did not differ between ambient and elevated CO<sub>2</sub> treatments. Root soluble concentrations increased an average of 11% and lignin concentrations decreased an average of 6% with elevated CO<sub>2</sub>, while nitrogen concentrations decreased an average of 21%. However, most tissue quality responses to CO<sub>2</sub> varied greatly among years, and C:N ratios were higher in only one year (22 ambient vs. 33 elevated). Among years, root nitrogen concentrations declined with increasing aboveground plant nitrogen yield, and increased over the study period. Estimates of root production by the ingrowth donut method were much lower than previous estimates in the shortgrass steppe based on <sup>14</sup>C decay. We discuss reasons why all ingrowth methods will always result in relative rather than absolute estimates of root production.

**Abbreviations:** NDF – neutral detergent fiber; ADF – acid detergent fiber.

### Introduction

Belowground primary production is often greater than aboveground production in perennial, native ecosystems (Coleman, 1976). This is particularly true in arid and semiarid grasslands (Sims and Singh, 1978). In the semiarid shortgrass steppe of North America, greater than 90% of plant standing biomass is belowground, and root production is 67% of total based on the isotope decay method (Milchunas and Lauenroth, 2001). Belowground inputs of carbon are, therefore,

important in predicting the effects of various climate change variables on native ecosystems, and this remains one of the least understood aspects of increases in atmospheric CO<sub>2</sub> (Norby, 1994; Curtis et al., 1994; Norby and Jackson, 2000). While there are fundamental differences in root systems among grasslands, forests, and agricultural systems, the data on effects of elevated atmospheric CO<sub>2</sub> on root dynamics is insufficient to make generalizations even when confined to native grassland communities (Arnone et al., 2000). The quality of roots is an important factor in the utilization of the inputs by the soil biota, decomposition and nutrient cycling rates, and soil carbon storage, and

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therefore the feedbacks to primary production as well as global carbon budgets (Zak et al., 2000). The broad objectives of this study were to assess both productivity and quality of roots produced during five years of exposing a native shortgrass steppe to elevated CO<sub>2</sub> treatment.

Early predictions of the effects of CO<sub>2</sub> on root production were based mainly on pot studies and indicated general increases in belowground production with elevated CO<sub>2</sub> that were greater than aboveground (Norby, 1994). Rogers et al. (1994) reviewed studies to date and found that 87% showed increases in root biomass with elevated CO<sub>2</sub> and 41% showed increases in root to shoot ratios. In a review specific to native grasslands, Arnone et al. (2000) summarized that seven of twelve studies showed little or no change in root-system size with elevated CO<sub>2</sub>, but five out of the eight studies that estimated root production showed increases (excluding sequential coring method). These authors concluded that insufficient data for native plant communities, the diversity of techniques used, and inconsistencies in responses hindered formulation of generalizations concerning effects of elevated CO<sub>2</sub> on root systems. Belowground primary production is a basic ecological process, but one where all methods have bias and errors (Milchunas and Lauenroth, 2001). Estimating root production rather than just biomass is labor intensive, and space limitations for destructive sampling of *in situ* CO<sub>2</sub> studies places further limits on obtaining data.

Several hypotheses concerning aboveground:belowground allocation in plants are relevant to potential effects of elevated CO<sub>2</sub> in different ecosystems. These concern both water availability and plant nitrogen concentrations. Root to shoot ratios generally increase when moving from mesic to more arid grasslands (Milchunas et al., 1988) and forests (Van Noordwijk et al., 1998; Joslin et al., 2000). This has led to the general perception that responses to dry conditions within a community would also lead to increases in root:shoot ratios (Joslin et al., 2000). In forests, these authors found 'little support' for increasing root:shoot ratios with drought. However, in a relatively drier grassland environment compared to forests, Milchunas and Lauenroth (2001) found some limited evidence that root:shoot ratios were greater during dry periods based on 13 years of root biomass data in the semiarid shortgrass steppe. One could hypothesize that the increased water conservation associated with elevated CO<sub>2</sub> (Owensby et al., 1993; Drake et al., 1997; Wullschleger et al., 2002; Morgan et al.,

2004) may result in a decrease in root:shoot ratios, and this may be greater in wet years. An alternative second hypothesis may be formulated based on the relationship between root:shoot ratio and plant internal nitrogen concentration. Root:shoot ratios are predicted to decrease with increasing plant nitrogen concentration (Agren and Ingestad, 1987; Levin et al., 1989). Since elevated CO<sub>2</sub> may decrease plant nitrogen concentrations (see below), one may hypothesize that root:shoot ratios may increase with elevated CO<sub>2</sub> even in semiarid environments, since an increased nitrogen demand increases the need for intensive root exploitation of the soil volume. Wet productive years can also increase nitrogen demand, whereby a greater increase in root:shoot ratios may be predicted. A third alternative hypothesis is based on a model incorporating both nitrogen-use-efficiency and water-use-efficiency concepts (Van Noordwijk et al., 1998), and predicts the root:shoot ratio response to elevated CO<sub>2</sub> depends on whether water or nutrients are limiting. No change in ratios is observed when water limits root function, increases in the ratios are found when nutrients are limiting, and a range of intermediate responses when both limit at times.

Nitrogen concentrations, C:N ratios, and the form of the carbon (carbohydrates, lignins) are important aspects of plant tissue quality in terms of decomposition and herbivory. Elevated CO<sub>2</sub> can in some situations lower root tissue quality by lowering N concentrations and thereby increase C:N ratios (Owensby et al., 1993; Jackson and Reynolds, 1996; Fitter et al., 1997; Pregitzer et al., 2000). Lignin:nitrogen ratios are sometimes better predictors of decomposition rates than are C:N ratios (Entry and Backman, 1995; Scott and Binkley, 1997), because lignins are the most recalcitrant of the primarily carbon, fiber constituents. A further breakdown of tissue constituents into solubles, celluloses (hemicellulose and cellulose), and lignin can be important to belowground herbivores, because these three components of the whole represent fast readily available, intermediately available, and slow or unavailable resources. Belowground herbivory in the shortgrass steppe is much greater than aboveground herbivory even in pastures where cattle consume 40% of annual aboveground net primary production. Changes in belowground tissue quality may have significant impacts on ecosystem productivity. Previous controlled environment studies including two important species of the shortgrass steppe indicate increased starch, sugars, and total nonstructural

carbohydrate concentrations of roots and crowns with elevated CO<sub>2</sub> (Skinner et al., 1999).

In this experiment, we used a modified root ingrowth method to estimate belowground net primary production, and used the material from the root ingrowth sampling to assess soluble, celluloses, and lignin fractions and nitrogen and carbon concentrations of tissue over each of four years of a five year CO<sub>2</sub> enrichment study in native grassland. Estimates of root tissue quality based on new annual growth can differ from estimates based on bulk sampling, especially in the semiarid shortgrass steppe where a total root turnover requires 6.9 years (Milchunas and Lauenroth, 2001). Depending upon live versus dead/decomposition phases in the slow turnover time, all roots may or may not have been newly grown under CO<sub>2</sub> treatment and all age classes of decomposing roots would not exist even after five years of CO<sub>2</sub> treatment. Bulk roots sampled through time would represent various stages approaching, but not attaining, a live-plus-dead-root equilibrium in the altered CO<sub>2</sub> environment. On the other hand, annually grown roots do not fully represent the potential differential life span and decomposition dynamics that can occur under the different CO<sub>2</sub> treatments. We discuss our results in comparison to some analyses of bulk roots from another study at this same site (King et al., 2004). We also describe a modified root ingrowth method that minimizes destructive sampling of the limited space in field CO<sub>2</sub> enrichment studies.

## Methods

The study was conducted at the Central Plains Experimental Range (lat. 40°49' N, long. 104°46' W) in north central Colorado. Mean annual precipitation is 321 mm, with a standard deviation of 98 mm (Lauenroth and Milchunas, 1991). Approximately 71% of the precipitation occurs during the May through September growing seasons. Mean monthly air temperatures range from 22 °C in July to below 0 °C in January.

Total vegetative basal cover at the site is typically 25–35% (Milchunas et al., 1989). Vegetation at this particular site has *Bouteloua gracilis* (H.B.K.) Lag., *Stipa comata* (Trin and Rupr.) and *Pascopyrum smithii* (Rybd.) as codominants (88% of total biomass), with other grasses and forbs and a subfrutescent half-shrub (*Artemisia frigida* [Willd.] comprising the remainder. Soil at the site is a Remmit fine sandy loam (Ustollic camborthids).

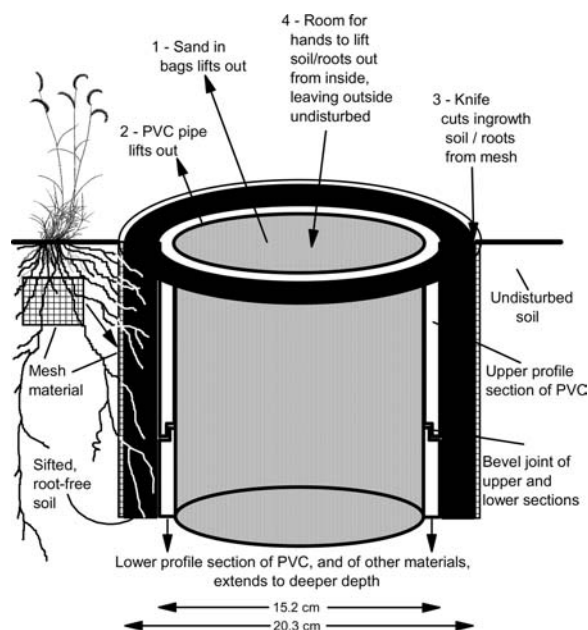


Figure 1. Design and materials for root ingrowth donuts, modified from root ingrowth cores to minimize destructive sampling and disturbance for use in experiments with limited space. Depth of sampling can vary, and is limited by capacity to remove ingrowth soil/roots. The diameters shown allowed both hands and both arms to reach into middle, empty space after removal of sand bags and PVC pipe and cutting of sifted soil, but lower limit may be determined by length of arm reach. Drawing is not to scale.

A portion of a pasture previously lightly to moderately grazed (about 30% annual forage removal) was divided into three blocks, with each block including ambient ( $360 \pm 20 \mu\text{mol mol}^{-1}$ ) or elevated ( $720 \pm 20 \mu\text{mol mol}^{-1}$ ) CO<sub>2</sub> large open-top chambers (4.5 m diameter by 3.8 m high, 15.5 m<sup>2</sup> ground area) and a non-chambered control of equal area. Each year, open-top chambers 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 open-top chambers. Comparisons of microclimatic and plant responses indicated significant chamber effects (Morgan et al., 2001, 2004). Canopy air and soil temperatures averaged 2.6 and 1.25 °C warmer inside than outside chambers throughout the experiment. Periodic measurements of photosynthetically active radiation indicated a 28% reduction by chambers, mostly due to the shading by chamber framework.

A new root ingrowth method was developed that minimized the impact of destructive sampling in the limited area inside the chambers by using permanent structures throughout the four years of study. We term the structures root-ingrowth donuts (Figure 1). Donut refers to the ring, or geometric toroid shape when viewing the concentric cylinders from above the ground. After removal of two cylinders of 20.3 cm outside diameter in each of the nine treatment-replicates, the surface of the smooth-faced sides of the holes were lined with 'plastic canvas' fabric (from large fabric roll; material is used for cross-stitch craft work) to a depth of 40 cm. Approximately 94% of the total root biomass is in the 0–40 cm depth, when based on a 60 cm total depth of sampling at a nearby site (Leetham and Milchunas, 1985). The rigid, hard plastic cloth had holes that were 2 × 2 mm square. Only five out of 48,192 roots at this site were larger than 2 mm diameter, based on minirhizotron measurements for three dates (unpublished data). Steel cylinders of 15.2 cm outside diameter were placed in the middle of the 20.3 cm holes and filled with sand bags. This created a donut space of 2.54 cm width all around the cylinder. The donut space between the cylinder and the ingrowth cloth was filled with root-free sifted soil from an adjacent area, and packed to a similar bulk density as outside soil using doweling. Roots were sampled by removing the sand bags and lifting out the cylinders using a towing chain, where the hooks were placed in holes cut on each side of the cylinder near the top (or using two vice-grip pliers as grips). A sharp, flexible bread knife was placed next to the hard plastic cloth and the root and soil donut cut away from the surface and into the space created by removing the cylinder. A large can placed at the bottom of the hole before cutting sped removal of the upper soil. New sifted soil was placed in the donuts each October, after removing the previous year's growth. The ingrowth plastic canvas fabric lasted four years and could have been used beyond that time, and can easily be replaced at any sampling time.

During the last year of this study, we modified the ingrowth donut method described above to permit sampling of two depths in the donut. Instead of placing solid cylinders in the middle of the larger hole, two pieces of poly-vinyl-chloride pipe of the same diameter were used in an additional two tubes per treatment-replicate plot. The two pieces were beveled on opposite sides to prevent movement (Figure 1). The top cylinder was 10 cm and the bottom 30 cm, representing 0–10 and 10–40 cm increments in the

soil profile when stacked. The double cylinder method allowed removal of the upper portion of the sample while the lower cylinder still held the lower portion of the sample in place. A small knife that could be held horizontally in the hole after removing the upper cylinder was used to cut around the top of the lower cylinder before cutting of sides of the upper portion of the donut sample.

Roots were separated from the soil by the floatation method of Lauenroth and Whitman (1971) using a 0.5 mm sieve, and dried at 55 °C before weighing and grinding through a Wiley mill. Root samples were analyzed for cell solubles, hemicellulose, cellulose, and lignin by the NDF, ADF, and sulfuric-acid lignin fractionation method (Van Soest, 1963, 1967, 1975), modified for block refluxing and without sodium sulfite. Some solubles may be lost during flotation of roots from soil, but this occurs more with older senescent roots that are fragile (Swinnen et al., 1994). Values were expressed on an ash-free basis (subsamples ashed in a muffle furnace at 600 °C). Hemicellulose and cellulose values were added together for statistical analyses and presentation, because of their nutritional similarity (Van Soest, 1975). Nitrogen and carbon were assessed using an automated C/N combustion analyzer (PDZ Europa).

CO<sub>2</sub> treatment effects were analyzed using SAS PROC MIXED (SAS Institute Inc., Cary, NC, USA) with 'Year' as a repeated measure variable; 'block' as a random effect (thereby removing the variability due to blocking); and block\*CO<sub>2</sub> treatment as the error term for CO<sub>2</sub> treatment comparisons. Where significant treatment effects were detected, treatment comparisons were conducted utilizing the Tukey's means comparison test at the 0.05 level of confidence.

## Results

A variety of seasonal precipitation patterns typical for the shortgrass steppe region occurred during the study (Figure 2A). The third year of the study had nearly twice the average annual precipitation (Figure 2B) due to both wet spring and autumn periods. Years two and five were only slightly above the average annual precipitation, but were very different seasonally. A long drought during the entire latter half of the growing season occurred in year five, but the very wet spring elevated the annual amount received. Year four was a little below annual average precipitation, but a very long drought occurred from spring until late summer,

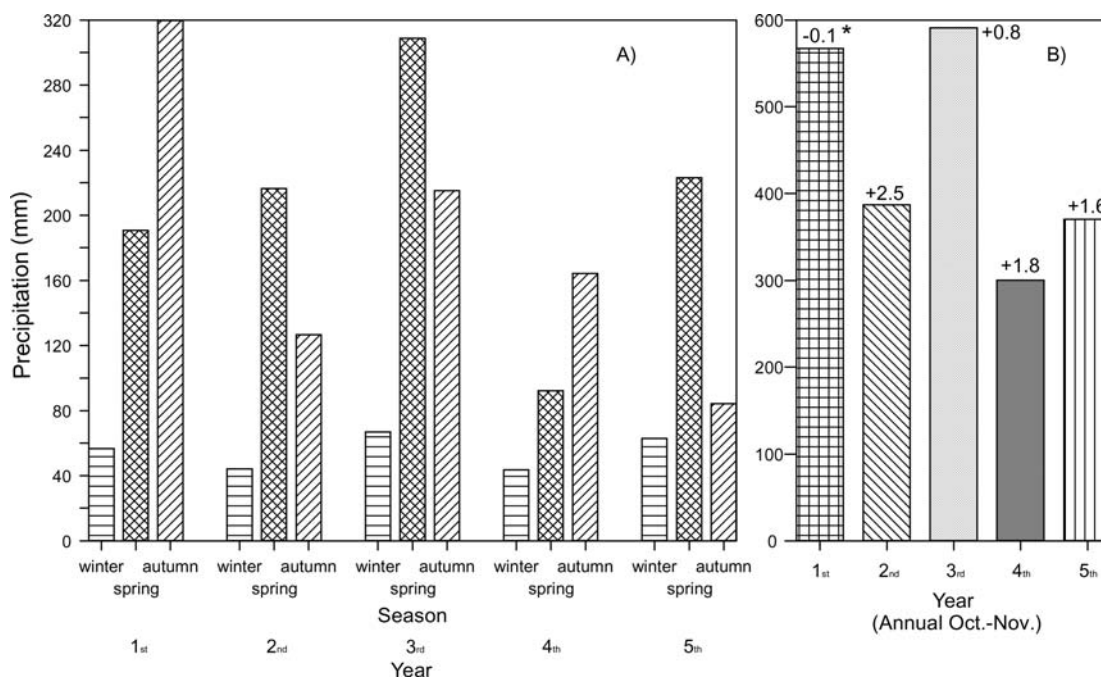


Figure 2. A) Seasonal and B) annual precipitation during the five years of CO<sub>2</sub> treatments at a shortgrass steppe site. Long-term average precipitation is 321 mm. Annual precipitation was calculated from November through October, since ingrowth cylinders were sampled in October after senescence. Winter was considered November–March, early growing season April–July 15, and late growing season July 15–October. July 15 is the approximate time of peak-green-standing crop. Years one through five were 1997–2001. \* = Difference between elevated and ambient CO<sub>2</sub> treatments in the percent gravimetric soil water overwinter carryover from the previous year (i.e., the differences between soil moisture of the treatments in early spring right after moving open-top chambers onto plots). Soil water data from Nelson et al. (2004).

when a few very heavy storms resulted in what appeared as a spring flush of growth in autumn. The first year of the CO<sub>2</sub> treatments was also very wet, but sampling for this study did not begin until year two. In addition to precipitation, differences in carryover of soil moisture from the previous year due to CO<sub>2</sub> effects on stomatal behavior could influence annual treatment effects. The difference between elevated and ambient CO<sub>2</sub> treatments in soil water carryover was most positive in the second year and least positive in the third year (Figure 2B).

Elevated compared to ambient CO<sub>2</sub> treatment resulted in greater root production in three out of four years (Figure 3A). No significant differences were observed among any treatments in the fourth year, when production was low on all plots due to the spring drought (Figure 2). The largest effects of elevated compared to ambient CO<sub>2</sub> treatment occurred in the second (+64%) and fifth (+66%) years with intermediate levels of precipitation. The very wet third year showed increased root production with elevated CO<sub>2</sub> treatment of only 34%. No significant differences in root production were observed between control and

ambient CO<sub>2</sub> treatments for years two, three, and five, even though precipitation patterns were different among the years and year three was very wet. The low production on all treatments during the fourth year with the spring drought did not occur during the fifth year with the autumn drought.

Increased root production was observed under elevated compared to ambient CO<sub>2</sub> treatment for both depths when assessed by depth in the fifth year, but it was significant only for the 10–40 cm depth (Figure 3B). There did not appear to be any shift in depth distribution with CO<sub>2</sub> treatments, because the proportion of the total root production that occurred in the 0–10 cm increment was 35, 37, and 36% for the control, ambient, and elevated CO<sub>2</sub> treatments.

Aboveground to belowground production ratios did not differ between ambient and elevated CO<sub>2</sub> treatments, but were both higher than the control (Figure 3C). Aboveground to belowground production ratios did not differ among years two through four, but were lower in the fifth year with the mid-growing season through autumn drought.

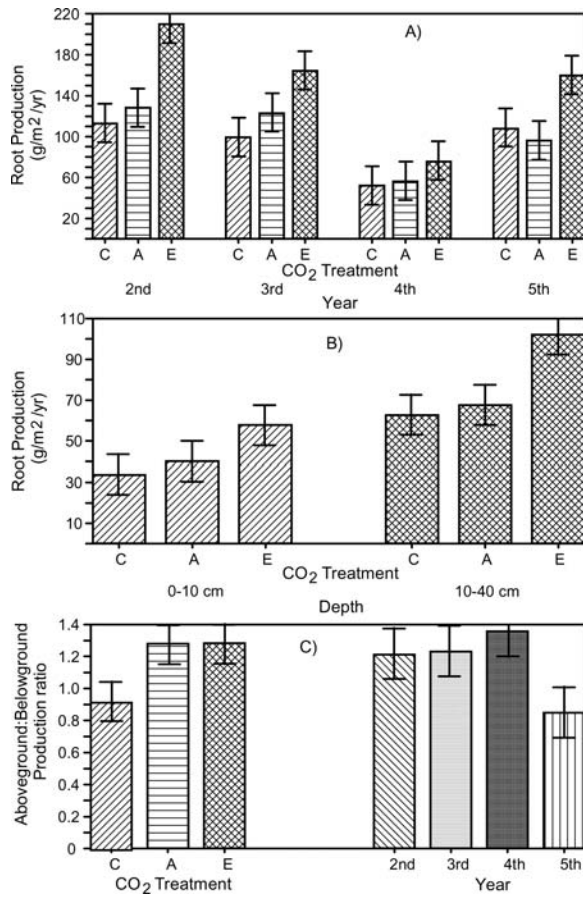


Figure 3. Root production ( $\text{g/m}^2/\text{yr}$ ) for control (C), ambient (A), and elevated (E)  $\text{CO}_2$  treatments for A) the second through fifth years of treatments for the 0–40 cm depth, B) the 0–10 cm and 10–40 cm depths during the fifth year of treatments, and C) ratios of aboveground to belowground production for the second through fifth years of treatments in a native shortgrass steppe plant community. Root values are ash corrected to an organic matter basis, and estimated from ingrowth donut-cylinders. Confidence intervals are Tukey's HSDs calculated at  $P = 0.05$ . Aboveground production data from Morgan et al. (2004).

Fiber analyses of the samples from the root ingrowth donuts showed some differences in tissue quality between ambient and elevated  $\text{CO}_2$  treatment. The soluble fraction of the new annual roots increased 11% with elevated  $\text{CO}_2$ , and this occurred as a main effect in all years (Figure 4A). No differences were observed between control and ambient  $\text{CO}_2$  treatments. Soluble concentrations varied much more among years than among  $\text{CO}_2$  treatments. The year with the extended spring drought (4<sup>th</sup>) had the lowest soluble concentrations, while the two years of intermediate precipitation (2<sup>nd</sup> & 5<sup>th</sup>) had the highest solubles. The year with the highest precipitation (3<sup>rd</sup>) did not produce more roots

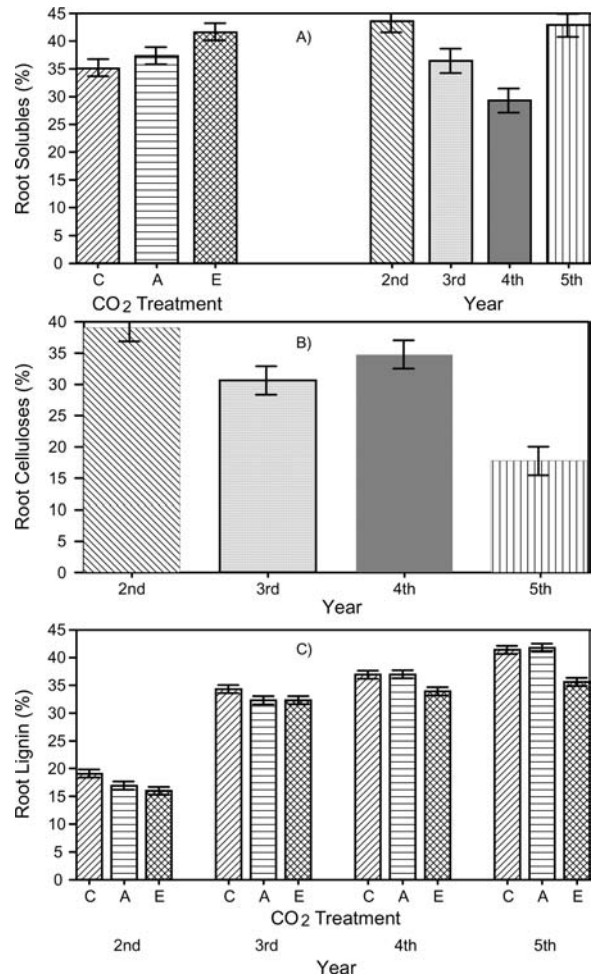


Figure 4. A) Cell solubles, B) hemicellulose plus cellulose, and C) lignin concentrations (%) of roots collected annually from ingrowth donut-cylinders in plots exposed to control (C), ambient (A), and elevated (E) levels of  $\text{CO}_2$  in the second through fifth years of treatment in shortgrass steppe. Roots were current-annual growth from the 0–40 cm depth and ash corrected to an organic matter basis. Fractionation of root biomass is based on NDF, ADF, and sulfuric acid procedures, and celluloses includes hemicellulose and cellulose combined. Confidence intervals are Tukey's HSDs calculated at  $P = 0.05$ .

nor were they higher in soluble concentration. No significant  $\text{CO}_2$  treatment effects were observed for celluloses. Concentrations of celluloses were lowest in the fifth year with the autumn drought (Figure 4B). A significant  $\text{CO}_2$  treatment by year interaction was found for lignin concentrations of the new annual roots. Lignin concentrations of roots were lower with elevated  $\text{CO}_2$  in treatment years four (–8%) and five (–15%), but only tended to be lower in years two (–5%) and three (–0.1%) (Figure 4C). Lignin concentrations in roots growing in ambient compared to

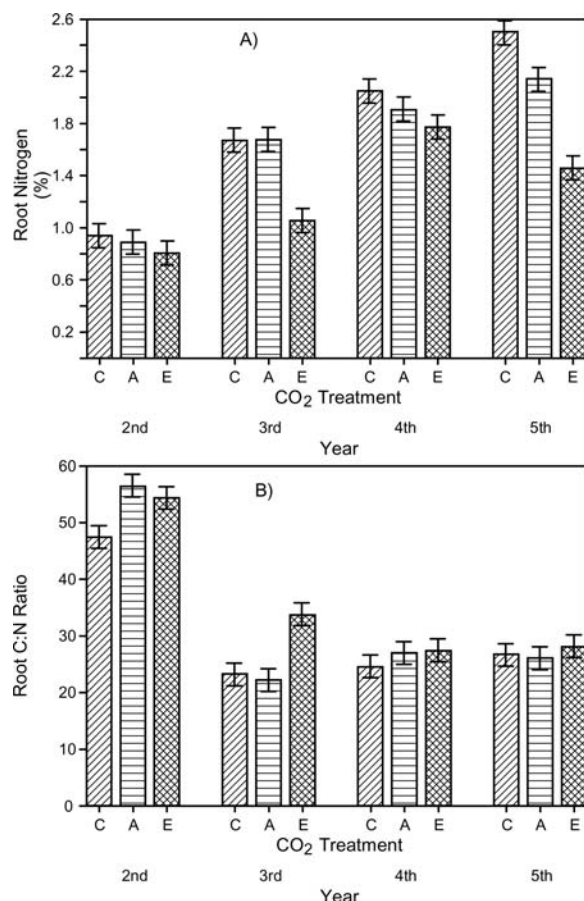


Figure 5. A) Nitrogen concentrations (%), and B) C:N ratios of roots collected annually from ingrowth donut-cylinders in plots exposed to control (C), ambient (A), and elevated (E) levels of CO<sub>2</sub> in the second through fifth years of treatment in shortgrass steppe. Roots were current-annual growth from the 0–40 cm depth and ash corrected to an organic matter basis.

control CO<sub>2</sub> treatments were slightly lower in two of the four years. Lowest lignin concentrations were in year two and highest in year five.

Nitrogen concentrations were lower in elevated compared to ambient CO<sub>2</sub> treatment in two out of four years (Figure 5A, significant CO<sub>2</sub> treatment by year interaction). The two significant years were the ones with intermediate productivity (Figure 3A). Nitrogen concentrations were 37% lower in the third year and 32% lower in the fifth year of elevated CO<sub>2</sub> treatment. Nitrogen concentrations increased each year in control and ambient treatments, but did not increase from year four to five in the elevated CO<sub>2</sub> treatment. One hypothesis for the temporal increase in root nitrogen concentrations is that aboveground nitrogen demand may have declined because of the general drying pe-

riod during the study, resulting in greater nitrogen storage in roots. A negative relationship was observed between annual aboveground nitrogen yield and annual root nitrogen concentration (Figure 6). The slope was most negative for the control treatment, and least negative for the elevated CO<sub>2</sub> treatment, but there was no significant difference between ambient and elevated CO<sub>2</sub> treatment.

Root C:N ratios were higher in elevated compared to ambient CO<sub>2</sub> treatment only in the third year of treatment, and were higher in all treatments in year two than other years (Figure 5B). Lignin:N ratios tended to be higher in, but were not significantly different between, ambient (39.0) compared to elevated (44.9) CO<sub>2</sub> treatments.

## Discussion

### *Root ingrowth donut method*

Estimates of root production based on root ingrowth were low compared to other methods used in nearby pastures. While all methods of belowground primary production have biases and assumptions, the isotope decay method for structural non-labile tissue has perhaps the least severe assumptions, is *in situ* and produces good estimates of aboveground net primary production where the validity of other methods is better understood (Milchunas and Lauenroth, 1992, 2001). Although impractical for short-term estimates or in sites where destructive sampling must be limited, the estimates based on isotope decay may be used as an integrated-over-years, long-term average to compare with other methods. Estimated average root production by isotope decay for this shortgrass steppe site was 223 g/m<sup>2</sup>/yr compared to an average of only 107 g/m<sup>2</sup>/yr for root ingrowth in this study (excluding the drought year).

There are several biases associated with root ingrowth methods, and their direction of bias can be opposite. First, the competition-free environment with an available soil water pool and the potentially less compacted soil of the root ingrowth space would tend to cause an overestimation of root production. Second, the horizontal wall of a section of a root ingrowth core or donut (outside plane of a cube with no possibility of a plant directly above) would be less likely to be intercepted by roots from adjacent plants than a similar small cube in the profile due to the architectural propensity of grass roots to have a greater

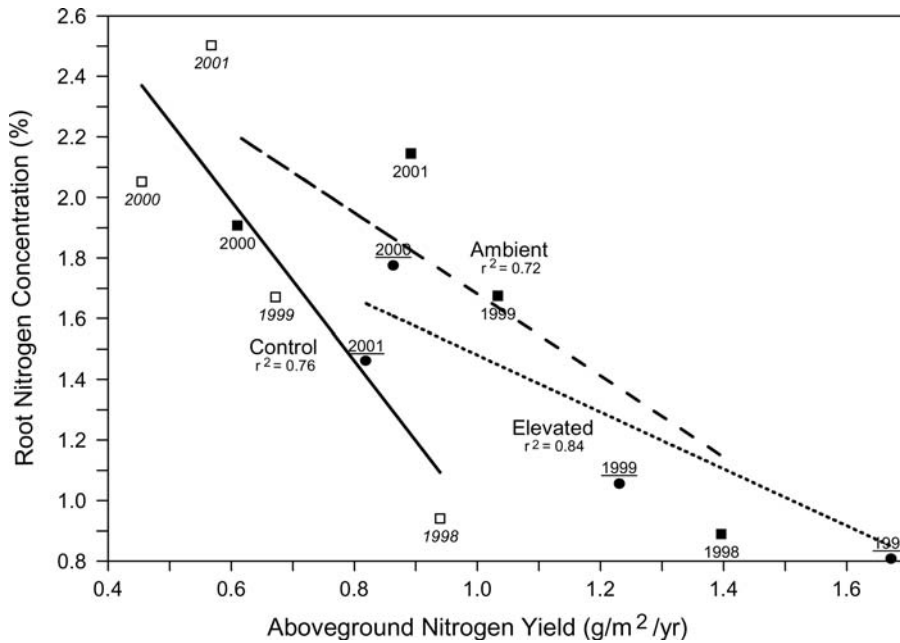


Figure 6. Relationship between annual aboveground plant nitrogen yield ( $\text{g/m}^2/\text{yr}$ ) and annual root nitrogen concentration for the control, ambient, and elevated  $\text{CO}_2$  treatments in a native shortgrass steppe. Aboveground plant nitrogen yield is the leaf and stem nitrogen concentration multiplied by the end-of-season, peak-standing crop. Aboveground plant nitrogen yield data from King et al. (2004) and Milchunas et al. (2004).

vertical-directional growth than horizontal-directional growth component (the opposite may be true for cacti and some species of trees and forbs than have a far reaching horizontal, near surface web architecture). Drawings of excavated root systems from this grassland (Lee, 1990) and other grassland species (Thorp, 1948; Weaver, 1958) suggest the predominant vertical spatial orientation of roots would result in an underestimation of root production from vertically oriented ingrowth walls, when established plants are horizontal to the ingrowth structure. Architectural bias is also a problem with the minirhizotron method of estimating root production from new length growth (Pages and Bengough, 1997), although the horizontal plane made by the top of the tube results in a greater likelihood of intercept, and growth over and along, by vertically compared to horizontally oriented root growth. However, preliminary estimates of root production in ambient compared to elevated  $\text{CO}_2$  in these same experimental plots based on minirhizotron new-length growth (unpublished data) gave similar treatment differences as those reported here for root ingrowth. A third additional factor influencing estimates of production/unit area from both ingrowth cores or donuts is that they are very sensitive to area sampled, i.e., the diameter of the ingrowth core or width of the donut

in relationship to potentially full exploitable area. The depth, and therefore volume, is obviously also important, but we here argue that horizontal space (area) is a critical component of potential bias. For example, an ingrowth core diameter greater than the horizontal root spread of a species would have some area without any root biomass, but that area would still be used in the calculation converting values to  $\text{g/m}^2$ , resulting in underestimation of production in root occupied areas. In contrast, a core so small as to be saturated with root growth before the end of the sampling period would automatically underestimate root growth. Since the optimum scaling of area and/or time can never be known, root ingrowth must 'by-definition' always be a relative and not an absolute estimate of root production. Fourth, some turnover of roots can occur within the period the ingrowth donuts are in place, and we sampled only once a year. This would be more of a bias in systems with shorter turnover than the long 6.9 years observed in this semiarid shortgrass steppe that has few annual plants (Milchunas and Lauenroth, 2001). This bias would tend to underestimate production, as would consumption by belowground herbivores which is large in this community (Lauenroth and Milchunas, 1991). Small diameter roots may turn over more rapidly, and this could potentially re-



sult in treatment bias as well if root diameter differs among treatments. However, grass roots are relatively uniformly small compared to ranges for other life-forms. Preliminary minirhizotron data from this CO<sub>2</sub> site indicate differences between ambient and elevated treatments of less than 0.5 mm (unpublished data). The elevated treatment sometimes had larger diameter roots and this could potentially cause an underestimation of CO<sub>2</sub> effects, but there were a greater number of small roots as well and this would bias in the opposite direction. Root production estimates from this study compared to isotope decay suggest that the potential biases for underestimation are greater than the potential biases for overestimation.

We did not in this study compare the modified 'ingrowth donut' method described in the methods section to ingrowth cores, but the potential biases described above should be similar for both. A potential problem with the ingrowth donut as executed here may be the use of the rigid plastic canvas as wall material. The use of a screen material with lower solid to hole ratio may prevent deflection out of incoming roots, which may have been another factor in addition to architecture and exploitable area/time for the apparent underestimates. The permanent placement of the ingrowth donuts for four years and the relatively large spatial area sampled compared to ingrowth cores minimized the amount of destructive sampling in the limited plot area of the CO<sub>2</sub> treatments. Once installed, sampling was fast, easy, and non-destructive. Tests of possible effects of sampling from permanent locations, and comparisons with other methods are certainly warranted. We developed the donut method to obtain samples of new-annual-growth root biomass for quality analyses in long-term plots with limited area for destructive sampling, as well as to provide comparative data to minirhizotrons in the same plots. In addition to minimizing destructive sampling, another advantage of the donut cylinder method over traditional ingrowth cores is that the large donut spans spatial variability better than a point sample provided by a core. This is particularly important in semiarid systems, such as the shortgrass steppe, where there are many large patches of bare ground that are not as densely occupied by roots (Milchunas and Lauenroth, 1989). The donut shape is an advantage over a similar diameter full core because of the sensitivity of the method to area sampled as discussed above, and because of the amount of soil that needs to be sieved free of roots prior to use as ingrowth medium and the amount of material to later remove roots from.

Twenty-one person-hours were required each year to sift the soil for the 18 ingrowth donuts used in this study. Sifting the soil for, and excavating, a similarly large, 20.3 cm diameter cylinder would require a substantially greater effort (approximately 20 kg soil/cylinder).

#### *Root production responses to CO<sub>2</sub>*

Very large increases in root production with elevated CO<sub>2</sub> were observed during years of intermediate levels of precipitation (a little above average annual), with smaller effects in a very wet year and no effects in a very dry year. Very wet years or very dry years may overwhelm the relatively small effects of CO<sub>2</sub> on water conservation through reduced stomatal opening. Very wet and very dry years may not be conducive to the water-use-efficiency response to CO<sub>2</sub> (Morgan et al., 2004); very little or no response in very wet years at a Kansas tallgrass prairie site (Owensby et al., 1993) and very dry years at a Nevada Mojave Desert site (Naumburg, 2003) further illustrate this. Using cylinder monoliths from this same shortgrass steppe site growing in controlled phytotron environment, Hunt et al. (1996) also observed the largest effects of elevated CO<sub>2</sub> on plant production occurred under an intermediate-level watering regime. Among years, root production in this study was lower in a drought year, with no difference among wet and intermediate years. Very wet years in a semiarid system may exceed the plasticity of plants to respond or eliminate the need to expand into unexploited micro-sites.

Because of problems with all field estimates of root production, results concerning aboveground to belowground production ratios need to be interpreted relative to treatments and years and not as absolute values. No differences in aboveground to belowground production ratios were observed between ambient and elevated CO<sub>2</sub> treatments, supporting the Van Noordwijk et al. (1998) prediction for water limited systems. Year effects indicated a reduction in aboveground to belowground ratios in the year with the autumn drought, but not the even drier overall year with the spring drought, thus only partially supporting the hypothesis of greater root relative to shoot allocation under dry conditions. The dry year with the spring-to-late-season drought was, however, an extreme case of extended severe drought. No relationship was observed between root nitrogen concentrations and annual differences in shoot to root ratios as suggested by Agren and Ingestad (1987) and Levin et al. (1989). However, the observed

increase in root nitrogen concentrations through time are unusual, and appeared to be due to the general decrease in aboveground plant nitrogen yield that would reduce nitrogen demand.

#### *Root tissue quality responses to CO<sub>2</sub>*

Quality of the roots produced showed different directions of response to elevated CO<sub>2</sub> when viewed in terms of soluble/fiber components compared to nitrogen. Root solubles increased and lignin concentrations decreased indicating an increase in quality, whereas nitrogen concentrations decreased indicating a decrease in quality. Overall however, quality decreased in only one year as indexed by an increase in C:N ratios, and lignin:N ratios showed only trends of an increase.

The effects of elevated CO<sub>2</sub> on the belowground plant component of this system were sometimes similar to effects aboveground. Aboveground nitrogen concentrations decreased 18% (Milchunas et al., 2004; King et al., 2004) compared to a decrease of 24% for roots, when averaged over all four years, and aboveground to belowground production ratios of ambient and elevated CO<sub>2</sub> treatments were also similar over the four years. Some differences between aboveground and belowground responses to elevated CO<sub>2</sub> were evident. In some years, solubles decreased, celluloses increased, and lignins did not change in aboveground plant tissue from elevated versus ambient CO<sub>2</sub> treatments at this shortgrass steppe site (Milchunas et al., 2004), compared to the increase in solubles, no change in celluloses, and decrease in lignin concentrations observed for roots.

In general, root production increases in response to elevated CO<sub>2</sub> were more consistent across years than were decreases in quality. However, the differences between elevated and ambient CO<sub>2</sub> treatments in nitrogen concentrations of current-year growth roots or of production from the ingrowth donuts observed in this study were not evident in nitrogen concentrations or biomass of bulk-sampled roots, where no significant CO<sub>2</sub> treatment effects were found during the first three years of the study (King et al., 2004). The improved soil moisture conditions under elevated compared to ambient CO<sub>2</sub> treatment (Nelson et al., 2004) may affect the life-span/decomposition and age-class distribution of bulk roots, and the long, seven year turnover of roots in this system (Milchunas and Lauenroth, 1995, 2001) compared to the only five years of CO<sub>2</sub> treatments would make detecting differences in bulk

roots more difficult. Pendall et al. (2003) observed significantly enhanced decomposition rates with elevated CO<sub>2</sub> at this shortgrass steppe study site, that suggested enhanced substrate induced priming (Diaz et al., 1993; Loiseau and Soussana, 1999). Although root:shoot production ratios did not change with elevated CO<sub>2</sub>, root:shoot biomass ratios decreased due to increases in aboveground production (Morgan et al., 2004). Long-term implications of an increase in the proportion of aboveground to belowground biomass could result in a system more susceptible to grazing by livestock and increased incidence and impact of fire (Milchunas et al., 1988; Burke et al., 1996), large changes in community species composition (Milchunas et al., 1995), and greater instability during drought and variability in climate.

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