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Root biomass of individual species, and root size characteristics after five years of CO_2 enrichment on native shortgrass steppe

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Abstract

Information from field studies investigating the responses of roots to increasing atmospheric CO_2 is limited and somewhat inconsistent, due partly to the difficulty in studying root systems *in situ*. In this report, we present standing root biomass of species and root length and diameter after five years of CO_2 enrichment ($\sim$720 μmol mol^{-1}) in large (16 m^2 ground area) open-top chambers placed over a native shortgrass steppe in Colorado, USA. Total root biomass in 100 cm long$\times$20 cm wide$\times$75 cm depth soil monoliths and root biomass of the three dominant grass species of the site were not significantly affected by elevated CO_2. Root biomass of *Stipa comata* in the 0–20 cm soil depth was nearly 100% greater in elevated vs. ambient CO_2 chambers, but this was not statistically significant ($P = 0.14$). However, there was a significant 37% increase in fine root length under elevated CO_2 in the 0–10 cm soil depth layer. Other reports from this study suggest that the increase in fine roots is primarily from improved seedling recruitment of *S. comata* under elevated CO_2. Few treatment differences in root length or diameter were detected in lower 10 cm depth increments, to 80 cm. These results reflect the root status integrated over two wet, two dry and one normal precipitation years and approximately one complete cycle of root turn-over on the shortgrass steppe. We conclude that increasing atmospheric CO_2 will have only small effects on standing root biomass and root length and diameter of most shortgrasss steppe species. However, the potential increased competitive ability of *Stipa comata*, a low forage quality species, could alter the ecosystem from the current dominant, high forage quality species, *Bouteloua gracilis*. *B. gracilis* is very well adapted to the frequent droughts of the shortgrass steppe. Increased competitive ability of less desirable plant species under increasing atmospheric CO_2 will have large implications for long-term sustainability of grassland ecosystems.

Abbreviations: OTC – open top chamber

Introduction

Increasing atmospheric CO_2 and resultant global climate change are predicted to cause significant changes in both natural and agricultural ecosystem processes. Arid and semi-arid ecosystems occupy 40% of the Earth's land surface (Dregne, 1991) and these ecosystems are expected to have relatively large responses to increasing CO_2 through changes in soil and plant water dynamics (Morgan et al., 2004a; Nelson et al., 2003; Volk

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et al., 2000). In the semi-arid shortgrass steppe of North America, about 90% of plant biomass and 67% of net-primary-productivity is below-ground (Milchunas and Lauenroth, 2001). Important ecosystem factors, such as plant competition, carbon cycling and the ability of grasslands to sequester carbon will be driven more by below-ground than above-ground processes in most grasslands (Norby and Jackson, 2000). Therefore, understanding the effects of elevated CO_2 on roots is critical.

To date there is insufficient information on root responses to elevated CO_2 to develop reliable predictive inputs needed to model ecosystem responses to global change (Arnone et al., 2000; Pendall et al., 2004b). This is primarily due to the difficulty in investigating roots *in situ* and to the limitations of the various methods used to measure root growth and production (Milchunas et al., 2005a, b). Studies performed in pots are severely limited for extrapolating to natural ecosystems (Norby and Jackson, 2000). Arnone et al. (2000) reviewed root studies performed on native grasslands either in the field or in monoliths placed in growth chambers or greenhouses. In five of these studies, root biomass was increased under elevated CO_2, while seven of the studies found little or no change (see Arnone et al., 2000 for references). This review highlights the shortage of studies investigating the dynamic responses of roots, such as production, decomposition and root characteristics (root diameter, length, area, depth distribution, branching). Also, there are no reports of below-ground responses of individual species to elevated CO_2 in a natural, undisturbed situation (Norby and Jackson, 2000).

We earlier reported on *in situ* root responses that were measured annually in a five year experiment, using open-top chambers (OTC) on the shortgrass steppe of Colorado, USA (Milchunas et al., 2005a, b; Pendall et al., 2004a). Large (20 cm diameter$\times$60 cm depth) soil cores showed that root biomass was increased by elevated CO_2 in only two of the five treatment years (Pendall et al., 2004a). However, root ingrowth and mini-rhizotron data showed a 46% and 52% increase in new root production (Milchunas et al., 2005a, b). These results reveal a discrepancy in root biomass vs. root productivity results which is a common issue in below-ground research, where pattern and rate of root mortality and decomposition may differ from that for root initiation and growth (Milchunas et al., 2005b). Bernston and Bazzaz (1996) also reported an increase in root production under elevated CO_2 in *Betula papyrifera*, which was offset by a nearly equal increase in root loss. The OTC study also showed an average increase in above-ground productivity of 41%, which was primarily attributed to one species, *Stipa comata* Trin and Rupr (Morgan et al., 2004b). Increased soil water and plant water status in the elevated CO_2 plots were the driving factors for increased above-ground productivity (LeCain et al., 2003; Nelson et al., 2003).

In order to add information to earlier reports of annual *in situ* root responses, this report presents a final characterization of the root zone at the end of five years of CO_2 enrichment on the Colorado shortgrass steppe experiment. These data represent the integrated response of this grassland, after two wet years, two dry years, and one normal precipitation year and nearly one full cycle of root turn-over (complete replacement of roots) (Milchunas et al., 2005a; Pendall et al., 2004a). Root biomass was measured in large, 100 cm long $\times$ 20 cm wide $\times$ 75 cm depth soil monoliths. Based on aboveground species responses, we attempted to separate roots by species from the large monoliths and present modifications to the method that help increase the proportion of roots that can be identified by species. Root length, diameter and surface area were also measured using soil cores and a digital scanning technique.

Based on previously reported increases in aboveground biomass and root productivity (Milchunas et al., 2005a, b; Morgan et al. 2004b) we hypothesized that there would be higher root biomass in the elevated vs. ambient CO_2 plots, primarily in the grass species *S. comata*. We also hypothesized that this increase in roots would be primarily in more fine roots under elevated CO_2, due to improved soil water content (Derner et al., 2001).

Materials and methods

Experimental site and design

The study site is the USDA-ARS Central Plains Experimental Range, lat. 40°50′ N, long.

104° 47′ W, in the shortgrass steppe region of northeastern Colorado, USA. Mean annual precipitation (55 years) is 320 mm yr^{-1}, and air temperatures averaged 15.6 °C in summer and 0.6 °C in winter, with maximum July temperatures of 30.6 °C (Milchunas et al., 1989). The year before beginning this experiment (1996) the dominant species at the site were *Bouteloua gracilis* [H.B.K.] Lag, (C_4 grass, comprising 45% of the vegetation), *Stipa comata* Trin and Rupr (C_3 grass, 25%) and *Pascopyrum smithii* [Rybd.] A. Love (C_3 grass, 18%). The soil at the experimental site is a Remmit fine sandy loam (Ustollic camborthids) to greater than one meter depth.

This experiment was established in spring of 1997 on a six-ha field of native grassland. The field was divided into three blocks, based on vegetation, and three plots per block were randomly chosen. From mid-March until late-October in 1997, 1998, 1999, 2000 and 2001 open-top chambers were placed on two plots in each of the three blocks. One chamber received ambient air CO_2 ($\sim$360 μmol mol^{-1}), the other received air with elevated CO_2 ($\sim$720 μmol mol^{-1}). Each block also had an ''unchambered control'' plot of equal ground area which was used to monitor the effect of the chamber.

The chambers were 3.8 m high and 4.5 m in diameter (16 m^2 ground surface area) with plastic walls (Lexan, Regal Plastics, Littleton, Colorado) and top, with a 0.75 m opening. An aluminum flange was buried to 0.75 m perpendicular to the soil surface, around the chamber wall. The chambers were aspirated at an exchange rate of one-and-a-half volumes min^{-1}. The CO_2 concentration was monitored with an infra-red gas analyzer (LI-COR LI6262; LI-COR, Lincoln, NE, USA) and was elevated with pure CO_2 in three of the chambers (720 ± 20 μmol mol^{-1}). Air and soil temperatures in the chambers were, on average, 2.6 and 1.3 °C higher than in the unchambered plots, but there was no difference in vapor pressure (LeCain et al. 2003). Photosynthetic photon flux was reduced about 28%, mainly due to chamber framework. Since the chamber top kept out much of the precipitation, rain was captured and returned to the plots with an automatic system. For a more detailed chamber description see Morgan et al. (2001).

Root monoliths and standing root biomass

At the end of the five year CO_2 enrichment period nine root monoliths (one per plot), measuring 100 cm long×20 cm wide×75 cm depth (150 L volume) were extracted from an area of the plots which had no previous soil disturbance. A modified ''nail-board'' method (Schuster and Wasser, 1964) was used to stabilize the monoliths and facilitate root washing. A back-hoe was used to dig a large trench to one meter depth on one side of the desired monolith. This allowed a person to work in the hole at the ''front'' of the monolith. A soil trencher was used to create a narrow trench on the ''back'' side of the monolith. Boards were placed on the front and back of the monolith. Then 20 cm nails were pushed through predrilled holes in the front board and through the soil width of the monolith. The sides and bottom were then cut with a reciprocating saw and straps used to secure the monolith between the boards. Monoliths were removed from the ground with a back hoe and transported to a root washing station.

The nail-board kept roots in their natural orientation, allowing root separation at 0–20, 20–45, and 45–75 cm depth intervals. Soil from the lowest depth increment was washed away first. Dislodged roots were captured in a 1 mm screen downstream of the root washer. All equipment was thoroughly cleaned and the second increment washed etc. After all soil was removed, the nail-board could be propped up with roots in their natural orientation. India ink was used to make a line on the roots at the desired root depth increments. Roots were separated by species by gently untangling roots that were attached to crowns which were identifiable by species. A commercial hair detangler (main ingredients: cetearlyl alcohol, cyclomethicone and polysorbate 20) was sprayed on and greatly aided the separation of roots. It is important to rinse the hair detangler off of the separated roots as the chemicals could have unknown effects on the drying and weighing steps. Roots were cut from the crowns, by depth increment, thoroughly washed and picked clean of litter, then dried at 60 °C and weighed. Dry weights were corrected for ash content. About 2/3 of the roots could not be identified by species. These were roots that came from crowns located

outside of the surface area of the monolith and roots that came off in the initial washing. These roots were added to the "total" root biomass data. In this report we present data from only the three dominant grass species. There were no significant treatment effects in the lesser species, and variability was very high.

Root size characteristics

In order to typify the resultant root characteristics after five years of CO_2 enrichment five 4.3 cm diameter×80 cm depth root cores were collected near each of the root monolith areas (45 total cores) using a soil coring device (Giddings Machine Co.; Fort Collins, CO). Cores were kept intact by lining the soil coring tube with a plastic sleeve (Giddings Machine Co.). Each soil core was cut in 10 cm depth increments; roots were washed from the soil by repeated flotation and sieving, picked clean of litter and kept moist prior to digital scanning. Root samples were placed on a tray and spread out to minimize overlap, then scanned using WinRHIZO software (Regent Instruments, Inc. Quebec, Canada, version 2002) and an Epson 1680 scanner at 1000 dpi resolution. Root diameter classes were set at 0.1 mm intervals; root diameter and length were measured and surface area and volume calculated. Data from the five cores was averaged for one data point per each of the nine plots.

Statistical analysis

The CO_2 * soil depth interaction was significant for both monolith and core data, therefore data were analyzed for each depth increment. Data in the figures are means ± standard errors. Data were analyzed as a completely randomized block design. Statistical significance was determined, within soil depth increments, using the SAS (SAS Inst. Inc., Cary, NC, USA) GLM procedure, with block as a random effect and block * treatment as the error term for treatment comparisons.

Results

Total standing and species root biomass

The total root biomass from the monoliths was not significantly affected by CO_2 enrichment, at any of the depth increments (Figure 1). The trend for more roots in the elevated vs. ambient CO_2 plots in the 0–20 cm soil layer appeared to be due to a decrease in the ambient CO_2 plots, rather than an increase in the elevated CO_2 plots, as root weight in the unchambered and elevated plots were nearly equal. About 60% of the total root biomass was in the 0–20 cm depth in all treatments. Root biomass of *S. comata* was nearly twice the amount in elevated vs. ambient CO_2 plots in the 0–20 cm layer (Figure 2). However, high variability made this not statistically

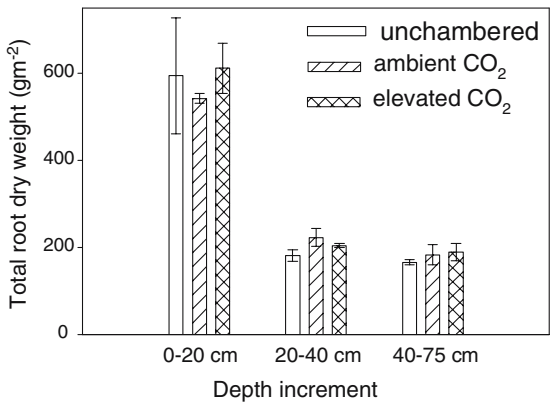

Figure 1. Total root dry weight from 100 cm long×20 cm wide×75 cm deep soil monoliths after five years in open-top-chambers with ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Data are means of three replications ± standard errors, within three soil depth increments. The treatment effect was not statistically significant.

significant ($P = 0.14$). Root biomass was not changed by elevated CO_2 in either *B. gracilis* or *P. smithii* (Figure 2). The root distribution by depth did differ among species, but CO_2 treatment did not affect root depth distribution within species. Root distribution in the 0–20, 20–45 and

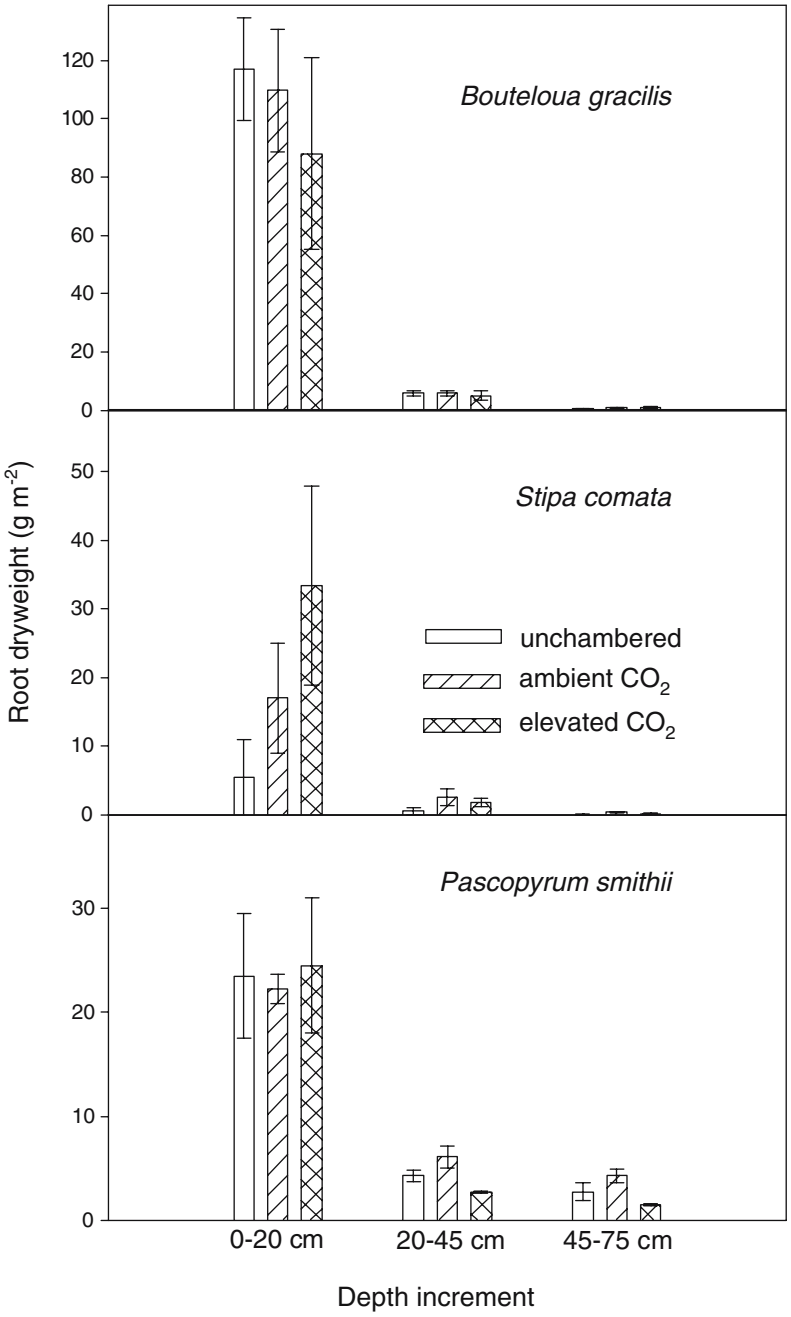

Figure 2. Root dry weight of the three dominant species from 100 cm long×20 cm wide×75 cm deep soil monoliths after five years in open-top-chambers with ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Data are means of three replications ± standard errors, within three soil depth increments. The treatment effect was not statistically significant.

45–75 cm depth increments, was 94, 5, and 1% for *B. gracilis*; 91, 8, and 1% for *S. comata*; and 77, 14, and 9% for *P. smithii*.

Root length, diameter and surface area

Roots washed from soil cores could not be identified by species. However, 88% of the above-ground biomass of these plots was composed of the three major grass species (Morgan et al., 2004b). Typical of grass roots (Norby and Jackson, 2000), there were very few roots with a diameter greater than 1.0 mm. 87% of the total root length was in the less than 0.4 mm diameter category and there were no treatment effects in the larger diameter classes. Therefore, only data from the fine (0–0.1, 0.1–0.2, 0.2–0.3 and 0.3–0.4 mm) root diameter classes are shown (Figure 3).

The only consistent elevated CO_2 effect was an increase in root length in the 0–10 cm soil depth (Figure 3). This was significant ($P < 0.07$) in three of the four root classes. Root length in the unchambered plots was lower than chambered plots in three depth/diameter classes. As suggested in the fine root diameter classes, root length in the 0–10 cm depth, summed over all diameters, was 34% higher in elevated vs. ambient CO_2 (Figure 4). However, this was not statistically significant ($P = 0.23$). The unchambered plots had significantly lower total root length in the 30–40 cm depth zone, but in all other cases roots were similar in the ambient CO_2 and the unchambered plots. Total root surface area was not affected by CO_2 treatment at any depth (Figure 4).

Discussion

A limited understanding of root responses remains one of the weakest links in predicting ecosystem responses to increasing atmospheric CO_2 and global change (Norby and Jackson, 2000). Studies in native grassland ecosystems have found both increased and no change in root amounts under elevated CO_2 (Arnone et al., 2000). Root responses in this report represent the integrated response to elevated CO_2 after two wet years, two dry years, and one normal precipitation year (Nelson et al., 2003), and after nearly one full cycle of root turn-over in a semi-arid grassland (Milchunas et al., 2005a; Pendall et al., 2004a).

Our hypothesis for higher root biomass after five years of elevated CO_2 was not well supported. This is noteworthy, as annual measurements from this study showed about a 50% increase in root production under elevated CO_2 (Milchunas et al., 2005a, b). Also, there was about an 87% increase in above-ground biomass in one of the major grass species, *S. comata* (Morgan et al., 2004b), but the CO_2 effect on *S. comata* root biomass was not significant.

Jastrow et al. (2000) conducted an experiment comparable to ours on the tallgrass prairie of Kansas, USA. At the end of an eight year period of CO_2 enrichment they found an average 34% increase in root mass in the 0–30 cm soil. In their report, the end of the study root response to elevated CO_2 was very similar to in-growth root production data. By contrast, annual in-growth root production data from our OTC study showed roughly a 50% increase under elevated CO_2 (Milchunas et al., 2005b), but there was very little difference in root mass in the monoliths. This apparent discrepancy can be explained by increased root mortality and decomposition rates under elevated CO_2. Milchunas et al (2005 a), using minirhizotrons, reported an average 37% increase in root-length losses under elevated CO_2. Pendall et al. (2003) reported evidence for increased soil substrate decomposition in the elevated CO_2 plots and attributed this to more labile C and higher soil water content and substrate availability under elevated CO_2, which stimulated microbial activity. Therefore, it appears that increased root production under elevated CO_2 is nearly balanced with increased root mortality and decomposition to result in little change in standing root biomass in this ecosystem. Similar results were reported for the tree species *Betula papyrifera*, which had an increase in root production under elevated CO_2 which was offset by an increase in root loss (Bernston and Bazzaz, 1996).

Although there was little effect of elevated CO_2 on standing root biomass in the monoliths, our hypothesis for an increase in fine roots under elevated CO_2 was confirmed, but only in the 0–10 cm soil depth zone. Supporting data suggest

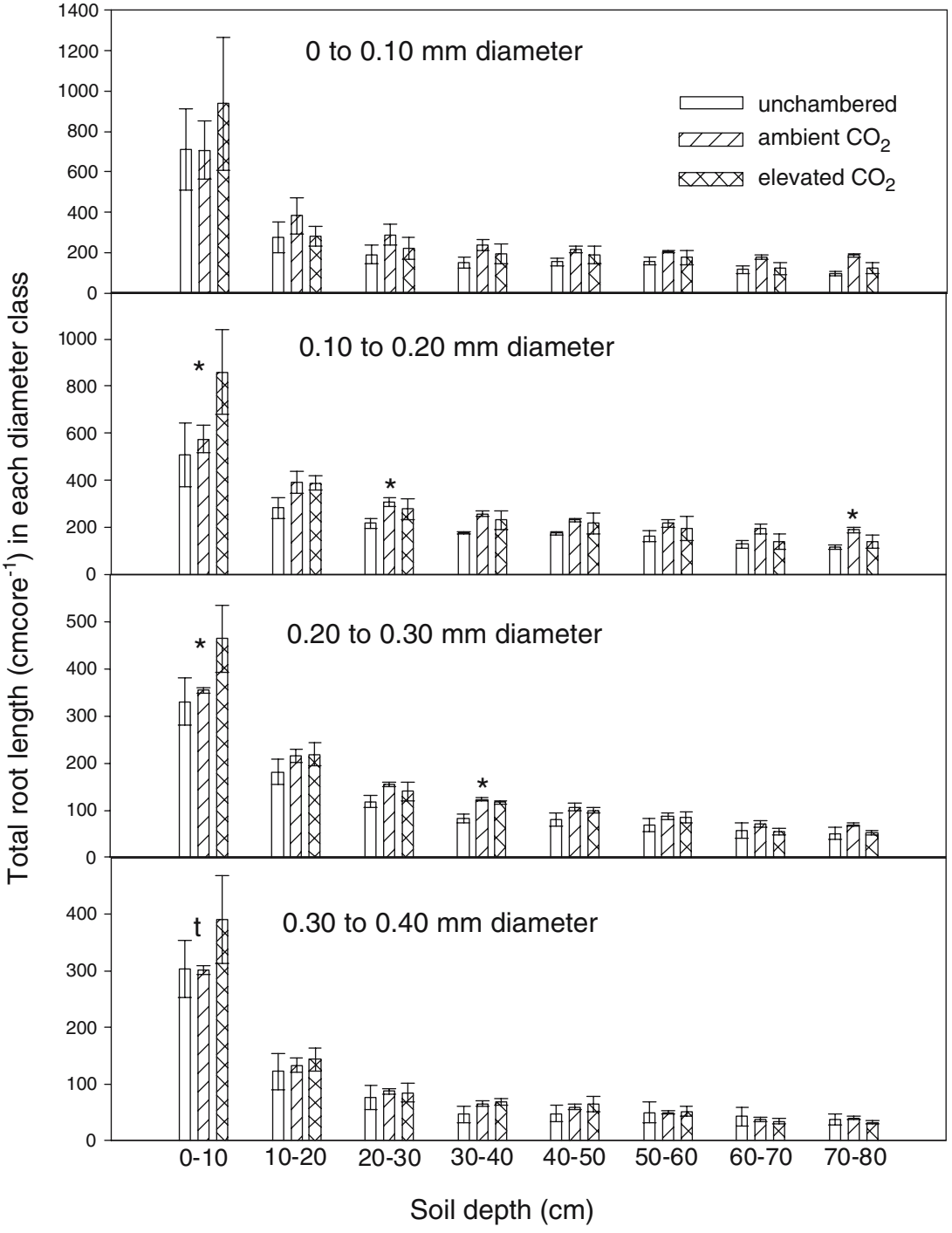

Figure 3. Root length in soil cores from open-top-chamber plots after five years with ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Cores were divided at 10 cm soil-depth intervals, and roots were divided into 0.1 mm diameter classes. Data are means of three replications ± standard errors. Treatment effects within each soil depth with an * are statistically significant at $P < 0.05$; with a 't' at $P < 0.07$.

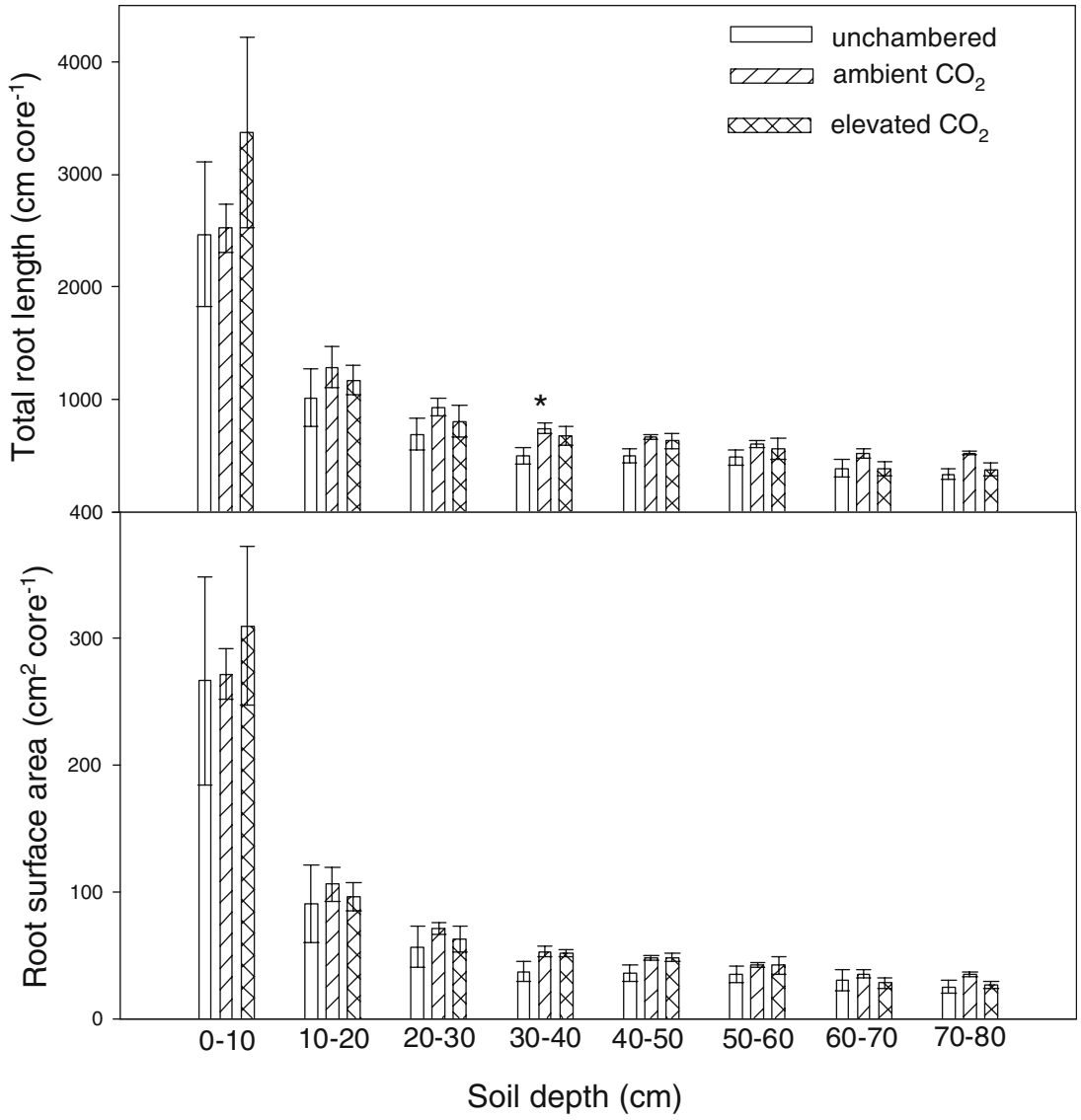

Figure 4. Root length summed over all root diameter classes, and the total root surface area in soil cores from open-top-chamber plots after five years with ambient (360 μmol mol^{-1}) and elevated (720 μmol mol^{-1}) CO_2 and in plots without chambers on the Colorado shortgrass steppe. Cores were divided at 10 cm soil-depth intervals. Data are means of three replications ± standard errors. Treatment effects within each soil depth with an * are statistically significant at $P < 0.05$.

that the increase in fine roots was largely *S. comata* roots. Morgan et al. (2004b) reported a nearly 400% increase in *S. comata* small seedling number in these same elevated vs. ambient CO_2 plots. Since seedling roots are primarily in the upper soil depth this would contribute to an increase in fine roots in the 0–10 cm soil layer. Also, though not significant, *S. comata* root biomass in the 0–20 cm soil (from monoliths) was about twice the amount in elevated vs. ambient CO_2 plots. Increases in fine roots with elevated CO_2 have also been reported in other ecosystems (Zak et al., 2000).

The observed increase in fine roots in the upper soil layer supports the findings of Pendall et al. (2004a) of an increase in new C inputs under elevated CO_2. Another study on the shortgrass steppe reported that fine roots turn-over

quickly, and therefore are major contributors to soil organic matter and soil C pools (Gill et al., 2002). Pendall et al. (2004a) reported no change in total mass of soil carbon, suggesting that the increase in fine roots under elevated CO_2 is contributing to reported higher net C flux from this ecosystem (Pendall et al., 2004c).

Root monoliths have often been used to map the root system of one species in its natural environment, but we could not find any studies in which roots were separated in a multi-species, natural ecosystem. In our study it was impossible to remove individual species roots from the large and entangled bulk root system without the use of hair detangler. With the hair detangler roots could be separated nearly completely intact. However, only about 1/3 of the total root mass could be identified by species, since much of the root system extended from crowns outside of the monolith surface area. It should be noted that the depth distribution of roots in the total root biomass vs. the individual species differs. The distribution of roots in the 0–20, 20–45 and 45–75 cm depths layers was 60%, 21% and 19% in the total root biomass, vs. 87%, 9% and 4% when averaged over the three grass species. It is likely that the deeper soil layers contain more roots that are not attached to the surface crowns (older roots), including roots that originated from plant crowns outside of the monolith surface area. Certainly a larger monolith surface area would improve the overall sample size for each species.

We conclude that elevated CO_2 causes increased production but also increased decomposition of roots, resulting in relatively small dif ferences in standing root pools of the dominant species of the shortgrass steppe. Increases in root decomposition and root mortality can be attributed to higher soil moisture and increased microbial activity under elevated CO_2 (Nelson et al., 2003; Pendall et al., 2003). Increased root turn-over is likely contributing to higher net C flux from this ecosystem (Pendall et al., 2004c). The increased aboveground (Morgan et al., 2004b) and suggested increased surface layer belowground productivity of *S. comata* has negative implications for the ecosystem. As discussed in Morgan et al. (2004b), *S. comata* is a low quality forage for livestock and wildlife. It also has limited drought hardiness and limited grazing tolerance. *B. gracilis*, the current dominant species of the shortgrass steppe, is a shallow rooted species, of high forage quality. *B. gracilis* is very drought hardy and was one of the key survivors of the dust bowl days in the 1930s (Allen-Diaz, 1995). Increased competitive ability of *S. comata* in the shallow soil layer could push the ecosystem to a less well adapted, poorer forage quality ecosystem. Changes in plant community composition will have large implications for nutrient cycling and long-term sustainability of grassland ecosystems.

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