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Root biomass and nutrient dynamics in a scrub-oak ecosystem under the influence of elevated atmospheric CO_2

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Abstract Elevated CO_2 can increase fine root biomass but responses of fine roots to exposure to increased CO_2 over many years are infrequently reported. We investigated the effect of elevated CO_2 on root biomass and N and P pools of a scrub-oak ecosystem on Merritt Island in Florida, USA, after 7 years of CO_2 treatment. Roots were removed from 1-m deep soil cores in 10-cm increments, sorted into different categories (<0.25 mm, 0.25–1 mm, 1–2 mm, 2 mm to 1 cm, >1 cm, dead roots, and organic matter), weighed, and analyzed for N, P and C concentrations. With the exception of surface roots <0.25 mm diameter, there was no effect of elevated CO_2 on root biomass. There was little effect on C, N, or P concentration or content with the exception of dead roots, and <0.25 mm and 1–2 mm diameter live roots at the surface. Thus, fine root mass and element content appear to be relatively insensitive to elevated CO_2. In the top 10 cm of soil, biomass of roots with a diameter of <0.25 mm was depressed by elevated CO_2. Elevated CO_2 tended to decrease the mass and N content of dead roots compared to ambient CO_2. A decreased N concentration of roots <0.25 mm and 1–2 mm in diameter under elevated CO_2 may indicate reduced N supply in the elevated CO_2 treatment. Our study indicated that elevated CO_2 does not increase fine root biomass or the pool of C in fine roots. In fact, elevated CO_2 tends to reduce biomass and C content of the most responsive root fraction (<0.25 mm roots), a finding that may have more general implications for understanding C input into the soil at higher atmospheric CO_2 concentrations.

Keywords Root biomass · Carbon dioxide · Carbon · Phosphorus · Nitrogen · Scrub-oak

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Introduction

Through burning of fossil fuels, deforestation, and cement manufacturing, humans have increased

the concentration of CO_2 in the atmosphere from pre-industrial levels of 280 ppm (Prentice et al. 2001) to current levels of 380 ppm (Sabine et al. 2004). Current and projected levels of atmospheric CO_2 have an unknown capacity to alter the future climate of the Earth due to the heat trapping nature of this greenhouse gas. Increasing CO_2 concentrations in the atmosphere could stimulate removal of CO_2 from the atmosphere by enhancing rates of photosynthesis and plant growth.

Root biomass is often stimulated by elevated CO_2 (Bernston and Bazzaz 1996; Jongen et al. 1995; Lipson et al. 2005; Matamala and Schlesinger 2000; O'Neill 1994; Wiemken et al. 2001). This can be driven by an increased need for belowground resources to support more rapid biomass accumulation (Chapin et al. 1987), causing plants exposed to elevated CO_2 to invest more carbon into root systems for further exploitation of the soil (Matamala and Schlesinger 2000). Carbon that is invested into the root system has a more direct route for entering long-term C storage in the soil than C deposited at the soil surface in the form of leaf and stem litter. Stimulation of plant activity by elevated CO_2 may lead to increased C residence time in the soil, increase the potential of the soil as a C sink (Canadell et al. 1996) and have a negative feedback on potential influence of elevated CO_2 on climate. Many studies on elevated CO_2 have investigated responses of the root system, but usually as a single unit, or at best distinguishing coarse from fine roots, less often live versus dead. More detailed information on how different root fractions respond to increased CO_2 are scarce. Because roots of different diameters may vary in functions like nutrient or water acquisition much like variations in the functions of leaves and stems, lumping roots together may miss key responses of the root system to elevated CO_2. Also, sampling has not often differentiated effects by depth.

In this study, we explored quantity and concentration of nutrients (C, N, P) in belowground biomass over a range of live root size classes and among live roots, dead roots and coarse organic matter in a Florida scrub-oak ecosystem where elevated concentrations of atmospheric CO_2 have been maintained since 1996. We hypothesized that root biomass would be stimulated by elevated CO_2, and consequently the total C contained in that biomass would increase under elevated CO_2. This was motivated by the finding that elevated CO_2 stimulated aboveground biomass in the scrub-oak (Dijkstra et al. 2002), indicating the plants under elevated CO_2 may have more C available to produce root biomass and store in larger roots, providing an advantage after fire. Initial responses of the root system indicated more rapid fine root growth and turnover (Dilustro et al. 2002; Langley et al. 2003).

We also hypothesized that there would be reduced N concentrations in roots, while total N content (g N m^{-2}) in roots would be unaffected by elevated CO_2. Reduced N concentrations would nullify a potential increase in total N content due to an increase in fine root mass. We based our hypothesis of decreased N concentration in roots on the findings of Dilustro et al. (2001), where there was a slight (but not significant) decrease in N concentration of roots grown in elevated CO_2 for 3 years, a trend that we expected to become more pronounced with prolonged exposure. Elevated CO_2 reduced extractable soil P at this site after 5 years of exposure (Johnson et al. 2003), suggesting a decline in P availability. For this reason, we also assessed the P content and concentration in fine roots, hypothesizing that the CO_2 effect would be similar to that of N. We also hypothesized there would be greater dead root mass, reflecting greater biomass of live roots and that altered N concentrations in live roots would not be present in dead roots. We based this on the evidence that live leaves in this system had altered C:N caused by an increase in simple carbon compounds, which were removed upon senescence, leaving C:N unaffected by CO_2 (Hall et al. 2005, 2006; Li et al. 1999).

Methods

Study site

A long-term experiment was established post burn in a Florida scrub-oak ecosystem in 1996

with the objective of examining elevated CO_2 effect on a water and nutrient stressed ecosystem. The height of the vegetation allows the use of open top chambers to elevate atmospheric CO_2. The experimental site is located on Merritt Island at Kennedy Space Center on the eastern coast of Florida, USA (28°38′ N–80°42′ W at an elevation of 0–3 m above sea level).

There are two soil types on the site, Paolo sand and Pomello sand (see Schmalzer and Hinkle 1990 for detailed descriptions). Their low pH, 3.8–4 in the top 15 cm and 4.3 in the 16–30 cm depth range (Schmalzer and Hinkle 1991), may limit nutrient availability in the system. Possible limitations at pH below 4 are N, P, S, B, C, Mg, Fe, Ca, and Mo (Taiz and Zeiger 1998).

The plant community is comprised of 76% *Quercus myrtifolia*, 15% *Q. geminata*, 7% *Q. chapmannii, Serenoa repense*, and *Lyonia ferreginea.* At the beginning of the current study, the community was 10 years post burn. It was burned again in February of 1996 prior to the construction of the chambers.

Experimental design

The treatments are manipulated in open topped chambers, which are octagonal, 365 cm high, 356.6 cm across and with sides of 139.9 cm. There are eight chambers with twice-ambient levels of CO_2 continuously blown in, eight chambers with ambient air and eight chamberless plots. Treatment was initiated on May 14, 1996. The type of pre-burn vegetation determined the chamber sites. Blocks were designated according to similarity of pre-burn vegetation composition. There was no chamber effect on the parameters measured in this study, so the data from the chamberless plots are not presented.

Root extraction and processing

In the spring of 2002, five points were randomly located along five radiating transects in each chamber from which 7 cm diameter by 100 cm deep cores were removed in 10 cm increments. Larger roots were removed from the soil with a 1 mm mesh sieve, and the sieved soil was divided among several researchers for different analyses; each core and soil subsample was weighed to enable scaling to the entire core. Sieved roots were dried for 2 h at 70°C to remove excess moisture that may have caused mold later and refrigerated at 4°C to prevent decay until further processing. The roots were sorted by hand into live root size classes of <0.25 mm, 0.25–1 mm, 1–2 mm, 2 mm to 1 cm, >1 cm, dead roots, and organic matter. If roots changed from one size class to the next along the length or a small root was connected to a large root, they were clipped and separated into different categories. Suppleness and springiness determined if the roots were dead or alive. In the case of the <0.25 mm roots, if they were still intact we assumed they were still alive at the time of harvest. The roots maintained their suppleness throughout the sorting process. The organic matter class included leaf litter and any unidentifiable organic particles.

All seven classes were dried at 70°C for 48 h and weighed. Because the root samples were not washed, a correction value for clinging soil was obtained using the methods described by Janzen et al. (2002). Root samples were not washed after core removal due to the need to retain the unaltered soil for other researchers doing microbial studies. To correct for sand, a subsample was washed and analyzed separately for C content and compared to unwashed samples (Janzen et al. 2002). These numbers were then used to obtain a conversion value that was applied to the 0.25–1 mm, 1–2 mm, 2 mm to 1 cm, and >1 cm root size classes, correcting for adhering minerals. This correction value was 97%, which was multiplied by the weight of the unclean roots to obtain a corrected weight. Organic matter, dead roots and <0.25 mm roots were corrected for sand contamination using the percentage of sand remaining after the samples were combusted. Organic matter was corrected for 23.18% sand content and the dead roots were corrected for 5.82% sand content. The finest root class (<0.25 mm) corrections were more complex. The fine roots from the top 10 cm were corrected using a 32.82% sand content, while the <0.25 mm roots from the 11–100 cm depths were corrected using the 3.00% correction from the Janzen et al. (2002) method. This seemingly high percent is due to the sand clinging to the fine roots and,

more likely, mycorrhizal filaments in the top 10 cm. These especially fine roots and mycorrhizae were not present in lower depths. The <0.25 mm roots were also removed from the soil portion that was saved from the sieved material. Only very fine roots passed through the 1-mm sieve because passage seemed to depend on the length of the root rather than the diameter. The volume used ranged from 5% to 10% of the original cores. The miniscule roots were removed using a dissecting microscope for each depth from a subset of chambers. Time limitations prevented the processing of all chambers in this manner. These fine roots were dried and weighed to approximate the root biomass that was underestimated by sieving. These estimates were extrapolated back to the full core and the subsample average by treatment was added to the <0.25 mm root size class to get a total estimate of biomass.

C and N analysis of roots and organic matter

Once root biomass was obtained from the cores, the roots were combined into depth classes of 0–10, 11–30, 31–60, 61–100 cm and then ground and analyzed for C and N by Dumas combustion (NC 2100; CE Elantech, Lakewood, New Jersey, USA), followed by continuous flow isotope ratio mass spectrometry (DELTA^{plus}–XL; Themoelectron Corporation, Bremen, Germany) at the Colorado Plateau Stable Isotope Laboratory. The percentages were applied to the biomass measurements to calculate C and N pools in the root system.

P analysis of roots and organic matter

We measured P only in the top 10 cm because the lower depths, in many cases, had insufficient material remaining after the other analyses, and thus were not analyzed for P. To ensure all replicates were present, we were unable to include depth as a factor and only looked at the top 10 cm. One-half gram of dried root material from the top 10 cm of soil taken in 2002 was measured into a crucible and ashed in a Thermoclyne muffle furnace at 500°C for 6 h. The cooled ash was suspended in 1 ml of concentrated H_2SO_4. Using phenolphthalein indicator, 50 µl of H_2SO_4 suspension was neutralized with 2 N NaOH solution. The neutralized solution was brought to a 10 ml volume with deionized distilled H_2O and divided into two 5 ml portions. The control portion was acidified with 1 ml 5 N H_2SO_4 acid. The phosphorus in the sample portion was assayed by adding 1 ml of ascorbic acid reagent (50 ml of 5 N H_2SO_4, 5 ml potassium antimonyl tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of 0.01 M ascorbic acid). The color was allowed to develop in the same period of time as a standard curve (more than 10 min and less than 30 min) before measurement on a spectrophotometer at 880 nm. The absorbance of the control sample was subtracted from the sample absorbance before conversion to P concentration. This adjusted absorbance was compared to a standard curve and converted back to µg P g^{-1} material.

Statistical analyses

A split-plot MANOVA was used to analyze root biomass. Vegetation composition at the initiation of the study in 1996 was used to determine the block, and one of the two chambers within each vegetation block was assigned as elevated CO_2 and the other ambient CO_2. The different size classes represented the multiple response variables. ANOVAs were used as a follow up for significant MANOVA results and the Least Means (LS) means procedure was used to interpret significant ANOVA interactions using SAS (SAS Institute 1990). Depth was not a factor in the phosphorus data sets, so the data were analyzed using a MANOVA.

Results

There was no CO_2 effect on total root and organic matter mass (Fig. 1), C and N content, C and N concentration or C:N ratio (Table 1). However, when these responses were examined at various depths, a treatment by depth interaction was found for all responses, except C concentration (Table 1). For the root classes affected (<0.25 mm, 1–2 mm, and dead roots), elevated CO_2 depressed the response (biomass,

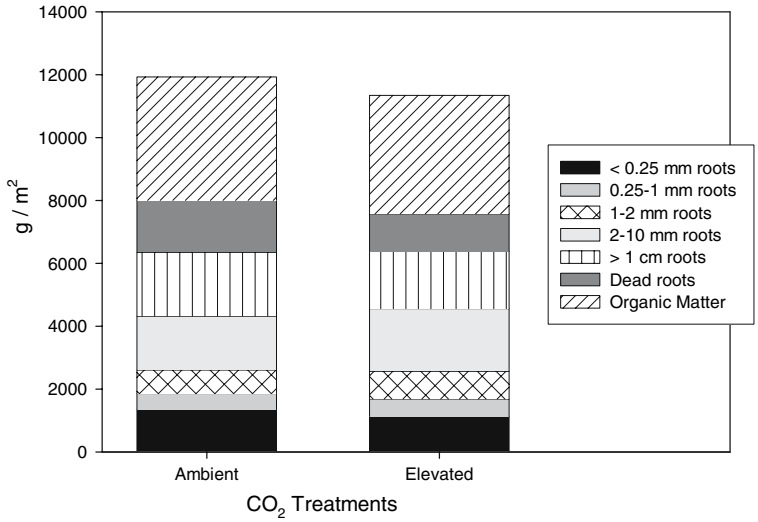

Fig. 1 Belowground biomass (g m^{-2}) to a meter depth from soil cores taken in May 2002. Live root size classes by diameter are <0.25mm, 0.25–1 mm, 1–2 mm, 2–10 mm, >1 cm. Dead roots were sorted into a separate category, while unidentifiable matter and leaf litter made up the organic matter

Table 1 Results of statistical analyses of plant response to elevated CO_2 from soil cores taken over a meter depth

	MANOVA results		Follow-up ANOVA results
	CO_2 treatment effect	CO_2 * depth interaction	Root categories of interest
Biomass	$P = 0.267$	$P < 0.001$	<0.25 mm roots ($P < 0.001$) Dead roots ($P = 0.068$)
C concentration (g C g^{-1} material)	$P = 0.067$	$P = 0.525$	None
N concentration (g C g^{-1} material)	$P = 0.238$	$P = 0.003$	<0.25 mm roots ($P = 0.018$) 1–2 mm roots ($P = 0.025$)
C content (g m^{-2})	$P = 0.545$	$P < 0.001$	<0.25 mm roots ($P < 0.001$) Dead roots ($P = 0.064$)
N content (g m^{-2})	$P = 0.088$	$P < 0.001$	<0.25 mm roots ($P < 0.001$) Dead roots ($P = 0.044$)
C:N of material	$P = 0.334$	$P = 0.163$	None

The response variables were <0.25 mm roots, 0.25–1 mm roots, 1–2 mm roots, 2–10 mm roots, >10 mm roots, dead roots and organic matter. The data were analyzed using a Wilk's Lambda statistic. $\alpha = 0.05$

C and N content, and N concentration) usually at the 0–10 cm depth, but in one case at the 11–30 cm depth increment. Further details are given below. Phosphorus concentration and content were only examined for the top 10 cm, but there was no significant effect of elevated CO_2 at the MANOVA level on the P concentration ($P = 0.568$) or P content ($P = 0.384$) for live roots, dead roots and organic matter. There was also no significant effect of elevated CO_2 on C:P ($P = 0.706$) or N:P ($P = 0.380$) for live roots, dead roots and organic matter in the top 10 cm of soil.

Root biomass

Elevated CO_2 reduced the biomass of roots <0.25 mm in diameter in the top 10 cm of soil. Statistically this is supported by a significant depth by treatment interaction for roots <0.25 mm in diameter (Table 1). The LS means analysis of the CO_2 by depth interaction showed that in the 0–10 cm depth increment, the elevated CO_2 plants had significantly less biomass (498 g m^{-2}) than the ambient CO_2 plants (737 g m^{-2}) (Fig. 2a). There was no significant difference at any other depth. The fine roots picked from the sieved soil were less

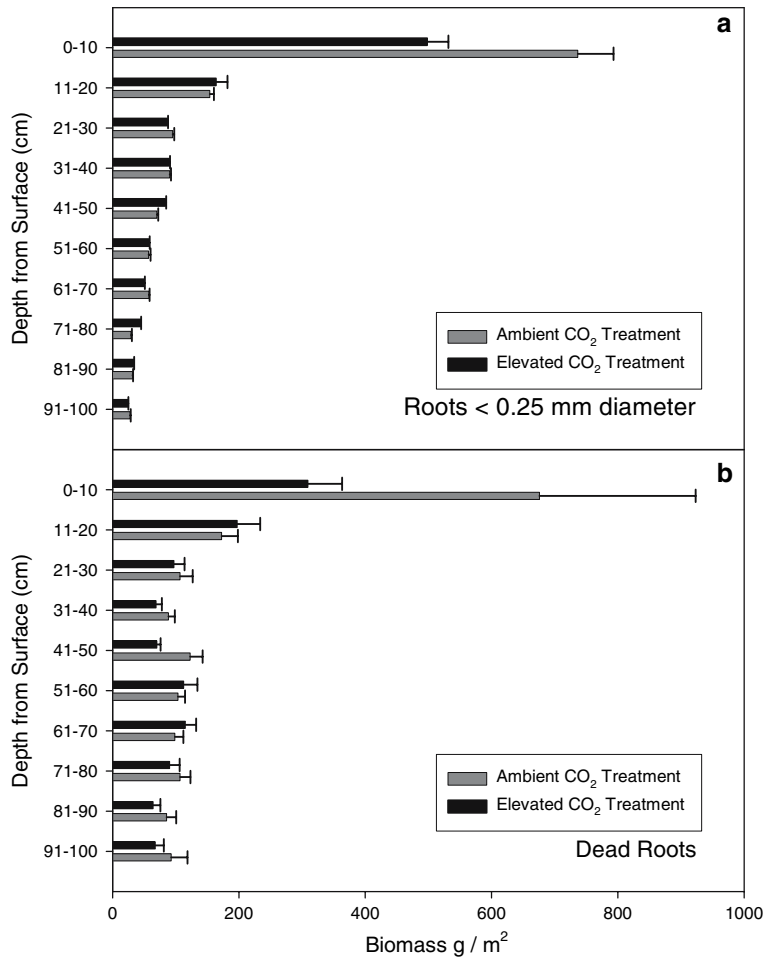

Fig. 2 Biomass of roots <0.25 mm in diameter (**a**) and dead roots (**b**) to a meter depth from soil cores taken in May 2002. Error bars represent one standard error

than 1 g m^{-2} to a meter depth. Since it was a subsample, there were not enough replicates to run a separate statistical analysis. However, the means reflected the patterns seen in the total biomass for this category with the ambient CO_2 treatment having 0.91 g m^{-2} and the elevated having 0.77 g m^{-2}. Due to the difficulty of recovering very fine roots in the sieved material, this portion is often neglected in belowground biomass studies. We found that in our study this sample constituted 0.01% of the root biomass, a negligible amount that will not affect the reliability of future studies at this site if not recovered.

The dead roots followed the same pattern of biomass distribution as the <0.25 mm roots, with decreased biomass in the 0–10 cm depth increment for the elevated CO_2 treated plants (Fig. 2b), even though the CO_2 by depth interaction was marginally significant (Table 1).

N concentration

There was a significant CO_2 by depth interaction for <0.25 mm and 1–2 mm roots (Table 1). The LS means analysis showed there was a significantly lower percent N in the <0.25 mm roots under elevated CO_2 in the 0–10 cm and 11–30 depths (Fig. 3a). There was also a similar trend in the 1–2 mm roots, but only in the 0–10 cm depth (Fig. 3b). Despite the differences in N concentration, the C:N ratio was unaffected (Fig. 4)

C and N content

The significant interaction (Table 1) for C and N content (g m^{-2}) in the <0.25 mm roots and dead roots showed a reflection of the biomass trends, with less C and N in the elevated CO_2 plots than the ambient CO_2 treated plots in the 0–10 cm

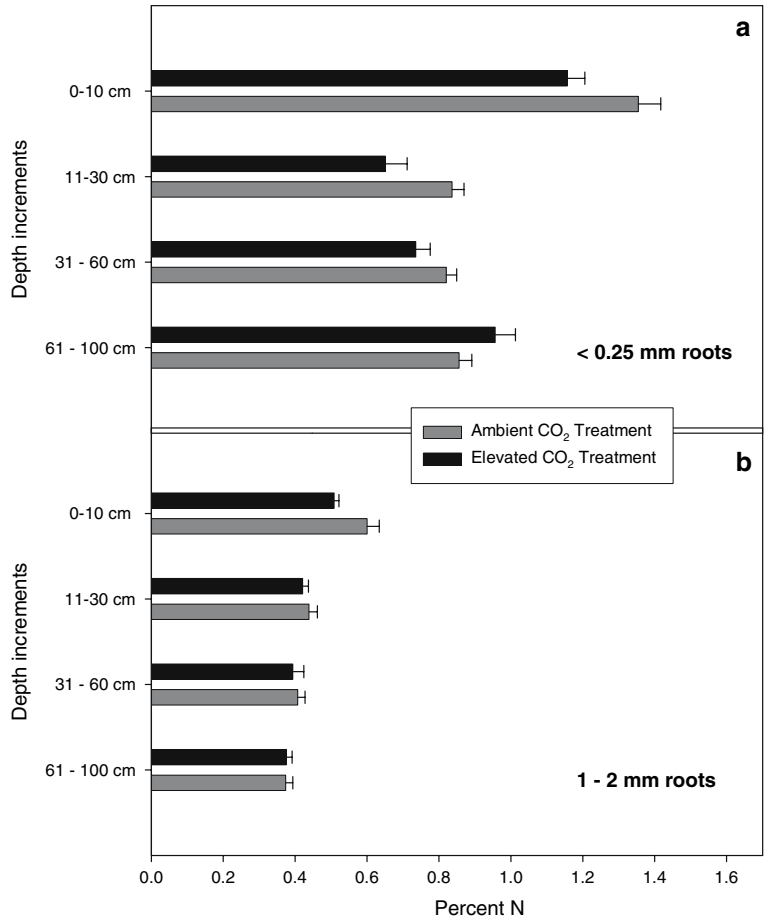

Fig. 3 Percent N concentration of roots <0.25 mm (**a**) and 1–2 mm (**b**) in diameter. Error bars represent one standard error

depth increment (Tables 2–4). There was no effect on C and N content for the other root and organic matter categories.

Phosphorus and N concentration across root diameters

Phosphorus content or concentration in root biomass was not affected by elevated CO_2 treatment. As roots increased in diameter, P concentrations also increased, whereas N concentrations decreased (Fig. 5).

Discussion

Biomass

Elevated CO_2 often increases belowground biomass. In a review by Rogers et al. (1994) of the 157 examples from studies of root biomass using several methods of CO_2 manipulation on various species, there were only 3 studies that exhibited negative responses to elevated CO_2 and 13 revealed no response. The remaining 141 studies showed a positive response of root biomass to elevated CO_2. More recent studies seem to follow the same trends. Root biomass roughly doubled for *Adenostoma fasciculatum* in a Chaparral system (Lipson et al. 2005); increased by 96% for *Populus tremuloides* and *Betula papyrifera* (King et al. 2001); increased by 50% for *Betula pedula* (Ineson et al. 1996); increased for *Pinus echinata* seedling fine roots (Norby et al. 1987) and increased for *Populus tremuloides* under a high nitrogen treatment (Pregitzer et al. 2000). Besides biomass, fine root production increased more than 100% for in growth bags for *Pinus sylvestris* seedlings (Janssens et al. 1998) and increased by 85% in calcareous soils and 43% in

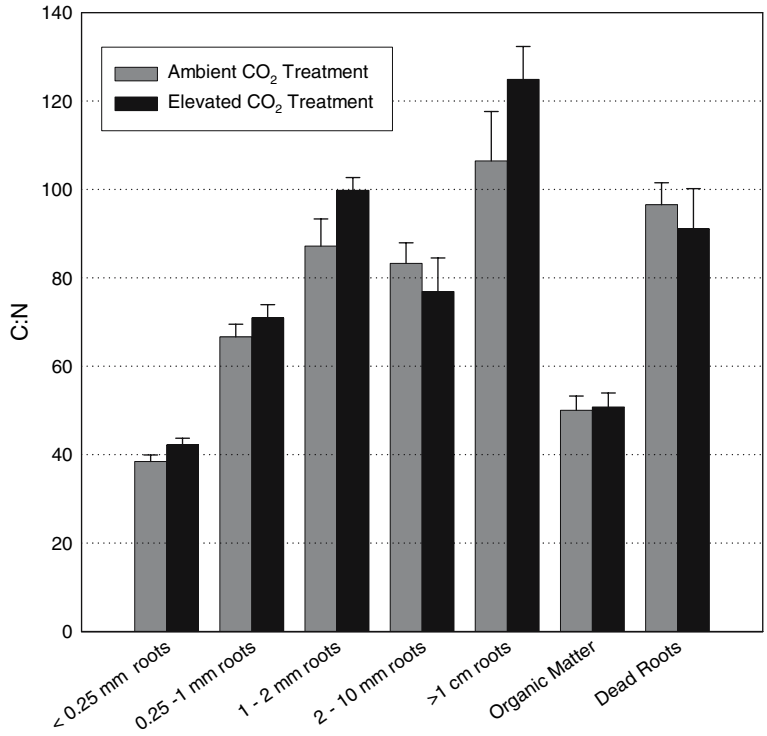

Fig. 4 C:N ratio of roots from the top 10 cm of soil. Error bars represent one standard error

siliceous soils for *Fagus silvatica* and *Picea abies* stands (Wiemken et al. 2001). Based on the findings of others and early minirhizotron measurements in the current project (Day et al. 2006), we expected to find high fine root biomass under elevated CO_2. Instead, our study falls among the minority where overall fine root biomass is unresponsive to elevated CO_2 treatment. Other research that has shown no significant effect of CO_2 on root biomass include a study of *Anthyllis vulneraria* and *Plantago media* (Ferris and Taylor 1993) and a study of *Populus tremuloides* under a low nitrogen treatment (Pregitzer et al. 2000).

Overall, the root biomass of this study was unaffected by CO_2 treatment, but when the root diameter sizes were examined separately by depth, it was found that the biomass of <0.25 mm roots was depressed under elevated CO_2 in the top 10 cm. The fine roots, such as those <0.25 mm, are responsible for nutrient and water uptake (Gordon and Jackson 2000), which are important factors in controlling exploitation of the soil (Fitter 1987; Waisel et al. 2002). This may influence the depression of <0.25 mm roots under elevated CO_2. Several studies have shown that under elevated CO_2, when high levels of N were available, root biomass and root production substantially increased (Pregitzer et al. 2000; Zak et al. 1993) as did mortality (Pregitzer et al. 2000). However, in the Florida scrub-oak ecosystem the nutrients are low and in the elevated treatment there are significantly lower levels of available N (Johnson et al. 2003), perhaps influencing fine root distribution.

Ectomycorrhizae are another possible influence on fine root mass. Scrub-oak is an ectomycorrhizal system and it has been shown in the past that oak root tips under elevated CO_2 had a higher frequency of ectomycorrhizal colonization, but there was no difference in the biomass of infected roots (Langley et al. 2003). Based on these findings, if ectomycorrhizae were to influence root biomass, it would have probably been the opposite of what we observed. The work on ectomycorrhizae was started in 1998, when the root system was still responding positively to elevated CO_2 (Dilustro et al. 2002), but ectomycorrhizal colonization frequency during the period in which we are observing a depression in the <0.25 mm roots at the surface was not measured.

Table 2 Carbon content of live roots (g C m^{-2}) for different diameter classes of <0.25 mm, 0.25–1 mm, 1–2 mm, 2–10 mm, >1 cm for 10 cm depth increments

C (g m^{-2})	<0.25 mm roots		0.25–1 mm roots		1–2 mm roots		2–10 mm roots		>1 cm roots	
Depth (cm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0–10	426 ± 37.4	266 ± 12.1	81 ± 5.7	94 ± 9.4	68 ± 9.7	95 ± 34.6	181 ± 26.0	207 ± 42.5	521 ± 179.9	480 ± 173.9
11–20	75 ± 3.7	56 ± 5.4	41 ± 3.5	53 ± 8.0	51 ± 7.0	69 ± 17.5	129 ± 22.1	141 ± 19.6	277 ± 190.3	263 ± 92.9
21–30	46 ± 1.2	31 ± 3.6	27 ± 3.6	26 ± 2.2	36 ± 3.5	35 ± 9.2	81 ± 18.8	69 ± 21.9	94 ± 54.6	9 ± 8.6
31–40	41 ± 1.7	34 ± 3.0	21 ± 1.8	23 ± 2.6	27 ± 3.5	39 ± 7.8	69 ± 16.1	45 ± 10.9	31 ± 21.8	14 ± 13.9
41–50	31 ± 1.5	32 ± 2.9	19 ± 2.2	19 ± 2.6	30 ± 5.5	33 ± 7.6	82 ± 30.4	54 ± 18.24	42 ± 30.9	23 ± 22.5
51–60	26 ± 1.8	22 ± 2.2	18 ± 1.2	21 ± 2.7	29 ± 4.6	41 ± 11.4	50 ± 21.3	96 ± 43.3	0	28 ± 21.5
61–70	28 ± 1.1	22 ± 0.8	18 ± 1.4	14 ± 1.7	27 ± 5.4	38 ± 11.6	61 ± 12.3	113 ± 27.8	0	0
71–80	14 ± 0.7	20 ± 0.6	13 ± 2.4	13 ± 2.2	27 ± 6.4	34 ± 9.4	64 ± 18.3	61 ± 13.2	0	18 ± 18.2
81–90	15 ± 0.8	15 ± 0.4	10 ± 1.0	12 ± 0.8	28 ± 9.7	33 ± 7.4	59 ± 17.9	75 ± 16.5	0	6 ± 6.4
91–100	13 ± 0.7	11 ± 0.3	15 ± 2.4	11 ± 2.5	46 ± 23.8	25 ± 6.0	53 ± 11.8	67 ± 9.8	0	0

Table 3 Nitrogen content of live roots (g N m^{-2}) for different diameter classes of <0.25 mm, 0.25–1 mm, 1–2 mm, 2–10 mm, >1 cm for 10 cm depth increments

N (g m^{-2})	<0.25 mm roots		0.25–1 mm roots		1–2 mm roots		2–10 mm roots		>1 cm roots	
Depth (cm)	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0–10	11.2 ± 1.04	6.4 ± 0.42	1.24 ± 0.10	1.34 ± 0.13	0.77 ± 0.09	0.95 ± 0.33	2.22 ± 0.32	2.55 ± 0.36	5.30 ± 1.97	4.06 ± 1.46
11–20	1.4 ± 0.10	1.2 ± 0.13	0.52 ± 0.06	0.66 ± 0.10	0.43 ± 0.05	0.56 ± 0.07	1.09 ± 0.17	1.07 ± 0.14	2.26 ± 1.59	2.01 ± 0.74
21–30	0.9 ± 0.03	0.6 ± 0.06	0.34 ± 0.05	0.33 ± 0.03	0.31 ± 0.03	0.28 ± 0.07	0.72 ± 0.17	0.56 ± 0.20	0.74 ± 0.46	0.07 ± 0.07
31–40	0.8 ± 0.03	0.7 ± 0.04	0.28 ± 0.03	0.27 ± 0.03	0.22 ± 0.03	0.28 ± 0.05	0.47 ± 0.11	0.30 ± 0.07	0.22 ± 0.15	0.12 ± 0.12
41–50	0.6 ± 0.03	0.7 ± 0.04	0.25 ± 0.03	0.23 ± 0.03	0.24 ± 0.05	0.23 ± 0.04	0.48 ± 0.14	0.37 ± 0.11	0.39 ± 0.30	0.15 ± 0.15
51–60	0.5 ± 0.02	0.5 ± 0.03	0.24 ± 0.02	0.25 ± 0.03	0.24 ± 0.05	0.29 ± 0.06	0.28 ± 0.09	0.65 ± 0.26	0	0.31 ± 0.24
61–70	0.6 ± 0.03	0.5 ± 0.02	0.22 ± 0.02	0.17 ± 0.02	0.21 ± 0.05	0.27 ± 0.08	0.32 ± 0.06	0.60 ± 0.14	0	0
71–80	0.3 ± 0.02	0.5 ± 0.02	0.18 ± 0.04	0.16 ± 0.02	0.21 ± 0.06	0.25 ± 0.06	0.31 ± 0.08	0.32 ± 0.06	0	0.16 ± 0.16
81–90	0.3 ± 0.02	0.4 ± 0.02	0.13 ± 0.01	0.16 ± 0.01	0.22 ± 0.08	0.24 ± 0.05	0.28 ± 0.08	0.41 ± 0.09	0	0.06 ± 0.06
91–100	0.3 ± 0.02	0.3 ± 0.01	0.19 ± 0.03	0.14 ± 0.03	0.36 ± 0.19	0.19 ± 0.04	0.27 ± 0.06	0.37 ± 0.05	0	0

Table 4 Nitrogen and carbon content of dead roots and organic matter for 10 cm depth increments

Depth (cm)	Dead roots				Organic matter			
	C (g m^{-2})		N (g m^{-2})		C (g m^{-2})		N (g m^{-2})	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0–10	349 ± 131	153 ± 29	3.34 ± 1.12	1.62 ± 0.19	1237 ± 146	1079 ± 94	25.15 ± 3.23	21.20 ± 1.39
11–20	89 ± 14	101 ± 20	0.83 ± 0.17	0.81 ± 0.14	255 ± 57	271 ± 51	3.43 ± 0.68	3.43 ± 0.66
21–30	54 ± 10	49 ± 9	0.48 ± 0.08	0.39 ± 0.06	174 ± 43	128 ± 23	2.31 ± 0.55	1.64 ± 0.33
31–40	46 ± 5	32 ± 4	0.36 ± 0.04	0.26 ± 0.03	92 ± 9	79 ± 12	1.19 ± 0.16	0.97 ± 0.15
41–50	65 ± 11	33 ± 3	0.51 ± 0.10	0.26 ± 0.02	78 ± 10	56 ± 5	1.04 ± 0.18	0.69 ± 0.08
51–60	54 ± 6	54 ± 11	0.41 ± 0.03	0.43 ± 0.09	69 ± 7	83 ± 10	0.91 ± 0.12	1.04 ± 0.14
61–70	49 ± 6	54 ± 9	0.39 ± 0.04	0.48 ± 0.08	75 ± 10	79 ± 8	0.86 ± 0.15	0.85 ± 0.09
71–80	53 ± 8	44 ± 9	0.43 ± 0.06	0.37 ± 0.07	73 ± 14	61 ± 7	0.83 ± 0.18	0.68 ± 0.09
81–90	43 ± 7	31 ± 6	0.34 ± 0.05	0.27 ± 0.05	61 ± 11	69 ± 9	0.67 ± 0.12	0.75 ± 0.11
91–100	46 ± 13	33 ± 6	0.37 ± 0.10	0.29 ± 0.07	68 ± 17	64 ± 8	0.75 ± 0.20	0.69 ± 0.10

Some studies that measure roots may not discern a difference in biomass because more rigorous methods of soil removal may destroy fine roots with a diameter <0.25 mm. The roots that are not destroyed are often lumped with larger roots that can mask differences that might exist. In the study of *Populus tremuloides*, where roots were separated into classes of <0.5 mm and 0.5–1 mm diameter, Pregitzer et al. (2000) showed that the smaller size class was more responsive to CO_2 and N treatments. Jach et al. (2000) found a shift in percentage of fine roots (<2 mm) and coarse roots (>2 mm) between CO_2 treatments. They showed that the percent of fine root biomass increased from 1% under ambient to 8% under elevated CO_2, whereas in our system there was a small increase from 27% to 29%.

Despite indications early in this study (Dilustro et al. 2002) that root biomass would respond positively to elevated CO_2, after 6 years of CO_2 treatment there was no effect on the biomass of fine and intermediate diameter roots over a meter depth. Our finding supports of Day et al. (2006) and indicates that the root system may have reached the point where further investment in increased root length no longer conferred any advantage in increased resource acquisition. Day et al. (2006) concluded that elevated CO_2 allowed the plant system to reach this point sooner than ambient CO_2 systems. Some other systems have exhibited more prolonged stimulation of CO_2 on root systems. Why did the scrub-oak reach root closure so quickly? Scrub-oak may be a more nutrient poor, xeric ecosystem than others examined for CO_2 responses. The most responsive fraction (<0.25 mm roots) was depressed under elevated CO_2 in the top 10 cm due to increased soil moisture (Hungate et al. 2002) or altered soil nutrient availability (Johnson et al. 2003).

Contrary to the findings of Jach et al. (2000), where root litter of *Pinus sylvestris* was greater under elevated CO_2, scrub-oak dead root mass in the top 10 cm constituted a smaller fraction under elevated CO_2. We know that the greater amount of dead root mass in the ambient CO_2 treatment is not from the <0.25 mm diameter roots that have died. One of our basic assumptions for sorting roots was that if they were <0.25 mm and still intact, they were alive or very recently dead and consequently were sorted into the live root fraction. Any dead roots of this size would have been unidentifiable and therefore sorted into the organic matter fraction.

Despite past evidence at this site of increased litter fall and increased forest floor mass (Hungate et al. 2006), we did not find any CO_2 effect on coarse particulate organic matter in the soil (>1 mm), which included surface leaf litter. Possibly, older particulate matter in the mineral soil horizons masked any influence of increased litter fall and forest floor mass on total soil particulate organic matter.

We found no evidence that plants under elevated CO_2 accumulated greater amounts of carbon in belowground biomass. One of our objectives was to sample roots that were larger

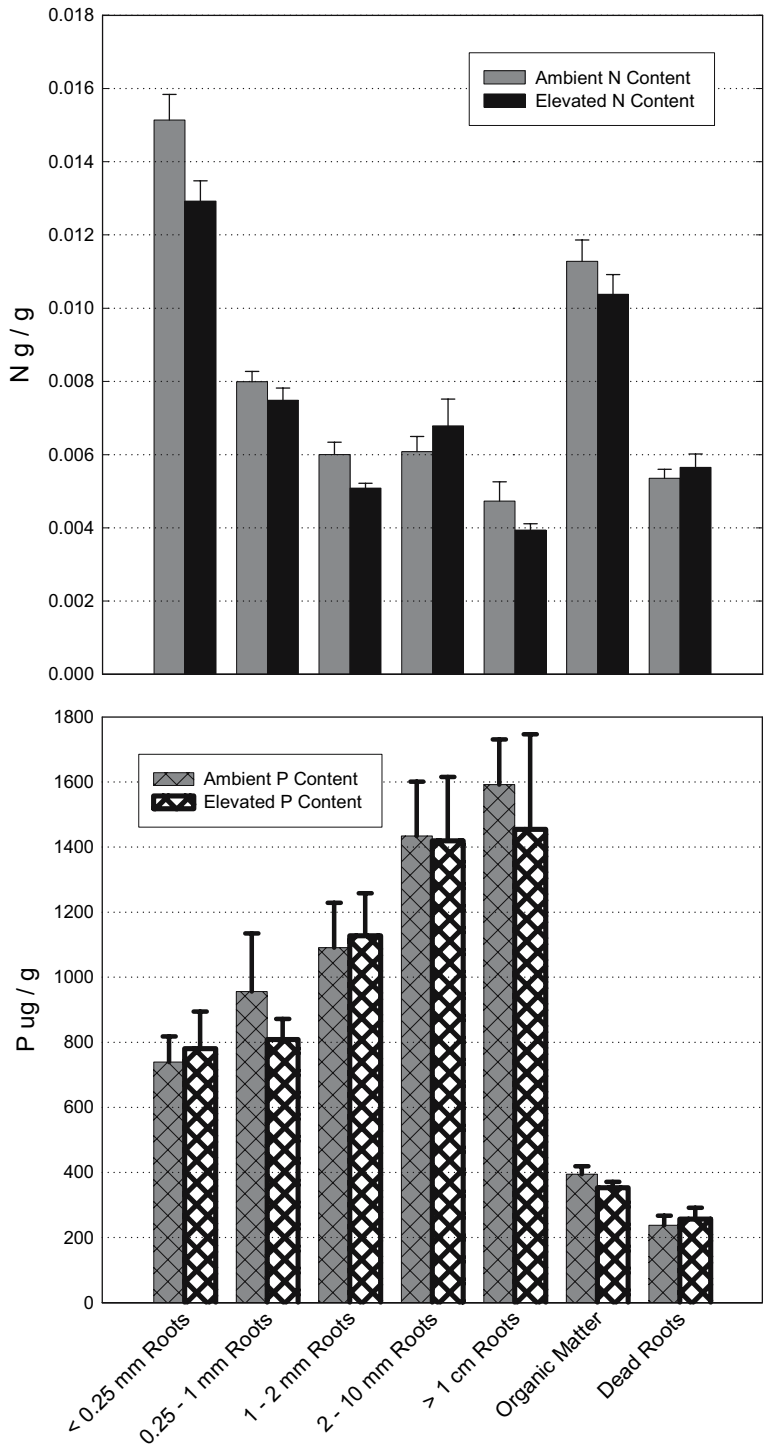

Fig. 5 N and P concentration of roots in the top 10 cm. Error bars represent one standard error

than the fine roots monitored by minirhizotrons. We were able to sample larger structural components of the root system, but our sampling methods were likely inadequate for roots >1 cm. The scrub-oak ecosystems contains lignotubers and rhizomes, large below ground structures likely important for storage and post-burn regeneration (Canadell et al. 1991; Canadell and

Lopez-Soria 1998). These structures have been encountered as large as 12.4–15 cm diameter using a 15 cm corer (P. C. Daniel Stover) outside of the chambers. The 7 cm corer we used did not capture these larger structures. Larger cores in the chambers would have been destructive to the long-term study and therefore were not feasible. Even though there was no significant effect, the largest size class of roots (>1 cm) were encountered in 20% of the cores below 50 cm in the elevated CO_2 chambers, while no roots of this size were encountered in the ambient CO_2 chambers below 50 cm. Further study is needed of these large roots and structures.

Element concentration and content

Elevated CO_2 reduced the mass of carbon and nitrogen contained in roots <0.25 mm in diameter, reflecting differences in biomass. Jach et al. (2000) investigated similar aspects of CO_2 effects on *Pinus sylvestris* on roots with diameters >2 mm, 1–2 mm and <1 mm. Pregitzer et al. (2000) investigated *Populus tremuloides* roots according to their order, which can be comparable to size. Both Jach et al. (2000) and Pregitzer et al.'s (2000) findings concerning the distribution of C and N were comparable to our findings. Similar to the findings of Pregitzer et al. (2000) we found no effect of CO_2 on C concentration in root biomass. Jach et al. (2000) found no significant effects of elevated CO_2 on N concentration in roots, but trends indicated that coarse roots (>2 mm) had lower N concentrations and fine roots (<1 mm) had higher concentrations of N under elevated CO_2. We found that the <0.25 mm diameter roots had significantly lower N concentrations under elevated CO_2 treatment near the surface. Because the fine roots of <0.25 mm diameter are the most labile components of the root system, this may indicate reduced N availability under elevated CO_2 (Hungate et al. 2006). The one root size class for which Jach et al. (2000) found no changes in N concentration was the 1–2 mm diameter roots. By contrast, we did find that elevated CO_2 significantly reduced N concentration in the 1–2 mm diameter roots. Despite these differences, our and Jach et al.'s (2000) results indicate that some effects of CO_2 would not have been elucidated had the roots been lumped into larger categories. Pregitzer et al. (2000) found a universal decrease in N concentration across all root orders of *Populus tremuloides*, qualitatively similar to what we found.

In a decomposition study in the scrub-oak, Dilustro et al. (2001) found that elevated CO_2 did not alter decomposition rates, however they used tissue that was living at the time of harvest. The material Dilustro et al. (2001) used had been exposed to 3 years of elevated CO_2, but did not have a significantly different % N. If we were to repeat his study, using roots that were living at the time of harvest, we may see different results based on the reduced N concentration in some roots and the changes in N availability and ecosystem N cycling observed by others (Hungate et al. 2006; Johnson et al. 2003). The N and lignin contents of leaf material have been shown to affect decomposition rates (Melillo et al. 1982), and there are numerous examples where elevated CO_2 has altered the N concentration of living leaf tissue (Körner and Arnone 1992; McGuire et al. 1995; Norby et al. 1992; Williams et al. 1994). However, using senesced leaf material instead of living material, several studies have shown that leaf litter decomposition is not affected by environment of tissue growth (i.e., grown under elevated or ambient CO_2 conditions) (Hall et al. 2006; Van Ginkel et al. 1996). How might growth under elevated CO_2 affect decomposition of roots after a programmed root death? The lack of a CO_2 effect on N concentration of dead root material in the scrub-oak leads to the conclusion that the effect that is present in some root categories while alive either disappears before root death or immediately after, or is masked by the other categories of unaffected roots. In any case, altered N concentration in those few roots is unlikely to affect long-term decomposition of root material.

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References

Bernston GM, Bazzaz FA (1996) The allometry of root production and loss in seedlings of *Acer rubrum* (Aceraceae) and *Betula papyrifera* (Betulaceae): implications for root dynamics in elevated CO_2. Am J Bot 83:608–616

Canadell J, Lopez-Soria L (1998) Lignotuber reserves support regrowth following clipping of two Mediterranean shrubs. Funct Ecol 12:31–38

Canadell J, Lloret F, Lopezsoria L (1991) Resprouting vigor of 2 Mediterranean shrub species after experimental fire treatments. Vegetatio 95:119–126

Canadell JG, Pitelka LF, Ingram JS I (1996) The effects of elevated (CO_2) on plant-soil carbon below-ground: a summary and synthesis. Plant Soil 187:391–400

Chapin FS III, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. Bioscience 37:49–57

Day FP, Stover DB, Pagel AL, Hungate BA, Dilustro JJ, Herbert BT, Drake BG, Hinkle CR (2006) Rapid root closure after fire limits fine root responses to elevated atmospheric CO_2 in a scrub oak ecosystem in central Florida, USA. Glob Change Biol 12:1047–1053

Dijkstra P, Hymus G, Colavito D, Vieglais DA, Cundari CM, Johnson DP, Hungate BA, Hinkle CR, Drake DP (2002) Elevated atmospheric CO_2 stimulates aboveground biomass in a fire-regenerated scrub-oak ecosystem. Glob Change Biol 8:90–103

Dilustro JJ, Day FP, Drake BG (2001) Effects of elevated atmospheric CO2 on root decomposition in a scrub oak ecosystem. Glob Change Biol 7:581–589

Dilustro JJ, Day FP, Drake BG, Hinkle CR (2002) Abundance, production and mortality of fine roots under elevated atmospheric CO_2 in an oak-scrub ecosystem. Environ Exp Bot 48:149–159

Ferris R, Taylor G (1993) Contrasting effects of elevated CO_2 on the root and shoot growth of four native herbs commonly found in chalk grasslands. New Phytol 125:855–866

Fitter AH (1987) An architectural approach to the comparative ecology of the plant root system. New Phytol 106:61–77

Gordon WS, Jackson RB (2000) Nutrient concentrations in fine roots. Ecology 81:275–280

Hall MC, Stiling P, Hungate BA, Drake BG, Hunter MD (2005) Effects of elevated CO_2 and herbivore damage on litter quality in a scrub oak ecosystem. J Chem Ecol 31:2343–2356

Hall MC, Stiling P, Moon DC, Drake BG, Hunter MD (2006) Elevated CO_2 increases the long-term decomposition rate of *Quercus myrtifolia* leaf litter. Glob Change Biol 12:568–577

Hungate BA, Reichstein M, Dijkstra P, Johnson D, Hymus G, Tenhunen JD, Hinkle CR, Drake BG (2002) Evapotranspiration and soil water content in a scrub oak woodland under carbon dioxide enrichment. Glob Change Biol 8:289–298

Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pagel AL, Moan JL, Day FP, Li J, Hinkle CR, Drake BG (2006) Nitrogen cycling during seven years of atmospheric CO_2 enrichment a scrub oak woodland. Ecology 87:26–40

Ineson P, Cotrufo MF, Bol R, Harkness DD, Blum H (1996) Quantification of soil carbon inputs under elevated CO_2: C3 plants in a C4 soil. Plant Soil 187:345–350

Jach ME, Laureysens I, Ceulemans R (2000) Above- and below-ground production of young scots pine (*Pinus sylvestris* L.) trees after three years of growth in the field under elevated CO_2. Ann Bot 85:789–798

Janssens IA, Crookshanks M, Taylor G, Ceulemans R (1998) Elevated atmospheric CO_2 increases fine root production, respiration, rhizosphere respiration and soil CO_2 effluz in Scotts pine seedlings. Glob Change Biol 4:871–878

Janzen HH, Entz T, Ellert BH (2002) Correction mathematically for soil adhering to root samples. Soil Biol Biochem 34:1965–1968

Johnson DW, Hungate BA, Dijkstra P, Hymus G, Hinkle CR, Stiling P, Drake BG (2003) The effects of elevated CO_2 on nutrient distribution in a fire-adapted scrub oak forest. Ecol Appl 13:1388–1399

Jongen M, Jones MB, Hebeisen T, Blum H, Hendrey GR (1995) The effects of CO_2 concentrations on the root growth of *Lolium perenne* and *Trifolium repens* grown in a FACE system. Glob Change Biol 1:361–371

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. Oecologia 128:237–250

Körner C, Arnone JA III (1992) Responses to elevated carbon dioxide in artificial tropical ecosystems. Science 257:1672–1675

Langley JA, Dijikstra P, Drake BG, Hungate BA (2003) Ectomycorrhizal colonization, biomass and production in a regenerating scrub oak forest in response to elevated CO_2. Ecosystems 6:424–430

Li J, Dijkstra P, Hinkle CR, Wheeler RM, Drake BG (1999) Photosynthetic acclimation to elevated atmospheric CO_2 concentration in the Florida scrub-oak species *Quercus geminata* and *Quercus myrtifolia* growing in their native environment. Tree Physiol 19:229–234

Lipson DA, Wilson RF, Oechel WC (2005) Effects of elevated atmospheric CO_2 on soil microbial biomass, activity, and diversity in a chaparral ecosystem. Appl Environ Microbiol 71:8573–8580

Matamala R, Schlesinger WH (2000) Effects of elevated atmospheric CO_2 on fine root production and activity in an intact temperate forest ecosystem. Glob Change Biol 6:967–979

McGuire AD, Melillo JM, Joyce LA (1995) The role of nitrogen in the response of forest net primary production to elevated atmospheric carbon dioxide. Annu Rev Ecol Syst 26:473–503

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626

Norby RJ, O'Niell EG, Hood WG, Luxmoore RJ (1987) Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under elevated CO_2 enrichment. Tree Physiol 3:203–210

Norby RJ, Gunderson CA, Wullschleger SD, O'Neill EG, McCracken MK (1992) Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. Nature 357:322–324

O'Neill EG (1994) Responses of soil biota to elevated atmospheric carbon dioxide. Plant Soil 165:55–65

Pregitzer KS, Zak DR, Maziasz J, DeForest JL, Curtis PS, Lussenhop J (2000) Interactive effects of atmospheric CO_2 and soil-N availability in fine roots of *Populus tremuloides*. Ecol Appl 10:18–33

Prentice IC, Farquar GD, Fasham MJR, Goulden ML, Heimann M, Jaramillo VJ, Kheshgi HS, Le Quéré C, Scholes RJ, Wallace DWR (2001) The carbon cycle and atmospheric carbon dioxide. Contributions of working group I to the third assessment report of the Intergovernmental Panel on Climate Change. In: Houghton JT et al (ed) IPCC climate change 2001: the scientific basis. Cambridge University Press, New York, pp 183–237

Rogers HH, Runion GB, Krupa SV (1994) Plant responses to atmospheric CO_2 with emphasis on roots and the rhizosphere. Environ Pollut 83:155–189

Sabine C, Freely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng T-H, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Schmalzer PA, Hinkle CR (1990) Geology, geohydrology and soils of Kennedy Space Center: a review. NASA Technical Memorandum 103813, NASA, Kennedy Space Center, Florida

Schmalzer PA, Hinkle CR (1991) Dynamics of vegetation and soils of oak/saw palmetto scrub after fire: observations from permanent transects. NASA Technical Memorandum 103817, NASA, Kennedy Space Center, Florida

Taiz L, Zeiger E (1998) Mineral nutrition in plant physiology. In: Taiz L, Zeiger E (eds) Plant physiology. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, pp 103–114

Van Ginkel JH, Gorissen A, Van Veen JA (1996) Long-term decomposition of grass roots as affected by elevated atmospheric carbon dioxide. J Environ Qual 25:1122–1128

Waisel Y, Eshel A, Kafkafi U (2002) Plant roots: the hidden half. Marcel Dekker, Inc, New York

Wiemken V, Laczko E, Ineichen K, Boller T (2001) Effects of elevated carbon dioxide and nitrogen fertilization on mycorrhizal fine roots and the soil microbial community in beech-spruce ecosystems on siliceous and calcareous soil. Microb Ecol 42:126–135

Williams RS, Lincoln DE, Thomas RB (1994) Loblolly pine grown under elevated CO_2 affects early instar pine sawfly performance. Oecologia 98:64–71

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117