

Root dynamics in an artificially constructed regenerating longleaf pine ecosystem are affected by atmospheric CO₂ enrichment

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Abstract

Differential responses to elevated atmospheric CO₂ concentration exhibited by different plant functional types may alter competition for above- and belowground resources in a higher CO₂ world. Because C allocation to roots is often favored over C allocation to shoots in plants grown with CO₂ enrichment, belowground function of forest ecosystems may change significantly. We established an outdoor facility to examine the effects of elevated CO₂ on root dynamics in artificially constructed communities of five early successional forest species: (1) a C₃ evergreen conifer (longleaf pine, *Pinus palustris* Mill.); (2) a C₄ monocotyledonous bunch grass (wiregrass, *Aristida stricta* Michx.); (3) a C₃ broadleaf tree (sand post oak, *Quercus margaretta*); (4) a C₃ perennial herbaceous legume (rattlebox, *Crotalaria rotundifolia* Walt. ex Gemel); and (5) an herbaceous C₃ dicotyledonous perennial (butterfly weed, *Asclepias tuberosa* L.). These species are common associates in early successional longleaf pine savannahs throughout the southeastern USA and represent species that differ in life-form, growth habit, physiology, and symbiotic relationships. A combination of minirhizotrons and soil coring was used to examine temporal and spatial rooting dynamics from October 1998 to October 1999. CO₂-enriched plots exhibited 35% higher standing root crop length, 37% greater root length production per day, and 47% greater root length mortality per day. These variables, however, were enhanced by CO₂ enrichment only at the 10–30 cm depth. Relative root turnover (flux/standing crop) was unchanged by elevated CO₂. Sixteen months after planting, root biomass of pine was 62% higher in elevated compared to ambient CO₂ plots. Conversely, the combined biomass of rattlebox, wiregrass, and butterfly weed was 28% greater in ambient compared to high CO₂ plots. There was no difference in root biomass of oaks after 16 months of exposure to elevated CO₂. Using root and shoot biomass as a metric, longleaf pine realized the greatest and most consistent benefit from exposure to elevated CO₂. This finding suggests that the ability of longleaf pine to compete with sand post oak, a

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common deciduous tree competitor, and wiregrass, the dominant understory herbaceous species, in regenerating ecosystems may be significantly enhanced by rising atmospheric CO₂ concentrations. © Published by Elsevier Science B.V.

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1. Introduction

Empirical evidence and models predict between 25 and 75% increases in photosynthesis and 30–40% increases in net primary productivity of plant systems as atmospheric CO₂ concentrations double in the next 100 years (Bernston and Bazzaz, 1996; Barrett et al., 1998). Long term stimulation of forest productivity, however, might be limited by nutrient limitations (Tingey et al., 2000).

Differential responses to elevated CO₂ exhibited by different species, or species of different functional types, may result in competitive shifts and, ultimately, changes in species composition, physiognomy, and ecosystem function (Reekie and Bazzaz, 1989; Ceulemans and Mousseau, 1994; Jifon et al., 1995). Several authors have reported that pine species are less responsive to CO₂ enrichment than broadleaf species (Tolley and Strain (1984), Kaushal et al. (1989), Ceulemans and Mousseau (1994), Gunderson and Wullschleger (1994), Pritchard et al. (1997, 1998); but see Saxe et al. (1998)); but studies are lacking that examine how competition between these two groups may change in a future higher CO₂ world.

Root systems often realize the most consistent and largest growth increase in plants grown with elevated CO₂ (Rogers et al., 1994; Batts et al., 1998). This phenomenon often, but not always, results in greater root:shoot and root:whole plant ratios (Wullschleger et al., 1995; Curtis and Wang, 1998). Greater C allocation to root growth is likely to cause significant shifts in belowground processes in forest ecosystems, including competition for soil resources (Bernston and Woodward, 1992; Jifon et al., 1995; Casper and Jackson, 1997; Pritchard et al., 1999). Although there is evidence that root growth of plants grown individually may differ from root responses of plants in nature (Casper and Jackson, 1997), few studies have examined root responses to CO₂ enrichment

within competitive arrays. In one such study, Jifon et al. (1995) found that CO₂ responses of sweetgum and loblolly pine roots differed in seedlings grown in monocultures compared to those grown in competitive mixtures. Even fewer studies have examined root responses to elevated CO₂ in either artificially constructed or intact forest ecosystems (Norby et al., 1999).

Root turnover is also likely to be affected by enhanced C allocation belowground. During periods of stress, most perennial plants periodically replace finer root elements when the cost of maintaining those roots will exceed the cost of constructing new roots upon the return of more favorable soil conditions (Caldwell, 1977; Van der Werf et al., 1988). Continuous root replacement (root turnover) allows plants to better explore soil by seeking out fertile microsites not accessible to existing roots. Accelerating temporal patterns of root initiation, growth, and senescence may represent a compensatory mechanism whereby forest ecosystems could enhance nutrient uptake efficiency in a higher CO₂ world. In the absence of such a mechanism, long-term increases in forest productivity will likely be constrained by nutrient limitations (Tingey et al., 2000).

Root turnover represents an important component of plant, ecosystem, and global C budgets (Pregitzer et al., 1995). Of all belowground C sinks, it has been hypothesized that periodic replacement of finer root elements might represent a greater energy demand than maintenance respiration, exudation, C transfer to symbionts, and belowground predation (Caldwell, 1977; Canadell et al., 1996). Caldwell (1977) suggested that at least 25% of root systems die and are replaced annually, accounting for as much as 50–80% of net production for a variety of perennial plant systems. From a global perspective, the total fine root C pool is about 5% the size of the atmospheric C pool; and biomass of fine roots has been

estimated to account for 33% of the global annual net primary productivity (Jackson et al., 1997). The temporal and spatial distribution of fine roots is increasingly being recognized as one of the most important and one of the least understood components of belowground C cycling, and perhaps the entire global C cycle (Torn et al., 1997). However, there are still too few data to make any generalizations concerning the impact of elevated CO₂ on root dynamics in crops (Pritchard and Rogers, 2000), grasslands (Arnone et al., 2000), or forests (Tingey et al., 2000).

The data reported herein were collected from an artificially constructed ecosystem consisting of five co-occurring species of varied functional types typical of the longleaf pine/wiregrass association of the southeastern USA. The use of artificially constructed ecosystems to examine real-world ecosystem responses to environmental change has

recently been advocated by several authors (Körrner and Arnone, 1992). Such idealized ecosystems provide an intermediate level of complexity compared to individual container grown plants and FACE (free air CO₂ enrichment) sites.

The objectives of this study were 2-fold: first, to use minirhizotron technology to characterize the influence of elevated atmospheric CO₂ on seasonal and spatial root dynamics in our idealized ecosystem, and second, to use soil cores to determine the impact of elevated CO₂ on root biomass of longleaf pine, sand post oak, and three understory species that are common competitors for resources in longleaf pine forests. Our hypothesis was that the increase in the air's CO₂ concentration would not provide longleaf pine with a competitive edge over these other species, based on the results of a number of prior monoculture studies.

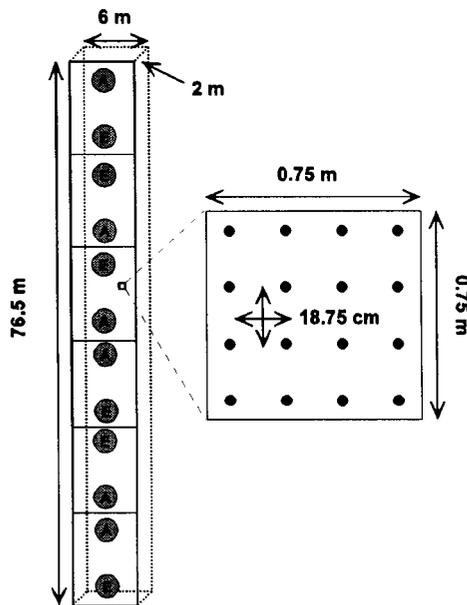


Fig. 1. Diagram of model regenerating longleaf pine ecosystem constructed at the USDA-ARS National Soil Dynamics Laboratory, Auburn, AL. The community was constructed in an outdoor soil bin 76.5 m long, 6 m wide, and 2 m deep. The experimental design was a randomized complete block design with six replications (total of 12 OTCs). The entire bin was divided into 0.75 m square quadrats as shown and individuals of each species were planted at densities reflective of nature into randomly assigned positions within each quadrat.

2. Materials and methods

2.1. Construction of the idealized ecosystem

The following five species typical of early successional longleaf pine savannahs throughout the southeastern USA were chosen for study: (1) a C₃ evergreen conifer (longleaf pine, *Pinus palustris* Mill.); (2) a C₄ bunch grass (wiregrass, *Aristida stricta* Michx.); (3) a C₃ broadleaf tree (sand post oak, *Quercus margaretta* Ashe); (4) a C₃ perennial herbaceous legume (*Crotalaria rotundifolia* Walt. ex Gemel); and (5) an herbaceous C₃ perennial (butterfly weed, *Aesclepias tuberosa* L.). These species differ in life form, growth habit, physiology, and symbiotic relationships.

The idealized ecosystem was constructed in an outdoor soil bin (2 m deep, 6 m wide, and 76 m long) at the USDA-ARS National Soil Dynamics Laboratory at Auburn, AL, USA. The bin contained a Blanton loamy sand (loamy, siliceous, thermic Grossarenic Paleudults) acquired from a longleaf pine area typical of the Southeastern Coastal Plains. The entire bin was divided into 0.75 m² quadrats, each possessing 16 equally spaced planting positions (Fig. 1). The community was constructed by planting individuals of each

species (three longleaf pine, three wiregrass, two oaks, one butterfly weed, and one rattlebox) into randomly assigned positions within each quadrat (planting was completed on 3 April, 1998). These plant densities reflect those of naturally regenerating longleaf pine-wiregrass ecosystems in the southeast (Hains, 1995; Jacqumain, 1996).

After planting, treatments were assigned in a randomized block design with six replications (total plots = 12). Open top chambers (OTCs) were constructed and installed in June 1998 to administer atmospheric CO₂ levels of 365 and 720 $\mu\text{l l}^{-1}$ (see Mitchell et al. (1995) for a thorough discussion of CO₂ dispensing and monitoring protocols). Plots received ambient rainfall and were watered only when necessary to prevent drought-induced mortality (water stress was assessed visually). A drip irrigation system was installed to allow for exact and consistent metering of water throughout the soil bin. For the first 2 months after planting, all dead plants were removed from plots and replaced with healthy plants from a greenhouse in order to maintain target plant densities. After 2 months, mortality was attributed to causes other than transplanting, and dead plants were not replaced.

2.2. *Minirhizotron analysis of root dynamics*

In May 1998 two minirhizotrons were installed randomly with respect to plants, but facing toward the door into each of the 12 plots in order to characterize root growth. Minirhizotrons are clear plastic tubes (outside diameter = 56 mm) that allow non-invasive measurement of root growth. The tubes were installed at an angle of 45° from vertical to a vertical depth of 32.5 cm. The portion of the minirhizotron tube extending above the ground was covered with a closed-cell polyethylene sleeve, and the end was sealed with a rubber cap to exclude light and minimize heat exchange between the air and the tube. A PVC cap was then installed over the end to protect the rubber cap from UV damage, and to further protect and insulate the tube. In order to prevent minirhizotron tubes from moving, customized aluminum brackets (design courtesy of Mark Johnson, EPA, Corvallis) were clamped to the

tubes and anchored into the ground with 40 cm stainless steel rods. No data were collected for the first 4 months after tube installation to allow time for roots to colonize the tube surface, and to allow soil adjacent to minirhizotrons to equilibrate with bulk soil.

Beginning in September 1998, a BTC-100x microvideo camera (Bartz Technologies, Santa Barbara, CA) was inserted into the minirhizotrons approximately every 3 weeks, and images of roots growing against the clear plastic tube were recorded. The camera was equipped with a indexing handle allowing precise and consistent camera placement over time (Johnson and Meyer 1998). Video images of roots were recorded 17 times from September 1998 to October 1999. Video frames were replayed in the laboratory where still images were digitized and root data were extracted using the image analysis program RooTracker (Dave Tremmel, Duke University Phytotron). A total of 17 frames representing depths from 0 to 32.5 cm were analyzed from all 24 tubes at each date for a total of 6936 images (17 depths \times 24 tubes \times 17 dates). Several variables were recorded for each minirhizotron frame at each date, including the number of live roots, number of dead roots, length of live roots, length of dead roots, total diameters of live roots, and total diameters of dead roots. We were unable to differentiate between roots of different species with the minirhizotrons. Therefore, all root data from minirhizotrons represent root lengths combined for all species. Roots were considered dead when they either disappeared or when significant structural damage (i.e. loss of integrity of root margins) was observed. From these data, root length production per day, root length mortality per day, and average root diameters were calculated. Relative root turnover (turnover index) was calculated by dividing mortality (length) at time x by standing root crop (length) at time $x - 1$. Therefore, turnover is a relative variable relating root flux to the total root standing crop averaged over 3 week sampling intervals. Because of the apparent confusion in the literature concerning the definition and use of the terms turnover, mortality, and absolute turnover, we

use the definitions set forth by Tingey et al. (2000).

2.3. Soil coring

Eight 10-cm diameter root cores (subsamples) were extracted from the soil in each chamber (replicate) to a depth of 30 cm in October 1998. The soil from the cores was placed in cold storage until the roots could be washed out and total root length determined (live and dead roots were not separated). The holes from the cores were then backfilled with the same soil series and a pinflag placed in the center of the hole. The following year (October 1999), smaller 3.9-cm cores were removed from the center hole of each of the backfilled holes from the previous year. Roots sampled from each date were separated from soil; pine and oak roots could be distinguished based on morphology and color. Therefore, data are presented for pine roots, oak roots and other roots (including wiregrass, rattlebox, and butterfly weed). Roots were dried at 55°C to a constant weight, weighed, ground, and homogenized to yield one sample per plot (one pine, one oak, and one other). Subsamples were then analyzed for total carbon and nitrogen with a LECO CHN-600 analyzer (LECO, Augusta, GA).

2.4. Statistical analysis

The experimental design was a split block with main units (CO₂ treatments) arranged in a randomized complete block design with six replications (six elevated OTCs and six ambient OTCs). A block consisted of two OTCs to which ambient and elevated CO₂ treatments were randomly assigned. Each OTC contained two subsample minirhizotron tubes, the results from which were averaged together for statistical analyses. Minirhizotron frames were grouped into six depth classes in order to determine treatment effects on vertical root distribution. The subunit factor depth is a split block, since depths are not randomly assigned within minirhizotron frames (Steele et al., 1997). Measurements were taken on each tube on 17 dates.

Initial analysis of variance was performed on the data combined over all dates. In this analysis, dates were treated as an additional split block sub-unit. If date did not interact with CO₂ or depth factors, then CO₂ and depth means averaged over all dates were presented based on this analysis. If date did interact with either CO₂ or depth factors, then a separate analysis of variance for each date was performed and the CO₂ and depth means were presented for each date. SAS PROC MIXED was used for the analysis of variance. Least squares means are presented and a least S.D. (LSD) value was used for mean comparisons (SAS, 1996).

3. Results

3.1. Aboveground growth/community composition

Total aboveground plant biomass (kg m⁻²) was greater in elevated CO₂ compared to ambient plots. Increases in biomass of pine ($P=0.006$) and oak ($P=0.10$) were observed in response to CO₂-enrichment (Fig. 2). No changes in aboveground biomass of rattlebox, wiregrass or butterfly weed were observed at the community level.

3.2. Root standing crop

In general, the greatest root densities were found at the lowest soil depths (Fig. 3). Peak root lengths for the three deepest depth classes occurred in mid to late spring, whereas peak root lengths for the shallower depths occurred in late summer (these data may also suggest that greater seasonal changes occurred with greater soil depths). Although there was 35% greater root length per minirhizotron frame in elevated compared to ambient CO₂ plots, this difference was not significant ($P=0.31$; Table 1). There was, however, a highly significant CO₂ x depth interaction ($P<0.0001$; Fig. 4). Exposure to high CO₂ resulted in 41, 29, 94, and 133% greater root length per minirhizotron frame in the third, fourth, fifth, and sixth depth classes, respectively (Fig. 4).

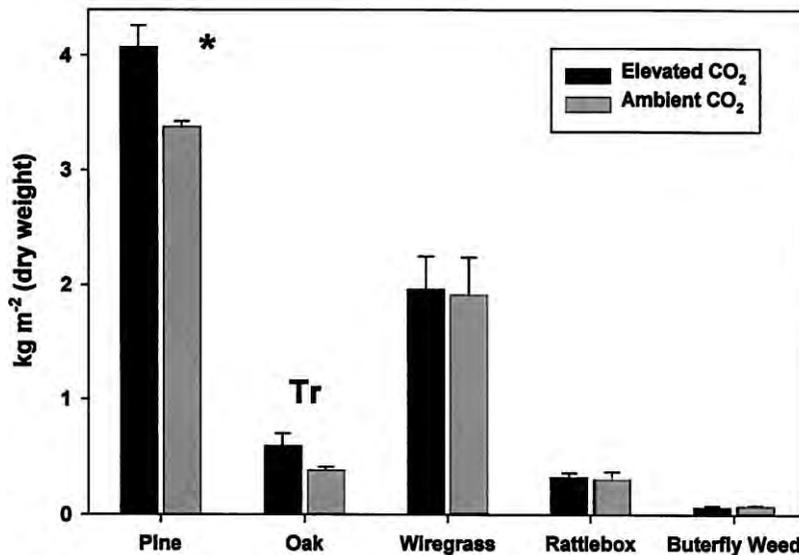


Fig. 2. Aboveground biomass estimates (\pm S.E.) (Spring, 1999) for the five species of the model regenerating longleaf pine ecosystem. Biomass was estimated from allometric equations relating height and stem dimensions (longleaf pine, oak, butterfly weed, and rattlebox), and tiller number (wiregrass) to total plant biomass. * $P < 0.05$; Tr, $0.05 < P < 0.15$.

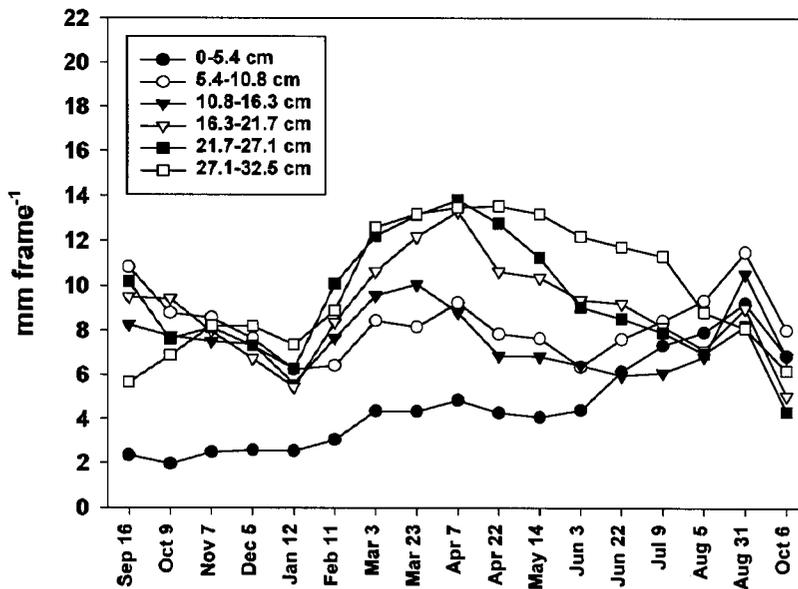


Fig. 3. Distribution of standing root length per minirhizotron frame at six depths from September 16, 1998, to October 6, 1999. Data are averaged over experimental and control plots.

Although the sum total of all root diameters per frame was 26% greater in the elevated compared to ambient CO₂ plots, this difference was

not significant ($P = 0.24$; Table 1). However, a significant CO₂ \times depth interaction was observed for this variable ($P < 0.0001$; Fig. 5). Significantly

greater total root diameters were observed for the fifth (+18%), and sixth (+124%) depth classes in high, compared to the low, CO₂ plots.

For soil cores harvested in October 1998, 5 months after the experiment was initiated, there was a trend for a main effect of CO₂ (Table 2). There was 38% greater root biomass in the high compared to ambient CO₂ plots averaged across all species ($P=0.14$). Increases of 135 and 53% were observed for biomass of oak and pine roots, respectively, in elevated compared to ambient CO₂ (Fig. 6). There was virtually no difference in root biomass in the ‘other’ category (containing butterfly weed, wiregrass, and rattlebox roots). There was also a significant main effect of species ($P<0.0001$) on the percentage of total root biomass comprised by each species; pine roots comprised 63%, oak roots 6%, and other roots 31% of total root biomass (data not shown). No species \times CO₂ interactions were observed for the percentage of total roots per species.

For soil cores harvested 1 year later (October

1999), there was no main effect of CO₂ on root biomass ($P=0.68$), but there was an interaction of CO₂ with species ($P=0.0002$; Table 2). There was 62% greater pine root biomass in the elevated CO₂ plots relative to ambient plots ($P=0.002$; Fig. 6). Conversely, in the other category, there was significantly greater biomass (28%) in ambient compared to high CO₂ plots ($P=0.02$). There was no difference in root biomass of oaks between CO₂ treatments ($P=0.66$). Finally, there was a significant main effect of species for root biomass in soil cores harvested in October 1999; pine roots comprised 34%, oak roots 13%, and other roots 53% of the total belowground root biomass.

3.3. Root production

There was a trend for enhanced root length production per day resulting from exposure to elevated atmospheric CO₂ concentrations; new root length produced per day was 37% greater in

Table 1

Main effects for root growth variables collected with minirhizotrons from October 1998 to October 1999 and from soil cores collected in October 1998 and 1999

	Elevated CO ₂ ^a	Ambient CO ₂	Probability
<i>Standing root crop characteristics</i>			
Root length (mm/frame ^b)	9.34 ^c	6.91	0.3079
Number of live roots (#/frame)	1.71	1.28	0.3711
Total root diameters per frame ^d (mm)	0.19	0.15	0.2417
Average diameter per live root (mm)	0.36	0.34	0.6297
<i>Production</i>			
Root length production per sampling interval (mm/interval $x-1$ to x)	1.37	0.98	0.0697
Root length production per day (mm/day)	0.060	0.044	0.0889
<i>Mortality</i>			
Number of dead roots (#/frame)	0.78	0.50	0.1484
Total dead root diameters per frame (mm)	0.092	0.067	0.1361
Average diameter per dead root (mm)	0.28	0.29	0.6224
Root length mortality per sampling interval (mm/interval $x-1$ to x)	0.65	0.43	0.2998
Root length mortality per day (mm/day)	0.028	0.019	0.3342
Root length turnover (unitless index)	0.062	0.062	1.0000

^a Elevated CO₂ = 725 $\mu\text{mol mol}^{-1}$; ambient CO₂ = 365 $\mu\text{mol mol}^{-1}$.

^b Minirhizotron frames represent a one dimensional area of soil 13.5 \times 18 mm (2.34 cm²).

^c All values represent main CO₂ treatment effects averaged over 17 dates and 17 depths/frames (from 0 to 30 vertical cm) from six replicates (each of the 12 OTC contained two minirhizotron subsamples).

^d Total root diameters represents the sum of the diameters of all roots present per frame, and is included as an index of total rooting density. Average diameter per live root represents the mean size, or diameter, of roots present within a given frame.

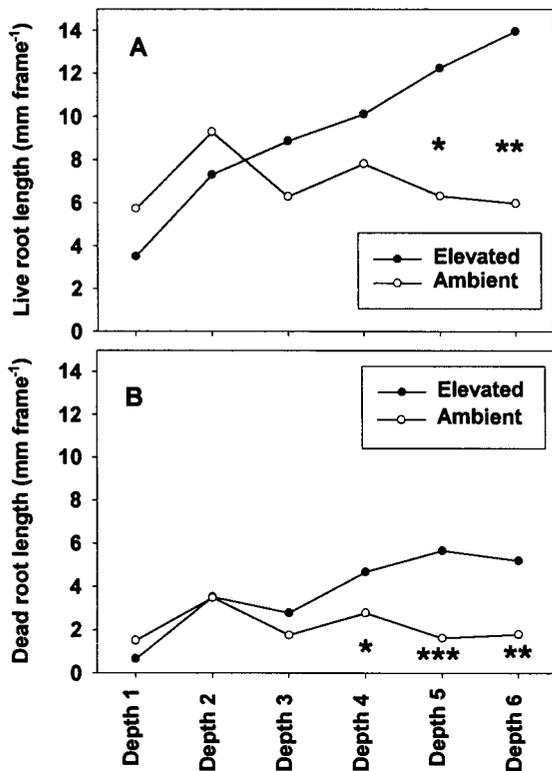


Fig. 4. Root variables from minirhizotron observation tubes. Values are averaged across dates and represent means from six replications with two minirhizotron tubes per replicate. Significant $\text{CO}_2 \times \text{depth}$ interactions were observed for both variables. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$.

the elevated plots compared to ambient plots ($P = 0.09$; Table 1). There were, however, significant $\text{CO}_2 \times \text{depth}$ ($P = 0.0003$) and $\text{CO}_2 \times \text{date}$ interactions ($P = 0.07$; Fig. 7). Root production per day was 62% greater in ambient plots for the shallowest depth interval. Conversely, root production was 27, 42, 30, 137, and 87% greater at the second, third, fourth, fifth, and sixth depths, respectively, in elevated compared to ambient CO_2 plots (Figs. 7 and 8). Consequently, cumulative root length production through the course of the experiment was greater in the elevated CO_2 plots at all depths except the shallowest (Fig. 8).

3.4. Root C and N content

There was no effect of CO_2 level on C content

of roots (Table 2). Carbon content between species, however, differed significantly ($P = 0.0009$). Oak roots contained 37%, pine roots 25% and other roots 34% C (data not shown). There was a trend for a significant main effect of CO_2 on root N content, ($P = 0.10$; Table 2). A significant CO_2 by species interaction revealed that N content was significantly reduced in oak grown in high CO_2 ($P = 0.01$), but was not changed in pine or other roots (data not shown). Similarly, there was a significant CO_2 by species interaction for C:N ratio of roots; this ratio was significantly higher in elevated compared to ambient CO_2 only for oak roots (Fig. 9).

3.5. Root mortality and turnover

Root length mortality per day was 47% higher in elevated than ambient CO_2 plots ($P = 0.33$; Table 1). There was also a significant $\text{CO}_2 \times \text{depth}$ interaction ($P < 0.0001$) and a trend for a significant $\text{CO}_2 \times \text{date} \times \text{depth}$ interaction ($P = 0.11$; Fig. 7). The interaction of CO_2 level with depth exhibited patterns consistent with other variables. Mortality per day was generally higher in ambient plots for the shallowest two depth classes, but was lower at greater soil depths (Fig. 7). This resulted in greater cumulative dead root lengths in elevated CO_2 plots at all soil depths except for depths one and two (Fig. 8). Relative root turnover was 0.062 for elevated plots and 0.062 for ambient plots ($P = 1.00$; Table 1). There were no interactions of CO_2 with depth or date for relative root turnover.

Total diameters of dead roots per minirhizotron frame were greater in elevated CO_2 plots (0.092 mm per frame) compared to ambient plots (0.067 mm per frame) ($P = 0.14$). A highly significant $\text{CO}_2 \times \text{depth}$ interaction for this variable showed patterns similar to other variables (Fig. 5). There were no consistent effects of CO_2 treatment on average root diameter per root (Table 1).

4. Discussion

In light of the significant enhancement in above- and belowground growth of longleaf pine

at elevated CO₂, this species appeared to have gained a competitive advantage over the other species 18 months after treatment initiation. Because longleaf has a large taproot and realized the greatest benefit from CO₂ enrichment, it is likely that the stimulation of root growth observed in minirhizotrons at greater depths resulted largely from enhanced longleaf pine root growth. Soil cores from the 16-month harvest indicated that longleaf pine root biomass was enhanced more by exposure to elevated CO₂ than the other species. In fact, elevated CO₂ had virtually no effect on oak roots; and the collective root biomass of

rattlebox, wiregrass, and butterfly weed was 28% greater in the *ambient* plots.

Longleaf pine's increase in whole plant growth, considered with its unchanged C:N ratio, suggests that enhanced root proliferation enabled it to sustain its greater growth by acquiring the extra N required to keep pace with enhanced carbon assimilation. In the oak species, although leaf level photosynthesis was stimulated (data not shown), the lack of root response was paralleled by a 53% increase in the root C:N ratio, suggesting that growth may have been constrained by N uptake. Soil core data showed that although root

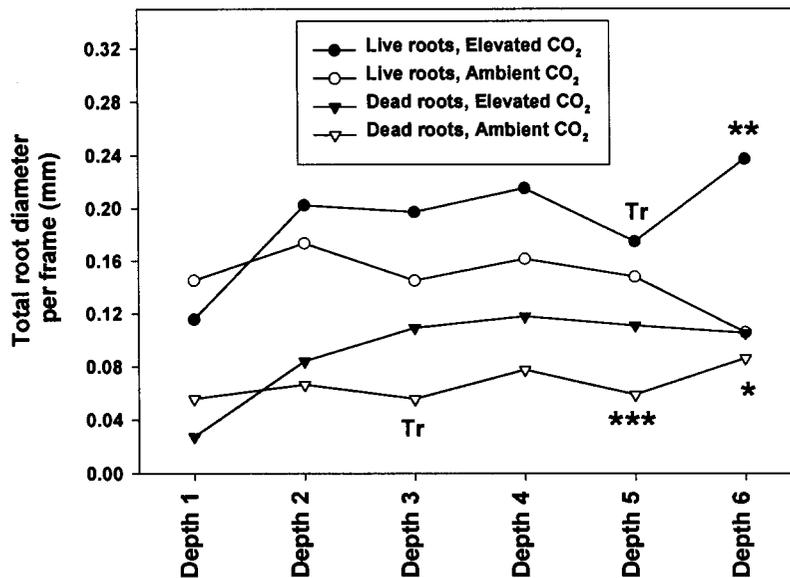


Fig. 5. Root variables from minirhizotron observation tubes. Values are averaged across dates and represent means from six replications with two minirhizotron tubes per replicate. Significant CO₂ by depth interactions were observed. Tr = 0.15 $\geq P \geq$ 0.05, * P 0.05, ** P 0.001, *** P 0.0001.

Table 2

Main effects for root growth variables collected with soil cores in October 1998 and 1999

Soil core data	Elevated CO ₂ ^a	Ambient CO ₂	Probability
Root biomass (g dw m ⁻³)—1998	29.4	21.3	0.1400
Root biomass (g dw m ⁻³)—1999	201.7	191.6	0.6761
C content (% dw)	33.0	31.0	0.4322
N content (% dw)	0.78	0.89	0.0994
C:N ratio	43.9	36.9	0.0189

^a Elevated CO₂ = 725 $\mu\text{mol mol}^{-1}$; ambient CO₂ = 365 $\mu\text{mol mol}^{-1}$.

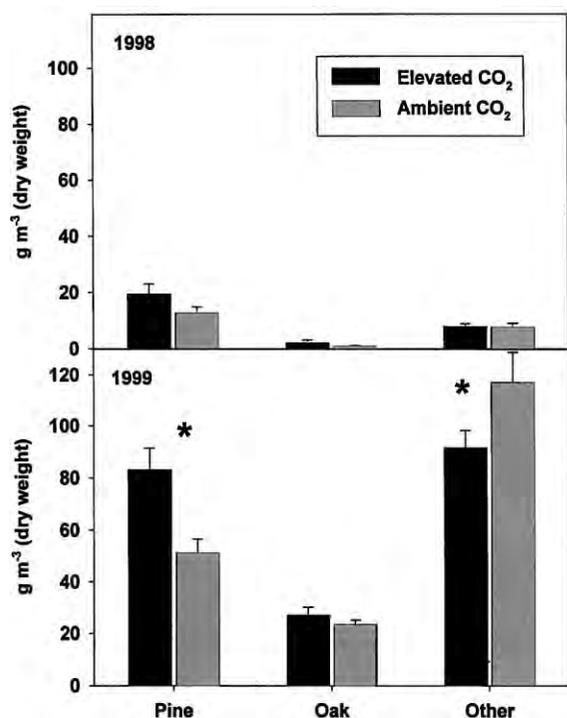


Fig. 6. Data from soil cores collected to a depth of 30 cm in November, 1998 and 1999. Values (\pm S.E.) are means of six replications (total of 12 OTCs) with eight cores collected per plot. * $P < 0.05$

biomass of oak was 135% greater in CO₂-enriched plots after 4 months, after 16 months there was no effect of CO₂ enrichment. These results underscore the importance of root growth patterns for N acquisition and, ultimately, whole plant competitive success.

The success of longleaf pine relative to oak forced us to reject our original hypothesis that the broadleaf tree would perform better under conditions of elevated atmospheric CO₂ conditions than the pine. Pines have historically been considered to exhibit very little anatomical, morphological and physiological plasticity (Esau, 1977). Such characterizations, considered with data from pines grown in pots, has prompted several authors to hypothesize that pine species will realize a smaller benefit from CO₂ fertilization than broadleaf species (but roots were not considered in these other studies) (Pritchard et al., 1997). In support of this hypothesis, Ceulemans and Mousseau (1994) re-

viewed the literature and found that increases in biomass and photosynthesis of conifers were 38 and 40% compared to increases of 63 and 61% in broadleaf species. In contrast to this earlier report Saxe et al. (1998) found a substantially larger average biomass increment of conifers (130%) compared to deciduous trees (49%) (stimulation of photosynthesis was similar for both groups). In order to resolve this apparent discrepancy, it may be necessary to group conifers that flush multiple times throughout the year (i.e. southern pines such as longleaf, loblolly, shortleaf and slash) separately from conifers adapted to colder climates that exhibit only one flush per season. In the case of southern pines such as longleaf, although leaf level stimulation of photosynthesis is often less than that observed for deciduous trees (M. Davis, unpublished data), C assimilation occurs in winter. So, while the enhanced C assimilation in oak was confined to a window of time between leaf flush in spring and senescence in the fall, longleaf pine fixed carbon throughout the year (M. Davis, unpublished data).

Results from this experiment suggest that root systems of wiregrass were larger in the ambient plots compared to elevated plots. At the second harvest date, there was 28% greater wiregrass + rattlebox + butterfly weed root biomass in ambient compared to CO₂-enriched plots. In general, rattlebox and butterfly weed root systems are dominated by a central taproot, having few lateral and fine roots. Wiregrass, however, has a fibrous root system with extensive horizontal growth confined mainly to the top 10 cm of soil (personal observation). It is likely, therefore, that wiregrass roots largely accounted for the increase in root biomass observed with cores, as well as the increase in root growth observed at shallow depths with minirhizotrons in the ambient compared to the CO₂-enriched plots. It is important to note however, that minirhizotrons often underestimate root growth at the most shallow soil layers and this may have affected our data. But regardless of methodological problems associated with minirhizotron studies, the fact that wiregrass has C₄ photosynthesis, a metabolic pathway repeatedly shown to be less responsive to changes in atmo-

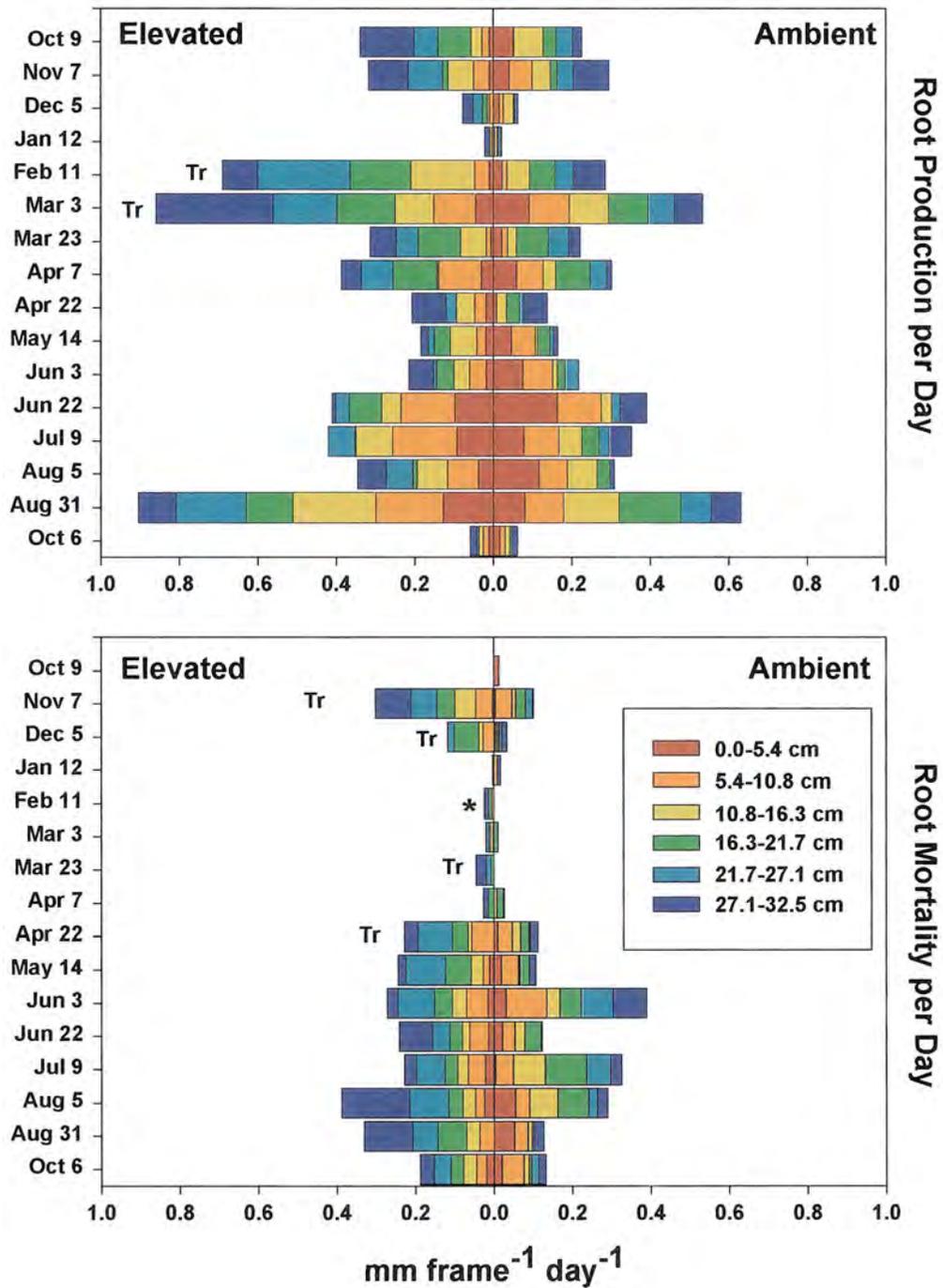


Fig. 7. Root production per day and mortality per day from minirhizotron tubes from October 9 1998 to October 6 1999. The interaction of CO₂ × date was significant ($P < 0.05$). Symbols indicated on the graphs represent significant differences between elevated and ambient CO₂ plots at given dates (values averaged for all depths). Tr = $0.15 \geq P \geq 0.05$, * $P < 0.05$.

spheric CO_2 , is consistent with the lack of stimulation we observed in high CO_2 . Other studies on grasses and grasslands have also reported a reduction in rooting at shallow depths in CO_2 -enriched atmospheres (Mo et al., 1992). Arnone et al. (2000) reviewed 12 studies in which grasslands were exposed to elevated CO_2 and found that five showed a marked increase in root growth, but that the other seven showed little or no change.

These results suggest, but by no means prove, that the ability of C_4 grasses to compete with more responsive C_3 plants may be compromised in a high CO_2 world both above- and belowground.

At the ecosystem level, exposure to elevated atmospheric CO_2 clearly contributed to a shift in vertical root distribution from shallow to deeper depths. Other studies have reported similar shifts (e.g. Day et al. (1996), Thomas et al. (1999)).

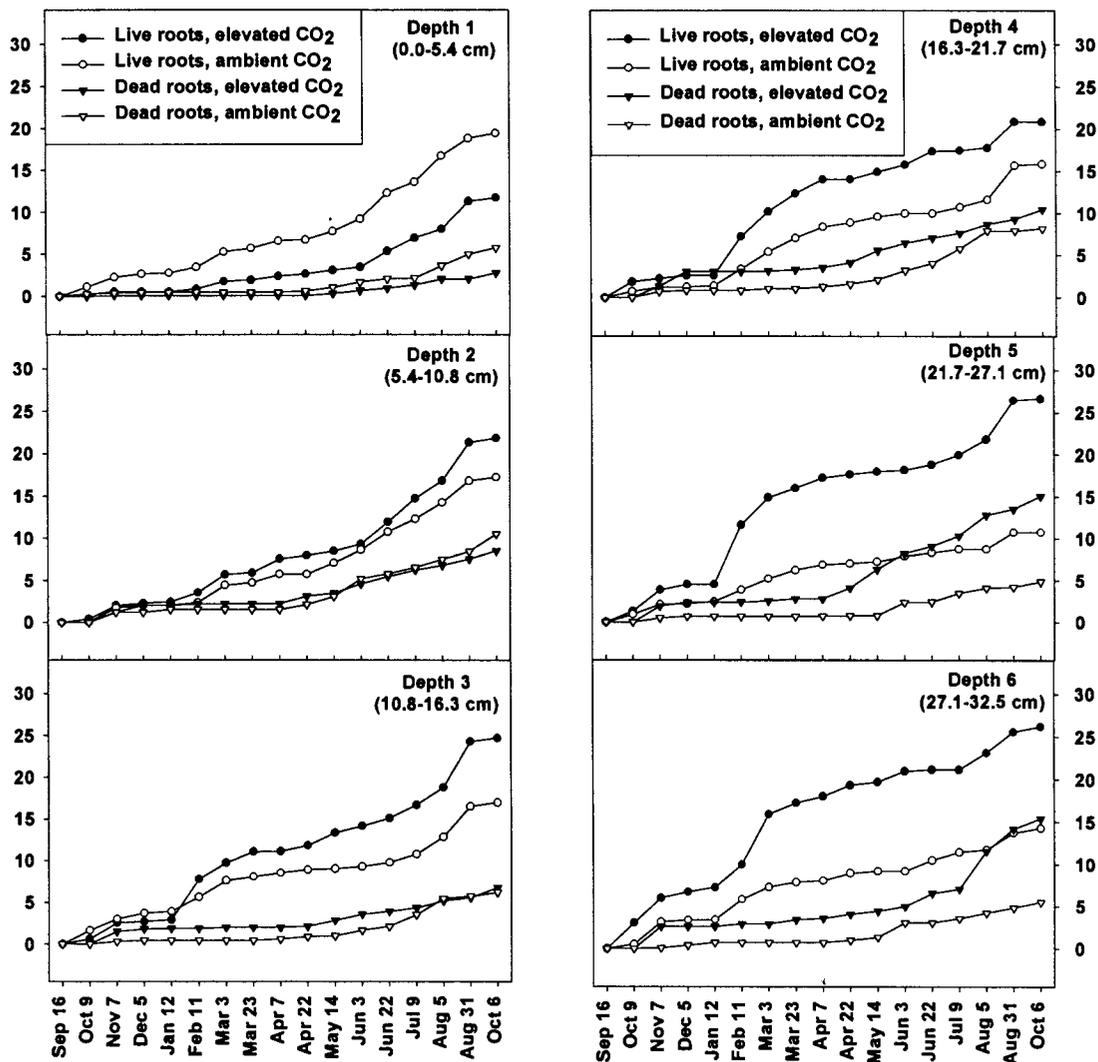


Fig. 8. Cumulative root length production and mortality (mm) per day at six depth classes from September 1998 to October 1999 determined from minirhizotrons.

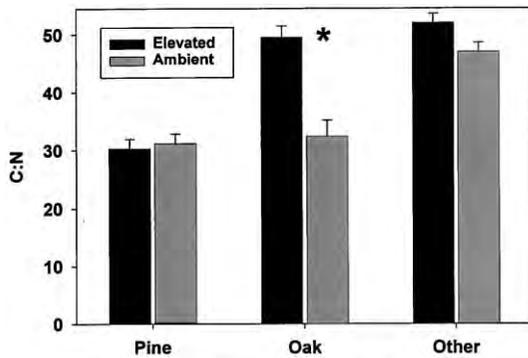


Fig. 9. Carbon to nitrogen ratio of root samples collected from soil cores at the November 1999 harvest date (\pm S.E). * P 0.05.

Also, a correlation often exists between spatial rooting patterns and soil environmental characteristics. For example, at the Duke forest free air CO₂ enrichment (FACE) site, where an intact loblolly pine forest is being studied, CO₂ enrichment resulted in an increase in root proliferation only at shallow soil depths. The authors attributed this shift to high mechanical resistance to root growth (soils are dense impervious clay-rich ultic Alfisols) in combination with accelerated nutrient cycling in the litter layer (Pritchard et al. unpublished data). In studies on pine seedlings growing in soils with lower mechanical resistance or impedance, i.e. sandy soils, CO₂ enrichment promoted greater root deployment only at greater soil depths (Thomas et al., 1999). Although soil physical properties surely play a role in shaping root architecture, shifts in spatial root deployment may also correlate with vertical distribution of available water or nutrients, or with effects of greater C availability on endogenous plant growth processes (Pritchard et al., 1999; Pritchard and Rogers, 2000).

Relative turnover was insensitive to CO₂ concentration. Canadell et al. (1996) summarized several studies in which the effects of elevated CO₂ on root turnover were measured; in four experiments on trees, increased turnover occurred in three and one showed a decrease. Tingey et al. (2000) reported that growth of conifers in elevated CO₂ resulted in enhanced absolute root turnover rates (mortality), but relative turnover rates (mor-

tality/biomass) ranged from an increase to a decrease. Currently, too few data are available to make any generalizations about how rising atmospheric CO₂ will change relative root turnover in forests (Norby and Jackson, 2000).

5. Conclusions

Our experiment and other recent studies suggest that production and mortality of fine roots in both idealized and natural CO₂-enriched forest ecosystems may significantly increase (Matamala and Schlesinger, 2000). Relative root turnover (turnover indices), driven by root longevity, did not change. CO₂ enrichment contributed to altered spatial and temporal root deployment within our idealized regenerating longleaf pine ecosystem. These changes in rooting patterns suggest shifts in belowground competition. Using root and shoot biomass as a metric, longleaf pine realized the greatest and most consistent benefit from exposure to elevated CO₂. This finding suggests that the ability of longleaf pine to compete with sand post oak, a common deciduous tree competitor, and wiregrass, the dominant understory herbaceous species, in regenerating ecosystems may be significantly enhanced by rising atmospheric CO₂ concentrations. Assuming that future fire regimes and precipitation patterns do not negate this response, such competitive shifts suggest that longleaf pine savannahs may flourish in a future CO₂-enriched world.

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