

# Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO<sub>2</sub> and water stress

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**Summary** Longleaf pine (*Pinus palustris* Mill.) seedlings were grown in 45-l pots and exposed to ambient or elevated (365 or 730  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) CO<sub>2</sub> concentration in open-top chambers for 20 months. Two water-stress treatments (target values of  $-0.5$  or  $-1.5$  MPa xylem pressure potential) were imposed 19 weeks after initiation of the study. At harvest, tissues (needles, stems, taproots, coarse roots, and fine roots) were analyzed for carbon (C), nitrogen (N), nonpolar extractives (fats, waxes, and oils), nonstructural carbohydrates (sugars and starch), structural components (cellulose and lignin), and tannins. The greatest dry weights and lowest N concentrations occurred in tissues of plants grown at elevated CO<sub>2</sub> or with adequate water.

Although allocation of C fractions among tissues was generally unaffected by treatments, concentrations of the analyzed compounds were influenced by treatments in needles and taproots, but not in stems and lateral roots. Needles and taproots of plants exposed to elevated CO<sub>2</sub> had increased concentrations of nonstructural carbohydrates. Among plant tissues, elevated CO<sub>2</sub> caused reductions in structural C concentrations and foliar concentrations of fats, waxes and oils.

**Keywords:** biomass, carbohydrate, carbon dioxide, longleaf pine.

## Introduction

The CO<sub>2</sub> concentration of Earth's atmosphere is increasing at a geologically unprecedented rate (Harrington 1987, Keeling et al. 1989, Post et al. 1990). It has been proposed that increases in CO<sub>2</sub> concentration and other trace gases will lead to increased temperatures and altered rainfall patterns, with increased drought likely in areas such as the southern United States (Perry et al. 1990, Ojima et al. 1991). Climatic changes will be particularly important to forest species, because of their long-lived nature and because forests often occupy marginal sites in terms of fertility and water (Perry et al. 1990).

Much is known about plant responses to both water stress and high CO<sub>2</sub>. Positive effects of elevated atmospheric CO<sub>2</sub> on plants, including increased growth and yield (Wittwer 1990,

Rogers and Dahlman 1993), photosynthetic rates (Radin et al. 1987, Long and Drake 1992), and water use efficiency (Rogers et al. 1983b, Sionit et al. 1984, Morison 1985) have been demonstrated. However, much less is known about plant responses to the combined effects of elevated CO<sub>2</sub> and drought. Evidence suggests that increasing atmospheric CO<sub>2</sub> may improve carbon assimilation and compensate for some of the negative effects of water stress (Rogers et al. 1983b, Tolley and Strain 1984, Samuelson and Seiler 1993, Tschaplinski et al. 1993, Liang and Maruyama 1995). Tschaplinski et al. (1993) found that elevated CO<sub>2</sub> increased the concentration of soluble sugars in roots of well-watered loblolly pine (*Pinus taeda* L.) seedlings, but water stress reduced the concentration of sugars in roots. This increased supply of photosynthate to roots could alter root tissue chemistry.

Many of the responses of terrestrial ecosystems to increasing atmospheric CO<sub>2</sub> concentration are likely to be indirect and manifested as changes in secondary reactions such as the production of secondary defense compounds that influence plant-herbivore and plant-microbe interactions (Kinney et al. 1997, Bezemer and Jones 1998). The CO<sub>2</sub>-induced changes in the quality of plant tissues influence insect herbivory (Lincoln and Couvet 1989, Fajer et al. 1992, Kinney et al. 1997) and the incidence and severity of plant diseases (Thompson et al. 1993, Thompson and Drake 1994). These changes also have the potential to alter decomposition processes (Melillo 1983, Coûteaux et al. 1991, Cotrufo and Ineson 1996, O'Neill and Norby 1996), which could impact C turnover and storage in soils (Prior et al. 1997c, Torbert et al. 1997). Despite the implications of such changes, little is known about effects of atmospheric CO<sub>2</sub> on C chemistry, particularly plant secondary metabolites, such as tannins.

Interacting effects of elevated CO<sub>2</sub> and water stress may be important for southern forest species, especially longleaf pine (*Pinus palustris* Mill.) trees that currently occupy the more xeric sites in the region. The objective of this study was to examine the effects of elevated atmospheric CO<sub>2</sub> combined with water stress on tissue chemistry of longleaf pine.

## Materials and methods

### *Plant growth and exposure system*

Longleaf pine seedlings, from a wild seed source, were lifted from a Florida nursery in February 1993. Seedlings were stored (2 °C) for less than one week, graded (mean root collar diameter  $\pm$  SD =  $13 \pm 2$  mm), and planted (three per pot) in 45-l plastic pots containing a coarse sandy medium (pH 5.1) that was low in mineral elements (P, K, Mg, and Ca = 0.9, 5.6, 6.9, and 26.6 mg kg<sup>-1</sup>, respectively).

Seedlings were exposed to ambient ( $\approx 365$   $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) or elevated ( $\approx 730$   $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ) CO<sub>2</sub> in open-top chambers similar to those described by Rogers et al. (1983a). The chambers, CO<sub>2</sub> supply, and CO<sub>2</sub> monitoring systems have been described for this study site (Mitchell et al. 1995). Carbon dioxide exposures were initiated on March 30, 1993. Mean daytime CO<sub>2</sub> concentrations ( $\pm$  standard deviation) during the experiment were  $372.3 \pm 19.0$   $\mu\text{mol}^{-1}$  in the ambient chambers and  $739.5 \pm 39.7$   $\mu\text{mol}^{-1}$  in the elevated CO<sub>2</sub> chambers.

Two water stress treatments (target values:  $-0.5$  or  $-1.5$  MPa xylem pressure potential) were imposed 19 weeks after initiation of the study. Water stress was facilitated by installing Teflon (5-mil FEP) rain covers over the top of each chamber. At initiation of the water stress treatments, all pots were flooded with water, allowed to drain overnight, and weighed; these weights were taken as field capacity values. New field capacity values were taken at the end of the first growing season (November 1993) and midway through the second growing season (July 1994), to compensate for changes in plant mass. Xylem pressure potential was measured periodically with a pressure chamber (Scholander et al. 1965), and values were correlated with gravimetric measurements collected with a pneumatic weighing device. Pots were watered to field capacity when gravimetric measurements indicated the appropriate water stress had been achieved. Xylem pressure potentials were measured periodically, to check the gravimetric readings. Immediately before watering, mean xylem pressure potentials were  $-0.6$  and  $-1.3$  MPa for seedlings in the well-watered and water-stress treatments, respectively. Deionized water was used throughout the study. Pots in the well-watered treatment received water every 3–4 days, and pots in the water-stress treatment received water every 12–20 days.

All nutrients were maintained at non-limiting concentrations in all pots by application of sulfur coated urea (400 kg N ha<sup>-1</sup> year<sup>-1</sup>) at three-month intervals. Sulfur coated potassium (80 kg K ha<sup>-1</sup> year<sup>-1</sup>) and MicroMax<sup>TM</sup> Plus (Scotts Co., Marysville, OH) (P = 280, Ca = 1140, Mg = 560, and S = 100 kg ha<sup>-1</sup> year<sup>-1</sup>, plus a complete complement of micronutrients) were mixed with sand at the time the pots were filled, with a second application at the end of the first year. Iron chelate (0.007 mg Fe g<sup>-1</sup> soil) was applied once in April 1993.

Treatments were arranged in a split-plot design with five replications. The CO<sub>2</sub> treatments (main plots) were randomly assigned to chambers. Water treatments (subplots) were randomly assigned to eight pots per chamber. To avoid within-chamber location effects, pot locations were re-randomized monthly.

### *Plant harvests*

Plants from one pot were destructively harvested from each treatment in each chamber in November 1994, corresponding to 20 months of CO<sub>2</sub> exposure. Plants were separated into tissue component parts (needles, stems, taproots, lateral roots, and fine roots), and oven dried to constant weight at 55 °C. Treatment effects on biomass production and morphological variables have been reported previously (Prior et al. 1997b).

### *Tissue analysis*

A 0.2-g subsample from each plant tissue was analyzed for C fractions. Soluble fats, waxes, and oils (FWO) were removed with a series of dichloromethane washes (TAPPI 1975). Non-structural carbohydrates (sugars and starches = NSC) were extracted and measured as described by Hanson and Moller (1975). Cellulose and lignin were determined gravimetrically (Effland 1977). Hot-water-extractable tannins were extracted and measured as described by Allen et al. (1974). Proximate C fractions were corrected for ash and presented as ash-free dry weight (Ryan et al. 1990). Total C (FWO + NSC + cellulose + lignin + tannins) and structural C (SC = cellulose + lignin + tannins or SC = TC – NSC) were calculated. Tissue N content was determined with a LECO CHN-600 analyzer (LECO Corp., St. Joseph, MI).

### *Data analysis*

Data were tested for homogeneity of variance and normality; transformations were not necessary. Data were analyzed by the general linear models procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC). In all cases, differences were considered significant at  $P \leq 0.05$ .

## Results

### *Concentration*

Biomass of all tissues (needles, stems, taproots, lateral roots, and fine roots) was greater for plants grown at elevated CO<sub>2</sub> than for plants grown in ambient CO<sub>2</sub> and greater for well-watered plants than for plants subjected to water stress (Table 1). Concentrations of fats, waxes, and oils (FWO) in needles were lower in plants exposed to elevated CO<sub>2</sub> than in plants exposed to ambient CO<sub>2</sub> and greater in well-watered plants than in plants subjected to water stress, whereas the concentration of sugars in needles followed the reverse pattern (Table 1). The concentration of sugars in stems was significantly lower in well-watered plants than in plants subjected to water stress. Starch concentrations were higher in needles and taproots but lower in fine roots of plants grown at elevated CO<sub>2</sub> compared to plants grown at ambient CO<sub>2</sub>. Starches were significantly higher in taproots and fine roots of well-watered plants than of plants subjected to water stress. No significant differences in cellulose concentrations were observed for either treatment. Lignin concentrations were lower in taproots of plants grown at elevated CO<sub>2</sub> than in plants grown at ambient CO<sub>2</sub> and lower in well-watered plants than in plants subjected to water stress. Lignin concentrations in stems were also significantly lower in

Table 1. Concentration of C fractions in *Pinus palustris* tissues after 20 months of growth at ambient and elevated CO<sub>2</sub> under well-watered or drought conditions in open-top chambers.

Tissue	[CO <sub>2</sub> ] (μmol mol <sup>-1</sup> )	Water stress (MPa)	Biomass (g)	Carbon fractions (mg fraction g <sup>-1</sup> tissue)					
				FWO <sup>1</sup>	Sugar	Starch	Cellulose	Lignin	Tannin
Needles	365	-0.5	153.6 a <sup>2</sup>	236.1 a	6.2 c	243.5 b	371.0 a	131.2 b	11.9 b
	720	-0.5	181.5 a	177.2 b	6.8 ab	283.2 a	368.8 a	149.2 ab	14.7 ab
	365	-1.5	57.1 c	175.7 b	6.6 bc	264.4 ab	383.6 a	155.6 a	14.1 ab
	720	-1.5	99.9 b	145.2 c	7.2 ab	281.0 a	394.0 a	155.0 a	17.7 a
Stems	365	-0.5	60.4 b	229.3 a	6.3 ab	207.4 ab	353.5 a	182.6 a	20.8 ab
	720	-0.5	97.7 a	270.1 a	6.8 a	213.0 ab	297.1 a	184.5 a	29.2 a
	365	-1.5	19.0 c	233.7 b	6.4 ab	234.6 a	327.1 a	166.8 ab	31.4 a
	720	-1.5	36.9 b	347.3 a	5.5 b	173.8 b	313.6 a	141.4 b	18.5 b
Taproots	365	-0.5	44.7 b	252.5 a	5.7 a	206.8 c	307.5 a	199.3 a	28.2 b
	720	-0.5	74.1 a	381.5 a	5.2 a	236.6 b	211.5 ab	131.6 bc	33.6 ab
	365	-1.5	17.2 c	278.9 a	5.8 a	241.1 b	276.7 ab	162.5 ab	34.7 ab
	720	-1.5	37.1 b	249.4 a	6.0 a	405.1 a	195.4 b	94.1 b	49.9 a
Lateral roots	365	-0.5	87.8 ab	346.4 a	5.3 a	204.9 a	254.4 a	172.9 a	16.1 a
	720	-0.5	140.9 a	392.7 a	5.0 a	214.8 a	222.3 a	144.6 a	20.6 a
	365	-1.5	25.6 c	315.4 a	5.5 a	250.2 a	240.3 a	167.2 a	21.4 a
	720	-1.5	81.5 b	387.0 a	5.2 a	220.9 a	231.0 a	140.8 a	15.1 a
Fine Roots	365	-0.5	36.2 b	388.3 a	5.9 a	135.3 b	274.9 a	186.2 b	9.4 a
	720	-0.5	46.0 a	371.9 a	8.2 a	93.7 c	246.0 a	267.6 a	12.6 ab
	365	-1.5	14.0 c	197.6 a	7.5 a	191.8 a	334.9 a	252.8 a	15.3 a
	720	-1.5	34.3 b	339.6 a	7.9 a	134.7 b	269.2 a	232.7 a	16.1 a

<sup>1</sup> FWO = fats, waxes and oils.

<sup>2</sup> For each tissue, in each column within each tissue type, values followed by the same letter are not significantly different as determined by the Least Square Means Test ( $P \leq 0.05$ ),  $n = 5$ .

well-watered seedlings than in plants subjected to water stress. Tannin concentrations in fine roots were significantly higher in well-watered plants than in plants subjected to water stress. Tannin concentrations in stems were lower for well-watered plants grown at elevated CO<sub>2</sub> than for well-watered plants grown at ambient CO<sub>2</sub>.

#### Allocation

There were few treatment effects on allocation of biomass or partitioning of N among tissues in longleaf pine seedlings (data not shown). There were no significant effects of water supply on C partitioning, although partitioning of FWO to stems tended to be higher and partitioning of sugars to fine roots tended to be lower in well-watered plants than in plants subjected to water stress. Partitioning of starch to fine roots was significantly lower in plants grown at elevated CO<sub>2</sub> than in plants grown at ambient CO<sub>2</sub> (data not shown). Partitioning of cellulose and tannins showed no response to CO<sub>2</sub>; however, partitioning of lignin to taproots was lower and partitioning to fine roots tended to be higher in plants grown at elevated CO<sub>2</sub> compared with plants grown at ambient CO<sub>2</sub> (data not shown).

#### Ratios among C fractions in tissues

The ratio of needle fats, waxes and oils to structural carbohydrates (FWO:SC) was significantly lower, whereas the cellulose:structural carbohydrates (Cel:SC) ratio was higher in

well-watered plants than in plants subjected to water stress (Table 2). The proportion of SC comprising lignin (Lig:SC) was significantly lower in taproots and lateral roots of plants grown at elevated CO<sub>2</sub> compared with plants grown at ambient CO<sub>2</sub>, but the proportion comprising tannins (Tan:SC) in needles and taproots showed the opposite responses to CO<sub>2</sub> concentration. Stem Lig:SC was significantly lower in well-watered plants than in plants subjected to water stress. The Tan:SC ratio in stems was lower in well-watered plants grown at elevated CO<sub>2</sub> than in well-watered plants grown at ambient CO<sub>2</sub>. The elevated CO<sub>2</sub> treatment increased the proportion of nonstructural carbohydrates to SC (NSC:SC) in needles and taproots, but decreased the NSC:SC ratio in fine roots, whereas the opposite responses to elevated CO<sub>2</sub> were observed for the relative proportion of SC to total C (SC:TC) in these tissues. Taproot and fine root NSC:SC were higher for well-watered seedlings than for seedlings subjected to water stress, whereas taproot and fine root SC:TC were lower for well-watered seedlings than for seedlings subjected to water stress.

#### Carbon/nitrogen ratios

Nitrogen concentration was significantly lower in all tissues in well-watered plants than in plants subjected to water stress and for most tissues in the elevated CO<sub>2</sub> treatment (Table 3). In general, the greatest biomass and the lowest N concentrations occurred in well-watered plants exposed to elevated CO<sub>2</sub>; however, these interactions were not statistically significant. In

Table 2. Ratios between C fractions in *Pinus palustris* tissues after 20 months of growth at ambient and elevated CO<sub>2</sub> under well-watered or drought conditions in open-top chambers.

Tissue	[CO <sub>2</sub> ] ( $\mu\text{mol mol}^{-1}$ )	Water stress (MPa)	Cel/SC <sup>1</sup>	Lig/SC	Tan/SC	NSC/SC	SC/TC	FWO/SC
Needles	365	-0.5	49.4 b <sup>2</sup>	17.5 b	1.6 b	0.33 b	75.0 a	31.4 a
	720	-0.5	51.9 ab	21.0 a	2.1 ab	0.41 a	71.0 b	25.0 ab
	365	-1.5	52.7 ab	21.4 a	1.9 b	0.37 ab	72.9 ab	24.0 ab
	720	-1.5	55.4 a	21.8a	2.5 a	0.41 a	71.2 b	20.3 b
Stems	365	-0.5	45.1 a	23.3 a	2.7 ab	0.27 a	78.6 a	28.9 a
	720	-0.5	38.1 a	23.6 a	3.7 ab	0.28 a	78.1 a	34.5 a
	365	-1.5	43.1 a	22.0 ab	4.2 a	0.32 a	75.9 a	30.8 a
	720	-1.5	38.3 a	17.4 b	2.3 b	0.22 a	82.1 a	42.1 a
Taproots	365	-0.5	38.9 a	25.3 a	3.6 b	0.27 b	78.8 a	32.1 a
	720	-0.5	28.4 a	16.9 b	4.8 ab	0.34 b	75.8 a	50.0 a
	365	-1.5	36.7 a	21.5 ab	4.6 ab	0.33 b	75.3 a	37.1 a
	720	-1.5	32.9 a	16.1 b	8.6 a	0.71 a	58.9 b	42.4 a
Lateral roots	365	-0.5	32.5 a	22.1 a	2.1a	0.27 a	79.0 a	43.3 a
	720	-0.5	28.5 a	18.4 a	2.7 a	0.29 a	78.0 a	50.4 a
	365	-1.5	32.5 a	22.6 a	2.9 a	0.35 a	74.4 a	41.9 a
	720	-1.5	29.6 a	17.9 a	2.0 a	0.30 a	77.4 a	50.5 a
Fine roots	365	-0.5	32.2 ab	21.8 a	1.1 b	0.17 b	85.9 ab	44.8 a
	720	-0.5	27.5 b	29.9 a	1.4 ab	0.11 c	89.8 a	41.3 a
	365	-1.5	41.8 a	31.6 a	1.9 a	0.25 a	80.1 c	24.7 a
	720	-1.5	31.5 ab	27.2 a	1.9 a	0.17 b	85.7 b	39.4 a

<sup>1</sup> Cel = cellulose, Lig = lignin, Tan = tannin, SC = structural carbohydrates (cellulose, lignin and tannins), TC = total carbon, NSC = nonstructural carbohydrates (sugars and starches).

<sup>2</sup> For each tissue, in each column within each tissue type, values followed by the same letter are not significantly different as determined by the Least Square Means Test ( $P \leq 0.05$ ),  $n = 5$ .

general, elevated CO<sub>2</sub> increased C:N ratios in well-watered plants, although there were a few cases where the ratio tended to be increased only in plants grown at elevated CO<sub>2</sub> under water-stressed conditions.

## Discussion

It has been hypothesized that increasing atmospheric CO<sub>2</sub> concentration will increase aridity over large areas of the world (Harrington 1987, Post et al. 1990, Chaves and Pereira 1992). An increase in biomass in response to elevated CO<sub>2</sub> in well-watered longleaf pine plants, with no interactions between CO<sub>2</sub> and water supply, is similar to results from other studies examining effects of water stress and elevated CO<sub>2</sub> on tree seedlings (Samuelson and Seiler 1993, Tschaplinski et al. 1993). Elevated CO<sub>2</sub> tended to decrease tissue N concentration, whereas water stress increased tissue N concentration, a finding similar to that reported for loblolly pine (Tschaplinski et al. 1993). Seedlings grown in elevated CO<sub>2</sub> and subjected to water stress partitioned more biomass below ground or into fine roots than well-watered seedlings grown in ambient CO<sub>2</sub>, but there were few significant changes in allocation of C compounds in response to either the CO<sub>2</sub> or water treatment, suggesting that functional relationships and balances among plant organs were not greatly altered by the treatments.

Few studies have examined CO<sub>2</sub>-induced changes in plant

chemistry, and these have mostly been restricted to foliar responses (Lindroth et al. 1993, Lindroth 1996, O'Neill and Norby 1996, Pritchard et al. 1997, Williams et al. 1997) and have shown high variability among plant species (Peñuelas et al. 1996, Poorter et al. 1997). Plants subjected to water stress had increased concentrations of needle FWO. A high wax content may be particularly important for reducing water loss in longleaf pine because the species currently occupies xeric sites. Prior et al. (1997a) also reported that drought increased epicuticular wax content of longleaf pine seedlings regardless of CO<sub>2</sub> concentration; however, they noted that epicuticular wax content of needles was lower for longleaf pine grown at elevated CO<sub>2</sub> when soil N was low. Although effects of soil N were not tested in our study, Entry et al. (1998) found that elevated CO<sub>2</sub> combined with high N fertility resulted in increased concentrations of starch, cellulose and tannins and decreased concentrations of lignin and FWO in roots.

Longleaf pine plants grown at elevated CO<sub>2</sub> had increased foliar nonstructural carbohydrate concentrations. Similar results have been obtained in other studies (e.g., Yelle et al. 1989, Tissue et al. 1995; Pritchard et al. 1997, Entry et al. 1998). Pritchard et al. (1997) reported increased tannin and total polyphenol contents in pine seedlings in response to elevated CO<sub>2</sub> and suggested that production of shikimate-derived compounds was driven by C availability. This suggestion supports the carbon nutrient balance hypothesis (Bryant et al. 1983) that

Table 3. Ratios between N and C fractions in *Pinus palustris* tissues after 20 months of growth at ambient and elevated CO<sub>2</sub> under well-watered or drought conditions.

Tissue	[CO <sub>2</sub> ] (μmol mol <sup>-1</sup> )	Water stress (MPa)	Nitrogen (mg N g <sup>-1</sup> )	FWO/N	Cel/N	Lig/N	Tan/N	SC/N	TC/N
Needles	365	-0.5	19.6 a	12.0 a	19.0 c	6.7 c	0.6 c	38.5 c	51.2 c
	720	-0.5	16.1 b	11.0 a	23.3 b	9.4 b	0.9 b	44.6 bc	62.8 b
	365	-1.5	14.5 b	12.1 a	26.5 b	10.8 b	1.0 b	50.4 b	69.1 b
	720	-1.5	10.9 c	12.4 a	36.2 a	14.3 a	1.6 a	65.5 a	92.0 a
Stems	365	-0.5	17.1 a	13.8 b	20.7 b	10.7 c	1.2 b	46.4 b	58.9 b
	720	-0.5	13.4 b	20.1 ab	22.2 b	13.8 b	2.2 ab	58.3 b	74.7 b
	365	-1.5	13.5 b	17.4 b	24.7 b	12.6 bc	2.4 a	57.1 b	75.3 b
	720	-1.5	8.7 c	42.9 a	38.0 a	16.4 b	2.1 ab	99.4 a	120.0 a
Taproots	365	-0.5	12.5 a	21.3 b	24.5 a	16.2 a	2.4 b	64.5 b	82.2 c
	720	-0.5	10.0 ab	38.8 a	22.1 a	13.7 a	3.3 b	78.0 ab	102.0 bc
	365	-1.5	8.8 b	31.7 ab	31.9 a	18.4 a	4.1 b	86.1 ab	115.0 ba
	720	-1.5	6.0 c	42.3 a	33.2 a	16.5 a	8.4 a	100.4 a	170.5 a
Lateral roots	365	-0.5	18.0 a	21.3 b	14.7 c	10.1 b	0.9 b	47.0 c	58.7 c
	720	-0.5	12.8 b	31.0 b	17.4 c	11.1 b	1.6 b	61.2 bc	78.6 bc
	365	-1.5	9.4 bc	33.1b	25.6 b	17.9 a	2.3 a	78.9 b	106.3 b
	720	-1.5	6.3 c	62.6 a	37.1 a	22.3 a	2.4 a	124.4 a	161.1 a
Fine roots	365	-0.5	18.4 a	20.9 b	15.2 c	10.3 b	0.5 b	46.8 d	54.6 c
	720	-0.5	11.5 b	31.8 a	21.8 bc	23.7 a	1.1 bc	78.5 b	87.5 b
	365	-1.5	13.4 b	15.5 b	25.2 ab	18.9 ab	1.2 a	60.7 c	75.8 b
	720	-1.5	7.9 c	42.3 a	34.7 a	29.9 a	2.1 b	109.0 a	127.3 a

<sup>1</sup> Abbreviations: Cel = cellulose, Lig = lignin, Tan = tannin, SC = structural carbohydrates (cellulose, lignin and tannins), TC = total carbon, and NSC = nonstructural carbohydrates (sugars and starches).

<sup>2</sup> For each tissue, in each column within each tissue type, values followed by the same letter are not significantly different as determined by the Least Square Means Test ( $P \leq 0.05$ ),  $n = 5$ .

states that, when carbohydrate production exceeds growth demands, the overflow is allocated to production of secondary C compounds. Because carbohydrate production should exceed growth demand to a greater extent when elevated CO<sub>2</sub> is combined with limiting soil resource availability, one might expect a significant interaction between CO<sub>2</sub> and water; however, this was not observed in our study. Kinney et al. (1997) suggest that foliar accumulations of “dynamic metabolites” do not follow the predictions of the carbon nutrient balance theory as well as do those of stable end products. It appears that plant parameters that are directly linked to CO<sub>2</sub> concentration (i.e., photosynthesis, growth, and foliar nonstructural carbohydrate content) respond more strongly and predictably to elevated CO<sub>2</sub> than do concentration of compounds that are indirectly affected.

Elevated CO<sub>2</sub> and water stress altered the C chemistry of the root systems of longleaf pine plants. Taproots of plants exposed to elevated CO<sub>2</sub> had lower concentrations of structural C components (cellulose and lignin), a lower ratio of lignin to other C compounds and higher nonstructural C (starch). The decrease in fine root starch concentration may have been a result of more rapid turnover of starch to sugars and utilization of these sugars to support growth (Runion et al. 1997). Because changes in plant tissue chemistry will affect decomposition rates (Meentemeyer 1978, Ryan et al. 1990, Cotrufo and Ineson 1996, O'Neill and Norby 1996), elevated CO<sub>2</sub> could

impact C turnover and storage in soils (Prior et al. 1997c, Torbert et al. 1997). Pregitzer et al. (1995) suggested that increased rates of root turnover may occur in response to elevated CO<sub>2</sub>, and if coupled with increased C:N, could lead to increased belowground C storage.

Changes in tissue chemistry may also affect plant susceptibility to insect or pathogen attack, or both (Lincoln and Couvet 1989, Waterman and Mole 1989, Entry et al. 1991, 1992, Fajer et al. 1992, Kinney et al. 1997). Changes in C chemistry in response to elevated CO<sub>2</sub> could alter nutrient leaching and turnover, tissue decomposition, as well as host-herbivore and host-pathogen interactions (O'Neill and Norby 1996, Prior et al. 1997a, Pritchard et al. 1997, Bezemer and Jones 1998). Our findings that elevated atmospheric CO<sub>2</sub> concentration and plant water status influenced longleaf pine tissue chemistry indicate potential alterations in susceptibility to insect and disease attack and rates of decomposition.

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