970

MASTER COPY

Plant and Soil **200**: 3–11, 1998.
© 1998 *Kluwer Academic Publishers. Printed in the Netherlands.*

Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings

James A. Entry[1], G. Brett Runion[2], Stephen A. Prior[2], Robert J. Mitchell[3] and Hugo H. Rogers[2]
[1]*USDA Agricultural Research Service, Northwest Irrigation and Soils Research Laboratory, 3793 North 3600 West, Kimberly, Idaho 83341*;* [2]*USDA-Agricultural Research Service, National Soil Dynamics Laboratory, Auburn, Alabama 36831, and* [3]*Joseph W. Jones Ecological Research Center, Newton, Georgia 31770, USA*

Received 5 March 1997. Accepted 2 December 1997

Key words: biomass, carbon concentrations, carbon fractions, *Pinus palustris*

Abstract

One-year old, nursery-grown longleaf pine (*Pinus palustris* Mill.) seedlings were grown in 45-L pots containing a coarse sandy medium and were exposed to two concentrations of atmospheric CO_2 (365 or 720 μmol^{-1}) and two levels of nitrogen (N) fertility (40 or 400 kg N ha^{-1} yr^{-1}) within open top chambers for 20 months. At harvest, needles, stems, coarse roots, and fine roots were separated and weighed. Subsamples of each tissue were frozen in liquid N, lyophilized at −50 °C, and ground to pass a 0.2 mm sieve. Tissue samples were analyzed for carbon (C), N, nonpolar extractives (fats, waxes, and oils = FWO), nonstructural carbohydrates (total sugars and starch), and structural carbohydrates (cellulose, lignin, and tannins). Increased dry weights of each tissue were observed under elevated CO_2 and with high N; however, main effects of CO_2 were significant only on belowground tissues. The high N fertility tended to result in increased partitioning of biomass aboveground, resulting in significantly lower root to shoot ratios. Elevated CO_2 did not affect biomass allocation among tissues. Both atmospheric CO_2 and N fertility tended to affect concentration of C compounds in belowground, more than aboveground, tissues. Elevated CO_2 resulted in lower concentrations of starch, cellulose, and lignin, but increased concentrations of FWO in root tissues. High N fertility increased the concentration of starch, cellulose, and tannins, but resulted in lower concentrations of lignin and FWO in roots. Differences between CO_2 concentrations tended to occur only with high N fertility. Atmospheric CO_2 did not affect allocation patterns for any compound; however the high N treatment tended to result in a lower percentage of sugars, cellulose, and lignin belowground.

Abbreviations: FWO – fats, waxes, and oils, Cel – cellulose, SC – structural carbohydrates, Lig – Lignin, Tan – tannins, NSC – nonstructural carbohydrates, TC – total carbon.

Introduction

Substantial research has demonstrated positive effects of increasing CO_2 in the atmosphere on plants, including increased growth and yield (Rogers and Dahlman, 1993; Wittwer, 1990), increased photosynthesis (Long and Drake, 1992; Radin et al., 1987), and decreased respiration (Amthor et al., 1992; Bunce, 1990; Wullschleger et al., 1994) for many plant species. It is this increased C uptake and assimilation which results in increased phytomass production; and, in many cases, the largest proportion of this extra phytomass is found belowground (Prior et al., 1994; Rogers et al., 1994; Wittwer, 1978). Increased C from elevated atmospheric CO_2 can also enter the rhizosphere via root growth, turnover, and exudation (Lekkerkerk et al., 1990; Norby et al., 1987; Pregitzer et al., 1995; Zak et al., 1993). There is increasing recognition that belowground responses to the CO_2 environment aboveground may be the critical missing link in our analysis of ecosystem responses to a changing atmosphere.

* FAX No: (334) 423-6555.
E-mail: jentry@kimberly.ars.pn.usbr.gov

The primary effect of elevated CO_2 is to increase the rate of photosynthesis, and enhanced photosynthesis is likely to increase the amount of C allocated to sinks, especially to root systems. In yellow-poplar (*Liriodendron tulipifera* L.) saplings grown in open top field chambers with elevated CO_2, photosynthesis was enhanced and remained so throughout three growing seasons (Norby et al., 1992). This did not result in a significant increase in plant mass, but rather a change in allocation from leaf production to fine root production and an apparent increase in C flux through the root system.

An increased supply of photosynthate to roots could alter root chemistry. Elevated CO_2 increased carbohydrate concentrations in loblolly pine (*Pinus taeda* L.) seedlings by 68% and compensated for the decline in carbohydrates caused by water stress (Tschaplinski et al., 1993). The concentration of non-structural carbohydrates in roots of cotton (*Gossypium hirsutum* L.) plants was 2 to 4 fold higher in elevated CO_2 (Wong, 1990). Carbon to nitrogen ratio (C:N) of roots of the marsh plant, American bulrush (*Scripus americanus* Pers.), was 22% higher under CO_2-enriched conditions (Curtis et al., 1990). Nitrogen concentration in roots also tended to be lower under elevated CO_2 in white oak (*Quercus alba* L.) and yellow-poplar fine roots from field-grown saplings (Norby et al., 1995) and in fine roots from potted *Quercus* spp. and *Liriodendron* spp. seedlings (Norby and O'Neill, 1989; Norby et al., 1986). Despite the increasing attention forest species are receiving in CO_2 effects research, there remain a paucity of data describing the effects of atmospheric CO_2 on the concentration of secondary metabolites, such as lignin and tannins, in plant tissues. The objective of this study was to examine the effects of elevated atmospheric CO_2, combined with soil N fertilization, on tissue chemistry of longleaf pine (*P. palustris* Mill.).

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 = 13 mm; standard deviation = 2), and planted on February 25, 1993 into 45-L plastic containers containing a coarse sandy medium (pH 5.1) which was low in mineral elements (P, K, mg, and Ca = 0.9, 5.6, 6.9, and 26.6 mg kg^{-1}, respectively).

Treatments were arranged in a split-plot design with five replications. Carbon dioxide treatments (main plots) were randomly assigned to chambers. Nitrogen treatments (subplots) were randomly assigned within each chamber. To avoid within chamber location effects, pot locations were re-randomized monthly.

Seedlings were exposed to ambient ($\approx$365 μmol CO_2 mol^{-1}) or elevated ($\approx$720 μmol CO_2 mol^{-1}) CO_2 within an open top chamber system similar to that described by Rogers et al. (1983). The chambers, CO_2 supply, and CO_2 monitoring systems have been previously described for this study site (Mitchell et al., 1995). Carbon dioxide exposures were initiated on March 30, 1993 and continued until November 28, 1994 (20 months). Average daytime CO_2 concentrations ($\pm$ standard deviation) through the duration of the experiment were 372.3 μmol^{-1} ($\pm$19.0) in the ambient chambers and 739.5 μmol^{-1} ($\pm$39.7) in the elevated CO_2 chambers.

Nitrogen treatments were similar to those described by Bazzaz and Miao (1993) with slight modification. Nitrogen treatments consisted of applying either 400 or 40 kg N ha^{-1} yr^{-1} at three-month intervals (as sulfur coated urea; 38-0-0) which correspond to high and low N treatments. Fertility of other nutrients was maintained in non-limiting levels in all pots by application of sulfur coated potassium (0-0-47 = 80 kg K ha^{-1} yr^{-1}) and MicroMax^{TM} Plus[1] (0-4-0; P = 280, Ca = 1140, Mg = 560, and S = 100 kg ha^{-1} yr^{-1}, plus a complete compliment of micronutrients) mixed into sand at the time containers were filled, with a second application at the end of the first year. Iron chelate (0.007 mg Fe g^{-1} soil) was applied once in April 1993. Nitrogen treatments were initiated at the time of planting and CO_2 treatments were initiated March 30, 1993.

Longleaf pine xylem pressure potential was measured periodically using a pressure chamber apparatus (Scholander et al., 1965). Gravimetric measurements of each container were made two times each week using a scale (Model LP-C4, 101 kg; Tri-Coastal Industries, Mukilteo, Washington, USA.). Gravimetric measurements were correlated with pre-dawn xylem pressure potentials, measured on excised needles at each weighing for one month. Trees received water

[1] Trade names and products are mentioned solely for information. No endorsement by the USDA is implied.

Table 1. Concentration of N and C fractions in *Pinus palustris* tissue after 20 months of growth at ambient and elevated CO_2 with high and low N soil fertility in open top chambers

Tissue	Treatment CO2 (μmol mol^{-1})	Nitrogen (kg ha^{-1}yr^{-1})	Biomass (g)	Nitrogen mg N g^{-1} tissue	Carbon Fractions FWO[a]	Sugar	Starch (mg fraction g^{-1} tissue)	Cellulose	Lignin	Tannin
Needles	365	40	22.9 b[b]	6.1 c	166.7 a	7.0 a	239.0 a	412.2 a	152.0 a	23.0 ab
	720	40	19.4 b	5.8 c	161.6 a	6.9 a	248.0 a	408.5 a	148.2 a	26.7 a
	365	400	153.6 a	14.5 a	175.7 a	6.6 a	264.4 a	383.6 a	155.6 a	14.1 b
	720	400	181.5 a	10.9 b	145.2 a	7.2 a	281.0 a	394.0 a	155.0 a	17.7 ab
Stems	365	40	12.8 c	2.6 c	279.6 a	6.1 a	164.5 a	370.2 a	158.5 a	21.0 a
	720	40	17.1 bc	3.5 c	355.4 a	5.4 a	146.2 a	322.4 a	151.8 a	18.9 a
	365	400	60.4 ab	13.5 a	233.7 a	6.4 a	234.6 a	327.1 a	166.8 a	31.4 a
	720	400	97.7 a	8.7 b	347.3 a	5.5 a	173.8 a	313.6 a	141.4 a	18.5 a
Taproots	365	40	16.8 c	3.1 c	226.5 a	6.2 a	311.8 ab	268.8 a	157.2 ab	29.6 b
	720	40	15.8 c	3.8 c	364.9 a	5.2 a	119.2 c	302.3 a	194.7 a	13.6 c
	365	400	44.7 b	8.8 a	278.9 a	5.8 a	241.1 b	276.7 a	162.5 ab	34.7 b
	720	400	74.1 a	6.0 b	249.4 a	6.0 a	405.1 a	195.4 a	94.1 b	49.9 a
Laterals	365	40	15.9 c	4.0 c	353.2 a	5.0 a	194.6 a	267.5 a	166.0 a	13.6 ab
	720	40	16.2 c	4.1 c	356.9 a	5.3 a	179.4 a	270.1 a	176.3 a	12.0 b
	365	400	87.8 b	9.4 a	315.4 a	5.5 a	250.2 a	240.3 a	167.2 a	21.4 a
	720	400	140.9 a	6.3 b	387.0 a	5.2 a	220.9 a	231.0 a	140.8 a	15.1 ab
Fine Roots	365	40	9.5 b	6.1 b	333.4 b	8.1 a	114.7 b	278.5 ab	251.7 a	13.4 a
	720	40	11.0 b	5.4 b	502.8 a	6.4 a	74.0 b	225.9 b	180.4 b	10.4 a
	365	400	32.6 a	13.4 a	197.6 b	7.5 a	191.8 a	334.9 a	252.8 a	15.3 a
	720	400	46.0 a	7.9 b	339.6 b	7.9 a	134.7 ab	269.2 b	232.7 ab	16.1 a

[a]FWO = fats, waxes and oils.
[b]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 ($p \leq 0.05$), $n = 5$.

sufficient to return them to field capacity weight when gravimetric measurements indicated a xylem pressure potential of −0.5 MPa had been reached. Pre-dawn xylem pressure potential continued to be taken a minimum of once each month as a check on gravimetric readings. Gravimetric measurements were again correlated with pre-dawn xylem pressure potentials to establish new field capacity values after November, 1993 (8 months) and July 1994 (16 months) to compensate for changes in plant mass; xylem pressure potential was measured on each container for one month following determination of new field capacity weights. Deionized water was used in order to ensure that fertility treatments remained unaffected by watering.

Plant harvests

One container from each N treatment in each chamber was destructively harvested in November 1994, corresponding to 20 months of CO_2 exposure. Plants were separated into tissue component parts (needles, stems, taproots, lateral roots, and fine roots), oven dried at 55 °C to a constant weight, and dry weight recorded. A detailed description of treatment effects on biomass production and morphological variables has been previously reported (Prior et al., 1997).

Tissue analysis

A 0.2 g subsample from each plant tissue was analyzed for C fractions. Soluble fats, waxes and oils were removed using a series of dichloromethane washes (TAPPI, 1975). Sugars and starches were removed

and measured using methods described by Hanson and Moller (1975). Cellulose and lignin were determined gravimetrically (Effland, 1977). Hot water extractable tannins were extracted and measured by methods described by Allen et al. (1974). Proximate C fractions were corrected for ash and presented as ash-free dry weight (Ryan et al., 1990). Tissue N content was determined with a LECO CHN-600 analyzer (LECO Corp., St. Joseph, MI).

Data analysis

Data were totaled for each seedling and averaged for each container prior to analysis. Data were tested for homogeneity of variance and transformations to achieve normality were not necessary. Data analysis was conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS, 1990). Error terms appropriate to the split-plot design were used to test the significance of main effects variables and their interactions. Residuals were equally distributed with constant variances. Significance was determined at $p \leq 0.05$ with the Least Square Means test.

Results

Concentration (mg g^{-1})

Biomass of all tissues (needles, stems, taproots, lateral roots, and fine roots) was usually greater when plants received the high N treatment regardless of CO_2 concentration (Table 1). In general, biomass of all tissues was greater when plants received both elevated CO_2 and N. The effects of elevated CO_2 and N on concentration of C fractions were observed for belowground, but not aboveground, tissues. Elevated CO_2 resulted in lower concentrations of starch, cellulose, and lignin, but increased the concentration of FWO in root tissues. High N fertility increased the concentration of starch, cellulose, and tannins, but resulted in lower concentrations of lignin and FWO in roots. Interactions between CO_2 and N often occurred with differences between CO_2 concentrations only with high N fertility. Nitrogen concentration (mg g^{-1}) was significantly greater in all tissues for plants grown in the high N treatment. Elevated CO_2 resulted in lower N concentrations for all tissues when grown with the high N treatment.

In fine roots, FWO were significantly higher when plants received elevated CO_2 with low N. Starches in fine roots were higher for plants receiving high N, particularly with ambient CO_2. Starches in taproots were greater for plants in elevated CO_2 and high N; however, in low N, starch concentration in taproots was greater for ambient CO_2-grown plants. Lignin concentration was lower while the concentration of cellulose was higher in fine roots in plants grown under ambient CO_2. Lignin concentration in taproots tended to be lower when plants received both elevated CO_2 and elevated N. Tannin concentration in taproots was higher for plants in elevated CO_2 and high N.

Allocation (% in tissue of total tree)

Biomass partitioning was higher in needles, but was lower in taproots and fine roots, when plants received high N (Table 2). Biomass partitioning to needles was greater for plants in ambient CO_2 in both N treatments. CO_2 concentration had no effect on N partitioning among tissues. A greater percentage of N was allocated to needles, and less to taproots and fine roots for plants receiving the high N treatment.

Partitioning of sugars to needles and lateral roots tended to be increased in plants receiving high N; however, allocation of sugars to taproots was lower for high N-grown plants (Table 2). Treatment effects on allocation of cellulose were seen only in needles, where allocation was greater for plants receiving high N. Allocation of tannins to taproots was lower in the low N treatment for elevated, compared to ambient, CO_2-grown plants; also, allocation was higher for plants grown in high N and elevated CO_2, compared to plants receiving low N and elevated CO_2. Allocation of tannins to lateral roots was greater for plants receiving high N, particularly for ambient CO_2-grown plants.

Ratios among C components in tissues

Treatment effects on ratios involving structural C (SC = cellulose + lignin + tannins) were infrequent and, with the exception of the ratio of tannins to total structural C (Tan:SC), were only observed for belowground tissues (Table 3). There were no treatment effects on the distribution of lignin as a percentage of total structural C (Lig:SC). The ratio of tannin to structural C (Tan:SC) was higher in taproots of elevated CO_2-grown plants receiving the high N, compared to low N, treatment. The ratio of nonstructural carbohydrates (NSC = starches + sugars) to total structural C (NSC:SC) tended to be higher in needles, taproots, and fine roots of plants receiving the high N treatment.

Table 2. Allocation of N and C fractions in *Pinus palustris* tissue after 20 months of growth at ambient and elevated CO_2 with high and low N soil fertility in open top chambers

Tissue	Treatment CO2 (μmol mol^{-1})	Nitrogen (kg ha^{-1}yr^{-1})	Biomass (%)	Nitrogen % of total	Carbon Fractions FWO[a]	Sugar	Starch	Cellulose	Lignin	Tannin
					(%of total in tissue)					
Needles	365	40	29.6 b[b]	41.1 ab	19.3 ab	32.6 bc	33.0 c	37.4 ab	26.8 b	32.6 a
	720	40	24.9 c	32.1 b	12.1 b	29.3 c	38.0 b	32.4 b	22.2 b	38.7 a
	365	400	40.5 a	47.4 a	30.7 a	42.4 a	43.3 a	48.1 a	37.4 a	27.2 a
	720	400	33.7 b	43.6 a	17.6 b	39.0 ab	37.7 b	44.9 a	36.1 a	27.8 a
Stems	365	40	16.4 a	9.6 a	18.1 a	15.7 a	12.3 a	18.5 a	15.2 a	16.5 a
	720	40	21.0 a	16.3 a	23.4 a	19.2 a	18.4 a	21.3 a	18.9 a	22.1 a
	365	400	16.0 a	17.4 a	16.3 a	16.5 a	15.4 a	16.1 a	15.7 a	24.2 a
	720	400	17.8 a	18.6 a	22.2 a	15.6 a	12.9 a	18.4 a	17.2 a	15.8 a
Taproots	365	40	21.7 a	14.7 ab	18.8 a	21.1 a	31.5 a	17.6 a	20.2 a	31.1 a
	720	40	19.4 ab	16.7 a	21.8 a	17.1 ab	14.6 b	18.6 a	21.9 a	15.8 b
	365	400	11.8 c	8.3 c	14.2 a	10.8 c	11.5b	9.9 a	11.2 b	19.3 ab
	720	400	13.6 bc	9.6 bc	12.3 a	13.2 bc	21.7 ab	9.6 a	8.8 b	31.1 a
Laterals	365	40	20.1 a	17.8 a	27.1 a	15.0 b	16.6 a	16.2 a	19.5 a	11.3 b
	720	40	20.6 a	18.8 a	21.6 a	18.7 ab	22.8 a	17.8 a	21.6 a	14.7 ab
	365	400	23.2 a	17.7 a	31.2 a	20.2 ab	23.2 a	17.2 a	22.9 a	23.1 a
	720	400	26.3 a	20.0 a	37.3 a	21.6 a	23.3 a	20.0 a	24.3 a	18.9 ab
Fine Roots	365	40	12.2 ab	16.8 a	16.7 ab	15.6 a	6.5 a	10.4 a	18.2 a	8.5 a
	720	40	14.0 a	16.2 a	21.1 a	15.8 a	6.3 a	9.9 a	15.4 a	8.7 a
	365	400	8.5 b	9.3 b	7.6 c	10.2 a	6.6 a	8.7 a	12.7 a	6.2 a
	720	400	8.6 b	8.2 b	10.7 bc	10.7 a	4.5 a	7.7 a	13.6 a	6.3 a

[a]FWO = fats, waxes and oils.
[b]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 ($p \leq 0.05$), $n = 5$.

In contrast to C, ratios involving tissue N concentrations were observed for all tissues except fine roots. Lignin to N ratios (Lig:N) and ratios of structural C to N (SC:N) were higher in plants growing under low N. Lig:N and SC:N ratios of belowground tissues were not affected by CO_2 treatment; however, Lig:N and SC:N of needles were higher for elevated CO_2-grown plants in the high N treatment, while Lig:N and SC:N in stems were higher for ambient CO_2-grown plants in low N.

Discussion

Effects of CO_2 and N on concentration of C compounds (FWO, sugars, starches, cellulose, lignin, and tannins) tended to occur belowground rather than aboveground, especially in the taproots and fine roots. A similar response was observed for biomass and this tendency for atmospheric CO_2 to enhance belowground more than aboveground growth has been previously noted (Prior et al., 1994; Rogers et al., 1994; Wittwer, 1978). Carbon dioxide by N interactions indicated that elevated CO_2 increased tissue dry weights only when combined with high N. This interaction of CO_2 with soil fertility has been commonly reported (Griffin et al., 1993; Pérez-Soba et al., 1995; Prior et al., 1997; Walker et al., 1995), although exceptions have been noted with some tree species (Johnson et al., 1995; Norby et al., 1995).

Although previous research has investigated CO_2 effects on plant biomass, few studies have examined changes in plant chemistry under increased levels of atmospheric CO_2, and these have been restricted to foliar effects (Lindroth, 1996; Lindroth et al., 1993; O'Neill and Norby, 1996). In this study, changes in the

Table 3. Allocation of C ratios in *Pinus palustris* tissue after 20 months of growth at ambient and elevated CO_2 with high and low N soil fertility in open top chambers

Tissue	Treatment CO_2 (μmol mol^{-1})	Nitrogen (kg ha^{-1}yr^{-1})	Cel/SC[a]	Lig/SC	Tan/SC	NSC/SC	SC/TC	Lig/N	SC/N
Needles	365	40	70.2 a[b]	25.9 a	3.9 ab	0.42 c	58.7 a	24.9 a	96.2 a
	720	40	70.0 a	25.4 a	4.6 a	0.44 bc	58.3 a	25.9 a	102.1 a
	365	400	69.3 a	28.1 a	2.5 b	0.49 ab	55.3 a	10.8 c	38.3 c
	720	400	69.6 a	27.3 a	3.1 ab	0.51 a	56.7 a	14.3 b	52.1 b
Stems	365	40	67.6 a	28.6 a	3.8 a	0.31 a	55.0 a	60.4 a	212.1 a
	720	40	65.1 a	31.1 a	3.8 a	0.31 a	49.3 a	43.2 b	146.2 b
	365	400	62.3 a	31.7 a	6.0 a	0.46 a	52.5 a	12.6 c	39.7 c
	720	400	66.2 a	29.9 a	3.9 a	0.38 a	47.3 a	16.4 c	56.4 c
Taproots	365	40	56.9 a	36.1 a	7.1 ab	0.75 ab	45.6 a	54.6 a	164.9 a
	720	40	59.4 a	37.9 a	2.7 b	0.24 b	51.1 a	51.0 a	134.2ab
	365	400	57.2 a	35.1 a	7.7 ab	0.55 ab	47.4 a	18.4 b	54.4 b
	720	400	57.2 a	27.8 a	15.0 a	1.24 a	33.9 a	16.5 b	58.1 b
Laterals	365	40	59.9 a	37.2 a	2.9 a	0.43 a	44.7 a	42.3 a	114.0 a
	720	40	59.3 a	38.2 a	2.6 a	0.40 a	45.8 a	42.6 a	111.3 a
	365	400	56.2 b	38.9 a	4.9 a	0.60 a	42.9 a	17.9 b	45.8 b
	720	400	60.0 a	35.9 a	4.2 a	0.64 a	38.7 a	22.3 b	61.8 b
Fine Roots	365	40	51.3 a	46.2 a	2.4 a	0.22 ab	54.4 a	41.7 a	90.0 a
	720	40	54.0 a	43.5 a	2.5 a	0.19 b	41.7 b	35.8 a	81.1 a
	365	400	55.7 a	41.8 a	2.5 a	0.33 a	60.3 a	18.9 a	45.2 a
	720	400	51.8 a	45.1 a	3.1 a	0.28 ab	51.8 ab	29.9 a	66.7 a

[a] Cel = cellulose, Lig = lignin, Tan = tannin, SC = structural carbohydrates (cellulose, lignin and tannin), TC = total carbon, NSC = nonstructural carbohydrates (sugars and starches).
[b] 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 ($p \leq 0.05$), $n = 5$.

concentration and allocation of C compounds in longleaf pine seedlings were observed to predominantly occur in belowground tissues, with N effects tending to be stronger than CO_2 effects.

In general, plant tissues receive photosynthate according to demand and plants will allocate a majority of this photosynthate to those tissues encountering the most limiting resource, i.e., greatest demand; this, of course, is dependent upon availability of photosynthate for allocation (Chapin et al., 1987; Eissenstat, 1992; Rogers et al., 1996). Both environmental variables tested in this study are undoubtedly important and plants will likely respond, if able, to attain the most limiting resource at any given time in order to maintain a balance of internal C and N pools (Rogers et al., 1996). These interactions indicate that only when sufficient N is available can the plants take advantage of the higher concentrations of atmospheric CO_2.

Ratios among C components were calculated to determine treatment effects on structural C allocation to plant tissues by removing the dynamic portion of the internal C pools (i.e., nonstructural carbohydrates). It has been previously shown that concentrations of nonstructural carbohydrates can be increased by elevated CO_2 (Yelle et al., 1989). Effects of CO_2 and N on partitioning of structural C occurred belowground only, as was observed for most data in this study.

Generally, CO_2-induced changes in the quality (higher C:N ratios and potential shifts in concentrations of plant defensive allelochemicals) of plant tissues is thought to influence insect herbivory (Fajer et al., 1992; Lincoln and Couvet, 1989) and could affect ecosystem structure and function (Lindroth et

al., 1993). Thus, the manner in which increases in atmospheric CO_2 will affect the major insect pests and diseases of the world's plants are largely unknown but may result in positive or negative impacts on plant health and productivity. Results of this study lead us to conclude that atmospheric CO_2 enrichment combined with elevated soil N may change plant defense compounds, such as lignin and tannins, thereby changing plant susceptibility to many insect and disease infestations (Entry et al., 1992, 1994).

By altering plant tissue quantity and quality, elevated atmospheric CO_2 will likely affect soil microbial composition and activity, changing C turnover and storage in soils (Goudriaan and de Ruiter, 1983; Lamborg et al., 1983). Melillo (1983) reported higher C:N ratios and higher levels of phenolics in sweetgum (*Liquidambar styraciflua* L.) leaves exposed to high CO_2 and hypothesized that this would result in reduced rates of decomposition and decreased soil fertility. Lekkerkerk et al. (1990) found that elevated atmospheric CO_2 increased the input of easily decomposable root-derived material in the soil of wheat (*Tritcum aestivum* L.) and reduced turnover of more resistant soil organic matter. Coûteaux et al. (1991) demonstrated similar results for an initial decomposition period and related the reduction in decomposition rate to lower N concentration and higher C:N ratios of CO_2-enriched plants. When decomposition was allowed to continue, changes in the composition of the microbial population resulted in an increased decomposition rate of CO_2-enriched material which led to an overall enhancement of C mineralization of 30%.

Effects of increasing CO_2 on biomass production will influence microbial composition and activity and, in turn, may impact mineralization/immobilization processes. Zak et al. (1993) reported significant increases in microbial biomass C in the rhizosphere and in bulk soil associated with plants grown under elevated CO_2. They also observed a significant increase in N mineralization which they related to possible increased turnover rate of microbial N and/or an increased N release from soil organic matter. We observed decreased lignin:N ratios for all tissues except fine roots under high N fertility which may increase the rate of decomposition and nutrient mineralization.

This study demonstrated that N fertility exerted a much stronger influence on longleaf pine tissue chemistry and C allocation among those tissues than did atmospheric CO_2. The majority of both N and CO_2 effects occurred belowground; however, effects on N concentration and lignin to N ratios were observed both above- and belowground. Also, the majority of treatment effects involved structural, rather than nonstructural, carbohydrates. Altered tissue C and N concentrations and altered lignin to N ratios suggest changes in tissue susceptibility to insect and disease attack and altered rates of decomposition and nutrient mineralization under elevated CO_2 and high N fertility.

Acknowledgements

This material is based upon work supported through the Southeastern Regional Center of the National Institute for Global Environmental Change by the US Department of Energy under Cooperative Agreement No. DE-FC03-90ER61010 and through the Experimental Program to Stimulate Competitive Research by the US Environmental Protection Agency under Contract No. R821826-01-1. Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Department of Energy or the US Environmental Protection Agency.

References

Agrios G N 1988 Plant Pathology. Third Edition. Academic Press, New York. 803 p.

Allen S E, Grimshaw H M, Parkinson J and Quermby C 1974 Chemical Analysis of Ecological Materials. Blackwell Scientific, Oxford. 386 p.

Amthor J S, Koch G W and Bloom A J 1992 CO_2 inhibits respiration in leaves of *Rumex crispus* L. Plant Physiol. 98, 757–760.

Bunce J A 1990 Short- and long-term inhibition of respiratory carbon dioxide efflux by elevated carbon dioxide. Ann. Bot. 65, 637–642.

Bazzaz F A and Miao S L 1993 Successional status, seed size, and responses of tree seedlings to CO_2, light, and nutrients. Ecology 74, 104–112.

Chapin F S III, Bloom A J, Field C B and Waring R H 1987 Plant responses to multiple environmental factors. Biosci. 37, 49–55.

Coûteaux M-M, Mousseau M, Célérier M-L and Bottner P 1991 Increased atmospheric CO_2 and litter quality: Decomposition of sweet chestnut leaf litter with animal food webs of different complexities. Oikos 61, 54–64.

Curtis P S, Balduman L M, Drake B G and Whigham D F 1990 Elevated atmospheric CO_2 effects on belowground processes in C_3 and C_4 estuarine marsh communities. Ecology 71, 2001–2006.

Effland M J 1977 Modified procedure to determine acid-insoluble lignin in wood and pulp. TAPPI 60, 143–144.

Eissenstat D M 1992 Costs and benefits of constructing roots of small diameter. J. Plant Nutri. 15, 763–782.

Entry, J A, K Cromack, Jr, R G Kelsey and N E Martin 1992 The response of Douglas-fir to *Armillaria ostoyae* infection after thinning or thinning plus fertilization. Phytopath. 82: 682–689.

Entry, J A, N C Vance and D L Copes. 1994 Resistance of graft compatible and incompatible *Pseudotsuga menziesii* rooted cuttings to *Phellinus weirii*. Can. J. For. Res. 24: 878–881.

Fajer E D, Bowers M D and Bazzaz F A 1992 The effect of nutrients and enriched CO_2 environments on production of carbon-based allelochemicals in *Plantago*: A test of the carbon/nutrient balance hypothesis. Amer. Natural. 140, 707–723.

Goudriaan J and de Ruiter H E 1983 Plant growth in response to CO_2 enrichment at two levels of nitrogen and phosphorus supply. 1. Dry matter, leaf area, and development. Neth. J. Agric. Sci. 31, 157–169.

Griffin K L, Thomas R B and Strain B R 1993 Effects of nitrogen supply and elevated carbon dioxide on construction cost in leaves of *Pinus taeda* (L.) seedlings. Oecologia 95, 575–580.

Hanson J and Moller I 1975 Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal. Biochem. 68, 87–94.

Johnson D W, Ball T, Walker R F 1995 Effects of elevated CO_2 and nitrogen on nutrient uptake in ponderosa pine seedlings. Plant Soil 168-169, 535–545.

Lamborg M R, Hardy R W F and Paul E A 1983 Microbial effects. *In* CO_2 and Plants: The Response of Plants to Rising Levels of Atmospheric CO_2, AAAS Selected Symposium 84. Ed. E R Lemon. pp 131–176. Westview Press, Boulder, CO.

Lekkerkerk L J A, van de Geijn S C and van Veen J A 1990 Effects of elevated atmospheric CO_2-levels on the carbon economy of a soil planted with wheat. *In* Soils and the Greenhouse Effect. Ed. A F Bouwman. pp 423–429. John Wiley & Sons, New York.

Lincoln D E and Couvet D 1989 The effect of carbon supply on allocation to allelochemicals and caterpillar consumption of peppermint. Oecologia 78, 112–114.

Lindroth R L 1996 CO_2-mediated changes in tree chemistry and tree-Lepidoptera interactions. *In* Carbon Dioxide and Terrestrial Ecosystems. Eds. G W Koch and H A Mooney. pp 105–120. Academic Press, New York.

Lindroth R L, Kinney K K and Plaz C L 1993 Responses of deciduous trees to elevated atmospheric CO_2: Productivity, phytochemistry and insect performance. Ecology 74, 763–777.

Long S P and Drake B G 1992 Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. *In* Crop Photosynthesis: Spatial and Temporal Determinants. Eds. N R Baker and H Thomas. pp 69–107. Elsevier, New York.

Melillo J M 1983 Will increases in atmospheric CO_2 concentrations affect decay processes? *In* Ecosystems Center Annual Report. pp 10–11. Marine Biology Laboratory, Woods Hole, MA.

Mitchell R J, Runion G B, Prior S A, Rogers H H, Amthor J S and Henning F P 1995 Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO_2 concentration. J. Exp. Bot. 46, 1561–1567.

Norby R J, Gunderson C A, Wullschlger S D, O'Neill E G and McCraken M K 1992 Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. Nature 354, 322–324.

Norby R J and O'Neill E G 1989 Growth dynamics and water use of seedlings of *Quercus alba* L. in CO_2-enriched atmospheres. New Phytol. 111, 491–500.

Norby R J, O'Neill E G, Hood W G and Luxmoore R J 1987 Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO_2 enrichment. Tree Physiol. 3, 203–210.

Norby R J, O'Neill E G and Luxmoore R J 1986 Effects of atmospheric CO_2 enrichment on the growth and mineral nutrition of *Quercus alba* seedings in nutrient poor soil. Plant Physiol. 82, 83–89.

Norby R J, O'Neill E G and Wullschlger S D, 1995 Belowground responses to atmospheric carbon dioxide in forests. *In* Carbon Forms and Functions in Forest Soils. Eds. W W McFee and J M Kelly. pp 397–418. SSSA, Madison, WI.

O'Neill E G and Norby R J 1996 Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment. *In* Carbon Dioxide and Terrestrial Ecosystems. Eds. G W Koch and H A Mooney. pp 87–103. Academic Press, New York.

Pérez-Soba M, Dueck T A, Puppi G and Kuiper J C 1995 Interactions of elevated CO_2, NH_3 and O_3 on mycorrhizal infection, gas exchange and N metabolism in saplings of Scots pine. Plant Soil 176, 107–116.

Pregitzer K S, Zak D R, Curtis P S, Kubiske M E, Teeri J A and Vogel C S 1995 Atmospheric CO_2, soil nitrogen, and turnover of fine roots. New Phytol. 129, 579–585.

Prior S A, Rogers H H, Runion G B and Mauney J R 1994 Effects of free-air CO_2 enrichment on cotton root growth. Agric, For. Meteorol. 70, 69–86.

Prior S A, Runion G B, Mitchell R J, Rogers H H and Amthor J S. 1997 Effects of atmospheric CO_2 on longleaf pine: Productivity and allocation as influenced by nitrogen and water. Tree Physiol. (*In press*).

Radin J W, Kimball B A, Hendrix D L and Mauney J R 1987 Photosynthesis of cotton plants exposed to elevated levels of carbon dioxide in the field. Photosyn. Res. 12, 191–203.

Rogers H H and Dahlman R C 1993 Crop responses to CO_2 enrichment. Vegetatio 104/105, 117–131.

Rogers H H, Heck W W and Heagle A S 1983 A field technique for the study of plant responses to elevated carbon dioxide concentrations. Air Pollut. Control Assoc. J. 33, 42–44.

Rogers H H, Runion G B and Krupa S V 1994 Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environ. Pollut. 83, 155–189.

Rogers H H, Prior S A, Runion G B and Mitchell R J 1996 Root to shoot ratio of crops as influenced by CO_2. Plant Soil (*In Press*).

Ryan, M G, Melillo J M and Ricca A 1990 A comparison of methods for determining proximate carbon fractions of forest litter. Can. J. For. Res. 20, 166–171.

SAS Institute INC 1990 SAS User's Guide: Statistics – Version 6.03 Edition. Statistical Analysis System (SAS) Institute Inc., Cary, NC. 584 pp.

Scholander P F, Hammel H T, Bradstreet E D and Hemmingsen E A 1965 Sap pressure in vascular plants. Science 148, 339–346.

TAPPI 1975 Water solubles in wood and pulp. T 207 os-69. Technical association of the pulp and paper industry, Atlanta, GA. USA.

Tschaplinski T J, Norby R J and Wullschleger S D 1993 Responses of loblolly pine seedlings to elevated CO_2 and fluctuating water supply. Tree Physiol. 13, 283–296.

Walker R F, Geisinger D R, Johnson D R and Ball J T 1995 Interactive effects of atmospheric CO_2 and soil N on growth and ectomycorrhizal colonization of ponderosa pine seedlings. Forest Sci. 41, 491–500.

Wittwer, S.H. 1978. Carbon dioxide fertilization of crop plants. p. 310–33. *In* Problems in Crop Physiology. Ed. U S Gupta. Haryana Agric. Univ., Hissar, India.

Wittwer, S.H. 1990. Implications of the greenhouse effect on crop productivity. HortScience 25: 1560–1567.

Wong S C 1990 Elevated atmospheric partial pressure of CO_2 and plant growth. II. Non-structural carbohydrate content in cotton plants and its effect on growth parameters. Photosyn. Res. 23, 171–180.

Wullschleger S D, Ziska L H and Bunce J A 1994 Respiratory responses of higher plants to atmospheric CO_2 enrichment. Physiol. Plant. 90, 221–229.

Yelle S, Beeson R C Jr, Trudel M J and Gosselin A 1989 Acclimation of two tomato species to high atmospheric CO_2. I. Starch and sugar concentrations. Plant Physiol. 90, 1465–1472.

Zak D R, Pregitzer K S, Curtis P S, Teeri J A, Fogel R and Randlett D L 1993 Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151, 105–117.

Section editor: E. Garnier