

# Altered growth and fine root chemistry of *Betula papyrifera* and *Acer saccharum* under elevated CO<sub>2</sub>

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**Abstract:** We investigated the effects of CO<sub>2</sub> enrichment on fine root chemical composition of two tree species common to northern hardwood forests. Two-year-old *Betula papyrifera* and 3-year-old *Acer saccharum* saplings were grown under ambient (400 µmol·mol<sup>-1</sup>) and elevated (700 µmol·mol<sup>-1</sup>) CO<sub>2</sub> in a glasshouse experiment. In both species, root/shoot ratios and fine root percentages (of total biomass) were unaltered by CO<sub>2</sub> enrichment. Tissue nitrogen concentrations decreased in the fine roots, and consequently, C/N ratios increased with elevated CO<sub>2</sub>. In birch, only condensed tannins increased with CO<sub>2</sub> enrichment, while root starch levels were conserved. In maple, neither condensed tannins nor hydrolysable tannins were positively influenced by elevated CO<sub>2</sub>. Both fine root biomass and chemistry responses of the tree saplings may be related to their successional status.

**Résumé :** Nous avons étudié les effets d'un enrichissement en CO<sub>2</sub> sur la composition chimique des racines fines de deux espèces d'arbres typiques des forêts de feuillus nordiques. Des jeunes plants de *Betula papyrifera* et d'*Acer saccharum* respectivement âgés de 2 et 3 ans ont été cultivés à des concentrations ambiantes (400 µmol·mol<sup>-1</sup>) et élevée (700 µmol·mol<sup>-1</sup>) de CO<sub>2</sub> dans une expérience en serre. Chez les deux espèces, le rapport racine/tige et le pourcentage de racines fines par rapport à la biomasse totale n'ont pas été modifiés par un enrichissement en CO<sub>2</sub>. La concentration en azote des tissus a diminué dans les racines fines et, conséquemment, le rapport C/N a augmenté avec une concentration élevée de CO<sub>2</sub>. Chez le bouleau, seuls les tannins condensés ont augmenté à la suite d'un enrichissement en CO<sub>2</sub> tandis que le niveau d'amidon dans les racines s'est maintenu. Chez l'érable, ni les tannins condensés, ni les tannins hydrolysables ont été positivement influencés par une concentration élevée de CO<sub>2</sub>. Les effets sur la biomasse des racines fines et les caractéristiques chimiques des jeunes tiges pourraient être reliés au stade de succession.

[Traduit par la Rédaction]

## Introduction

Greater growth and biomass accrual have been demonstrated in CO<sub>2</sub>-enriched plants compared with plants grown under ambient CO<sub>2</sub> levels (Curtis and Wang 1998; Curtis et al. 2000). Atmospheric CO<sub>2</sub> enrichment has been shown to increase carbon/nitrogen ratios of both leaves and roots (Cotrufo and Ineson 1996; Couteaux et al. 1999; Pregitzer et al. 2000), and to raise levels of carbon-based secondary metabolites, particularly phenolics, in the foliage of woody plants (Peñuelas and Estiarte 1998; Koricheva et al. 1998). CO<sub>2</sub>-fertilization trials also have resulted in increased fine root concentrations of total phenolics and condensed tannins, but these pot-based experiments have focused mainly on cultivated, warm temperate conifers (e.g., King et al. 1997; Entry et al. 1998; Gebauer et al. 1998; Runion et al. 1999) and subtropical broad-leaved species, such as cotton (Booker

2000). Moreover, few explicit comparisons of tissue chemistry have been made regarding CO<sub>2</sub> effects on trees of differing successional stages, and these stem mainly from the tropics (Kanowski 2001; Coley et al. 2002). Although rising atmospheric CO<sub>2</sub> concentrations certainly will alter carbon allocation to above- and below-ground tissues of cool temperate and boreal woody perennials, how fixed carbon will be partitioned among storage, structural, and defense compounds, and whether these responses are easily generalized across species is not known. Apportioning of excess fixed-carbon between roots and shoots is likely dictated by complex interactions between plant demand and the availability of light, moisture, and nutrient resources, together with plant developmental stage and species life history characteristics.

Sugar maple (*Acer saccharum* Marsh.) and paper birch (*Betula papyrifera* Marsh.) are common components of Great Lakes region forests, but these northern hardwoods differ greatly in their geographic range and life histories. Sugar maple is a slow growing, shade-tolerant, late-successional species restricted to the northeastern United States and adjacent Canada. Paper birch, in contrast, is a fast growing species that is distributed throughout boreal North America and as a primary colonizer of disturbed habitats, it is shade-intolerant and nutrient-sensitive (Burns and Honkala 1990). We compared root system responses of these two hardwood species to CO<sub>2</sub> enrichment following a 4-month-long glasshouse study. We recognize that glasshouse studies cannot be used to predict the long-term response of native, open-grown

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adult trees to elevated atmospheric CO<sub>2</sub>, but such short-term experiments still have utility in garnering basic data on root growth and chemistry at seedling and sapling stages, when plant growth rates are generally highest and where resource competition and acquisition is likely to be very intense (Ceulemans and Mousseau 1994).

In this study, we were concerned with the quality rather than the quantity of fine roots (<2 mm in diameter) produced under elevated CO<sub>2</sub> concentrations, since fine root biomass, root respiration, and exudate production have been demonstrated to increase with CO<sub>2</sub> fertilization without changing allometric relationships such as root/shoot ratios (e.g., Rogers et al. 1994; Janssens et al. 1998; Pregitzer et al. 2000). Phenolic compounds in general, and condensed tannins in particular, are the major carbon-based secondary compounds of both paper birch and sugar maple, the foliar levels of which can increase with CO<sub>2</sub> enrichment (Lindroth et al. 1993, 2001; Lindroth and Kinney 1998; Kopper 2001). We predicted increases in fine root concentrations of condensed tannins in both the early-successional birch and late-successional maple grown under elevated CO<sub>2</sub>. Other secondary metabolites, such as hydrolysable tannins and terpenoids (Koricheva et al. 1998), and the phenolic derivative lignin (Booker and Maier 2001) have been shown to be either less responsive or insensitive to supplemental CO<sub>2</sub> treatments. Roth et al. (1998) demonstrated that foliar concentrations of hydrolysable gallotannins, but not ellagitannins, in sugar maple rose in response to elevated CO<sub>2</sub>. We expected a similar response for hydrolysable tannin concentrations in maple fine roots. Under enriched CO<sub>2</sub>, we expected concurrent increases in the biomass of fine roots and in the C/N ratios of those tissues, but no increase in the concentrations of structural carbon-based compounds, such as lignins.

## Materials and methods

### Experimental design and set-up

Alterations in birch and maple fine root chemistry were assessed as part of a larger CO<sub>2</sub>-enrichment experiment conducted in the University of Wisconsin Biotron (Kopper 2001). The larger experiment dealt with the interaction between CO<sub>2</sub> enrichment and foliar herbivory by the whitemarked tussock moth (*Orgyia leucostigma* J.E. Smith). Only the root chemistry responses of undamaged trees are reported here.

Birch and maple saplings were obtained, respectively, as 2- and 3-year-old nursery stock. All saplings were of similar size when they were planted into 16-L pots containing a 70:30 sand-loam mixture and 32 g of Osmocote fertilizer (8- to 9-month release, 16:8:12 (N-P-K) with micronutrients, Scotts, Marysville, Ohio). Sixteen dormant plants of each species were randomly assigned to each of six glass-house rooms. From early May until late August 1998, CO<sub>2</sub> levels were continuously monitored in the six rooms, half of which were maintained under ambient conditions (400 μmol CO<sub>2</sub>·mol<sup>-1</sup>), while the others received supplemental CO<sub>2</sub> (ambient + 300 μmol CO<sub>2</sub>·mol<sup>-1</sup>). Day length increased over the course of sapling growth, but day and night air temperatures were maintained at 26°C and 18°C, respectively, throughout the experiment. Photosynthetically active radiation did not differ among CO<sub>2</sub> treatments, averaging 928.3 ±

25.6 μmol·m<sup>-2</sup>·s<sup>-1</sup> (mean ± SE) when measured 1 m above pots at five locations in each room (solar noon, 31 July 1998). Saplings were watered as needed.

The trees were harvested 25 and 26 August 1998. Root and shoot data were collected from trees that had not been subjected to herbivory ( $n = 4$  individuals per room, except in five cases, where  $n = 3$ ). Roots were gently freed of adhering soil by repeated submersion in running water. The intact root systems, together with fresh roots retained on the sieves after pouring the wash water over 5-, 2-, and 0.5-mm mesh sieves, were cut and separated into coarse (>5 mm), small (2–5 mm), and fine (<2 mm) diameter classes. The fine roots were flash-frozen in liquid N<sub>2</sub>; these frozen samples were lyophilized for subsequent chemical determinations. The remaining root classes and shoots were oven-dried to constant mass (65°C for 72 h).

### Root chemistry

The freeze-dried roots were ground to pass a 20-mesh sieve prior to analysis. Tissue nitrogen was determined by Nesslerisation following Kjeldahl digestion (Allen 1989). Total nonstructural carbohydrates (free sugars and starch) were determined colorimetrically as glucose equivalents via a modification of the dinitrosalicylic acid method (Lindroth et al. 2001). Free sugars were extracted by sonicating ground fine roots in 80% ethanol (3 × 2 mL, 25 mg tissue). Starch was enzymatically converted to glucose prior to the colorimetric assay (Prado et al. 1998).

Secondary metabolites in the sapling root tissues consisted of a variety of tannin compounds and lignin. These phenolics included condensed tannins, which occurred in both tree species, together with hydrolysable tannins in maple (ellagitannins and gallotannins). Tannins were exhaustively extracted from ground tissues (100 mg) in 70% acetone (4 × 1 mL). Condensed tannin concentrations in the extracts were determined colorimetrically following hydrolytic conversion of proanthocyanidins to anthocyanidins in butanol – hydrochloric acid (Porter et al. 1986). Hydrolysable ellagitannins and gallotannins were determined as the respective acid-liberated ellagic and gallic acid monomers, following the methods of Wilson and Hagerman (1990) and Inoue and Hagerman (1988). Acid-detergent fibre content was measured gravimetrically following tissue digestion in a 0.5 M H<sub>2</sub>SO<sub>4</sub> solution of 5% hexadecyl-trimethylammonium bromide (Rowland and Roberts 1994). Klason (acid insoluble) lignin also was determined gravimetrically, following incubation of the acid-detergent fibre residues in 72% H<sub>2</sub>SO<sub>4</sub>.

Chemical concentrations (mg·g<sup>-1</sup> tissue) were corrected to an ash-free dry mass basis to account for soil contamination. Ash content was determined gravimetrically following loss-on-ignition (LOI: 550°C for 6–12 h). Carbon content was determined from ash-free dry mass, assuming that 50% of the organic material was elemental carbon (Allen 1989).

### Statistical analyses

The root chemistry data were subjected to analysis of variance in SAS (PROC MIXED, Littell et al. 1996). A split-plot experimental design was used, with the two levels of CO<sub>2</sub> as the whole plot factor, and the two tree species as levels of the subplot factor. Means were calculated using the LSMEANS statement in SAS (Littell et al. 1996) and are

**Table 1.** Biomass responses of paper birch and sugar maple to ambient (400  $\mu\text{mol}\cdot\text{mol}^{-1}$ ) and elevated (700  $\mu\text{mol}\cdot\text{mol}^{-1}$ )  $\text{CO}_2$  concentrations.

Variable	Birch		Maple	
	Ambient	Elevated	Ambient	Elevated
Whole-plant biomass (g)	101.8 (10.5)a	157.8 (9.6)b	145.3 (4.5)b	188.9 (13.8)c
Total root biomass (g)	35.3 (4.2)a	52.0 (2.5)b	80.4 (2.8)c	98.9 (5.6)d
Fine root biomass (g)	18.0 (2.2)a	29.2 (1.6)b	24.7 (3.0)a	27.5 (0.3)b

**Note:** Values represent means derived from three replicate glasshouse rooms (SE in parentheses). Within a row, means followed by the same letter do not differ significantly at  $P = 0.10$ .

**Table 2.** Root chemistry responses of birch and maple to ambient and elevated  $\text{CO}_2$ .

Variable	Birch		Maple	
	Ambient	Elevated	Ambient	Elevated
Nitrogen ( $\text{mg}\cdot\text{g}^{-1}$ )	21.9 (0.9)b	19.48 (1.3)a	29.8 (0.4)d	23.4 (1.5)c
Starch ( $\text{mg}\cdot\text{g}^{-1}$ )	57.2 (1.1)b	59.0 (6.0)b	41.5 (4.8)a	41.2 (3.9)a
Condensed tannins ( $\text{mg}\cdot\text{g}^{-1}$ )	60.1 (2.5)b	76.6 (2.8)c	53.9 (3.3)a	49.5 (1.6)a
Gallotannins ( $\text{mg}\cdot\text{g}^{-1}$ )	—	—	nd	nd
Ellagitannins ( $\text{mg}\cdot\text{g}^{-1}$ )	—	—	nd	nd
C/N	23.0 (0.9)b	26.1 (1.8)b	17.1 (0.3)a	22.2 (1.7)a
Fibre ( $\text{mg}\cdot\text{g}^{-1}$ )	504 (15)a	579 (43)b	572 (11)b	605 (18)c
Klason lignin ( $\text{mg}\cdot\text{g}^{-1}$ )	237 (10)a	228 (28)a	253 (5)b	264 (10)b
Lignin/nitrogen	10.9 (0.8)a	12.1 (2.1)a	8.9 (0.2)a	11.8 (1.4)a

**Note:** Values represent means derived from three replicate glasshouse rooms (SE in parentheses). Within a row, means followed by the same letter do not differ significantly at  $P = 0.10$ .

reported for each  $\text{CO}_2$  by species combination. Prior to analysis, responses of the individual trees, which were the measurement units, were averaged within rooms, the true replicated units in the model. Because there were only three replicate rooms per treatment,  $\alpha$  was set at  $P = 0.10$  to reduce potential type II statistical errors (Filion et al. 2000).

## Results and discussion

### Plant growth

Whole-plant biomass increased significantly under elevated  $\text{CO}_2$  (Table 1;  $P = 0.005$ ), consistent with expectation and regardless of species. Increases in total root mass with  $\text{CO}_2$  enrichment were significantly higher in birch compared with maple ( $P = 0.039$ ). Mean masses of the fine root fraction did not differ between species ( $P = 0.325$ ).

Within each species,  $\text{CO}_2$  enrichment did not alter root/shoot ratios (R/S), which were estimated as slope coefficients from the least-squares regression of total root mass against total shoot mass. The mean R/S, or slope coefficient (plus standard error of the estimate), of birch ( $0.50 \pm 0.02$ ) was significantly lower (species,  $P < 0.001$ ) than that of maple ( $1.15 \pm 0.04$ ); our estimate for the maple R/S was, in turn, at least 40% higher than that reported by Bazzaz et al. (1990). Moreover, in our experiment, fine roots constituted 14.5–21.5% of plant mass, regardless of  $\text{CO}_2$  treatment. These large percentages likely are not representative of fine root contributions to the biomass of naturally grown trees. Thus, the responses of glasshouse-grown, fertilized, and potted saplings can only be cautiously extrapolated to larger scale studies of open-grown plants in field soils.

Despite these disparities, our results agree in the direction of response with those of Lindroth et al. (1993) and Bazzaz

et al. (1990), who also found no differences in R/S with  $\text{CO}_2$  enrichment. The latter authors concluded that the growth of shade-tolerant and shade-intolerant species is enhanced differently by elevated  $\text{CO}_2$  (Ceulemans and Mousseau 1994). With its slower inherent growth rate, sugar maple showed a smaller relative whole-plant response to elevated  $\text{CO}_2$  than did birch (i.e., 30% for maple and 55% for birch, respectively, over ambient biomass values). Under  $\text{CO}_2$  enrichment, the shade-tolerant maple also maintained carbon allocation to belowground rather than aboveground biomass, while the converse was true in the shade-intolerant birch.

### Fine root chemistry

Chemical analyses revealed strong differences between species, and several significant effects of  $\text{CO}_2$  enrichment on root chemistry (Table 2). Nitrogen decreased with elevated  $\text{CO}_2$  exposure ( $P = 0.090$ ), but levels did not change uniformly between species (species  $\times$   $\text{CO}_2$ ,  $P = 0.028$ ). Overall, tissue nitrogen concentrations were highest in sugar maple and lowest in paper birch, which may simply reflect higher rates of fine root turnover in the shade-tolerant maple species (Ceulemans and Mousseau 1994). Indeed, Hendricks et al. (1993, cited in Gordon and Jackson 2000) have hypothesized that fine root life-span generally decreases as tissue nitrogen increases.

Tissue nitrogen concentrations in the fine roots of our potted saplings were 25–300% higher than those reported for the same northern hardwood species in both native forest stands (Gordon and Jackson 2000) and  $\text{CO}_2$  enrichment experiments (Pregitzer et al. 1995). Despite these differences between our study and others, N decreased in birch and maple fine roots in response to elevated  $\text{CO}_2$ , both in accordance with expectation and consistent with aboveground

responses of the same plants, as reported by Kopper (2001). Declines in N (12–22%) were comparable to those observed for fine roots in other CO<sub>2</sub> enrichment studies, including *Populus tremuloides* (Pregitzer et al. 2000; Zak et al. 2000a).

We observed a concomitant trend toward increased C/N ratios, consistent with our initial hypothesis, although values did not differ significantly between the two CO<sub>2</sub> treatments (Table 2;  $P = 0.163$ ). Birch roots had the highest C/N, regardless of CO<sub>2</sub> level (species,  $P < 0.001$ ). The slight increases in C/N that we observed with CO<sub>2</sub> enrichment mirrored relative tissue N declines observed for each species (Table 2). Moreover, C/N ratios were similar to those reported by Zak et al. (2000a) for nutrient-absorbing aspen roots <0.3 mm in diameter. Unlike Zak et al. (2000a), we did not subdivide fine roots into additional diameter classes. We were able to recover larger diameter, fleshy roots as well as woody roots 0.5–2.0 mm in diameter, but some of the very finest nutrient-absorbing roots were likely lost, despite care taken in washing the root systems. Nevertheless, fine root N concentrations differed significantly between our CO<sub>2</sub> treatments, while Pregitzer et al. (2000) found significant differences in N among treatments only for aspen roots <0.5 mm in diameter.

Fine roots also contained measurable quantities of starch (Table 2), while free sugars were not detectable (data not shown). Starch content of roots was not affected by CO<sub>2</sub> enrichment ( $P = 0.488$ ), but levels in birch were significantly greater than those in maple ( $P < 0.001$ ). Our results are consistent with those reported by King et al. (1997) for *Pinus taeda* and *Pinus ponderosa*, and by Pregitzer et al. (2000) for *Populus tremuloides*, which grew in low-nitrogen soil under high CO<sub>2</sub> concentrations. However, belowground responses did not completely accord with aboveground responses for the same trees. Kopper (2001) found that elevated CO<sub>2</sub> significantly increased the foliar starch concentrations of paper birch and sugar maple.

Our fine root results were partly consistent with the inverse relationship commonly observed between total non-structural carbohydrates and nitrogen (Mooney et al. 1995, cited in Zak et al. 2000b). Starch synthesis and amino acid production likely represent competing sinks for photosynthate, and as such, tissue concentrations of the two primary metabolites should be negatively correlated. Fine root starch and nitrogen were indeed negatively correlated when all data were combined (Spearman rank correlation:  $r_s = -0.556$ ,  $P < 0.003$ ,  $n = 43$ ), but strengths of the correlations differed among the various species and treatment combinations. Fine root starch and nitrogen in birch were negatively correlated at ambient CO<sub>2</sub> ( $r_s = -0.794$ ,  $P = 0.017$ ,  $n = 10$ ). This negative relationship disappeared under elevated CO<sub>2</sub> ( $r_s = 0.083$ ,  $P = 0.814$ ,  $n = 9$ ) suggesting that, at least in birch, competition for photosynthate was alleviated by CO<sub>2</sub> enrichment. No significant starch–nitrogen correlations were noted for sugar maple.

CO<sub>2</sub> enrichment produced few shifts in the concentrations of secondary metabolites. Supplemental CO<sub>2</sub> elicited species-specific responses in condensed tannin concentrations (CO<sub>2</sub> × species,  $P = 0.083$ ), consistent with our initial hypothesis, with levels increasing in birch but not in maple (Table 2). Condensed tannins were highest in birch, regard-

less of treatment condition (species,  $P = 0.033$ ). Hydrolysable tannins could not be detected in maple roots, irrespective of CO<sub>2</sub> level; levels of ellagic and gallic acid never exceeded the detection limit of 1 mg·g<sup>-1</sup>. Acid-detergent fibre content of the fine roots increased slightly with CO<sub>2</sub> enrichment (Table 2,  $P = 0.057$ ), and did not differ significantly between species ( $P = 0.245$ ). Lignin concentrations, in contrast, did not differ between CO<sub>2</sub> treatments but were 11% higher in maple than in birch (species,  $P = 0.045$ ). The same trends that were observed for C/N were evident for lignin/nitrogen, i.e., higher ratios under elevated CO<sub>2</sub> and highest ratios overall in birch, although neither CO<sub>2</sub> nor species effects were statistically significant ( $P = 0.412$  and  $P = 0.358$ , respectively). The absence or weakness of allelochemical responses in maple may reflect the lack of significant correlations between the primary metabolites starch and nitrogen at either CO<sub>2</sub> concentration. Moreover, starch concentrations in maple roots were not significantly and negatively correlated with condensed tannins or lignin concentrations, a result contrary to the observations made by Runion et al. (1999). They found that doubling of atmospheric CO<sub>2</sub> resulted in increased lignin and tannin concentrations while decreasing starch concentrations in the fine roots of *Pinus palustris*. In our study, starch was positively but weakly correlated with Klason lignin in maple roots grown at ambient CO<sub>2</sub> ( $r_s = 0.517$ ,  $P = 0.086$ ), and correlated with neither lignin ( $P = 0.898$ ) nor tannins ( $P = 0.353$ ) at elevated CO<sub>2</sub>.

The responses of the tree saplings to elevated CO<sub>2</sub> may be related to their successional status. As Bazzaz et al. (1990) have noted, early-successional species like paper birch should show greater plasticity of response to varying environmental conditions than late-successional species such as sugar maple. Paper birch added greater height and aboveground biomass than did sugar maple when both species were exposed to elevated CO<sub>2</sub>, but birch also showed stronger responses than maple in terms of chemical quality of the fine root tissues. Both species had the same amount of fine root biomass at the conclusion of the pot experiment. However, under elevated CO<sub>2</sub>, the higher condensed tannin concentrations that were present in the birch fine roots may offer these tissues greater protection against soil-borne pathogens and herbivores, and consequently, a competitive advantage to this faster growing species over slower growing, less well-defended species, such as sugar maple. Such small changes in species response to elevated CO<sub>2</sub> at an early life stage might, as Ceulemans and Mousseau (1994) have suggested, lead to important differences in response by the time these individuals have achieved adult status and attained a position in the forest canopy.

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