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Wood properties of trembling aspen and paper birch after 5 years of exposure to elevated concentrations of CO_2 and O_3

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Summary We investigated the interactive effects of elevated concentrations of carbon dioxide ($[CO_2]$) and ozone ($[O_3]$) on radial growth, wood chemistry and structure of five 5-year-old trembling aspen (*Populus tremuloides* Michx.) clones and the wood chemistry of paper birch (*Betula papyrifera* Marsh.). Material for the study was collected from the Aspen FACE (free-air CO_2 enrichment) experiment in Rhinelander, WI, where the saplings had been exposed to four treatments: control, elevated $[CO_2]$ (560 ppm), elevated $[O_3]$ (1.5 × ambient) and their combination for five growing seasons. Wood properties of both species were altered in response to exposure to the treatments. In aspen, elevated $[CO_2]$ decreased uronic acids (constituents of, e.g., hemicellulose) and tended to increase stem diameter. In response to elevated $[O_3]$ exposure, acid-soluble lignin concentration decreased and vessel lumen diameter tended to decrease. Elevated $[O_3]$ increased the concentration of acetone-soluble extractives in paper birch, but tended to decrease the concentration of these compounds in aspen. In paper birch, elevated $[CO_2]$ decreased and elevated $[O_3]$ increased starch concentration. The responses of wood properties to 5 years of fumigation differed from those previously reported after 3 years of fumigation.

Keywords: Betula papyrifera, carbon dioxide, cell wall, climate change, FACE, ozone, Populus tremuloides, wood chemistry, wood structure.

Introduction

Atmospheric concentrations of carbon dioxide ($[CO_2]$) and tropospheric ozone ($[O_3]$) are predicted to continue increasing during this century (IPCC 2001). Although it is well known that these greenhouse gases have opposing effects on the growth of trees as single factors, the long-term interactive effects of elevated $[CO_2]$ and $[O_3]$ on tree growth are less well documented. Previous field studies, performed as chamber experiments with trembling aspen (*Populus tremuloides* Michx.) and silver birch (*Betula pendula* Roth), showed compensation of the negative effect of elevated $[O_3]$ on growth by elevated $[CO_2]$ (Riikonen et al. 2004) depending on genotype (Dickson et al. 2001). Data from a larger aspen field study using the free-air CO_2 enrichment (FACE) technique showed that elevated $[O_3]$ negated or offset the positive growth responses to elevated $[CO_2]$ (Isebrands et al. 2001, Karnosky et al. 2003, King et al. 2005). In four FACE experiments conducted in forest stands, a 23% increase in forest net primary productivity was observed as $[CO_2]$ was elevated to 550 ppm, a value expected within the next few decades (Norby et al. 2005).

Effects of elevated $[CO_2]$ (e.g., Ceulemans and Mousseau 1994, Rey and Jarvis 1997, Norby et al. 1999) and elevated $[O_3]$ (e.g., Matyssek and Innes 1999, Kolb and Matyssek 2001) can depend on the stage of ontogeny because growth and resource allocation patterns change as a result of physical (e.g., tree size) and environmental (e.g., resource availability, competition) constraints or metabolic changes during ontogeny (e.g., changes in gene expression) (Kolb and Matyssek 2001). Sensitivity to O_3 may increase with increasing exposure time and tree size, as has been reported for silver birch (Oksanen 2003). These findings emphasize the importance of long-term experiments in understanding the effects of greenhouse gases on forest trees.

Most studies on the effects of elevated $[CO_2]$ and $[O_3]$ have been made on small trees, in chambers, and free of competition (Long et al. 2004). Only a few CO_2 (Ceulemans et al. 2002) and O_3 exposure studies have been conducted with forest trees, and rarely have data been obtained on how secondary xylem structure and the chemical composition of hardwoods respond to combined exposure to elevated $[CO_2]$ and $[O_3]$ (Kaakinen et al. 2004, Kostiainen et al. 2006). A thorough understanding of the effects of future climate on stem wood prop-

erties is critical because tree stems have important biological roles in water, nutrient and solute transport, as storage organs and in providing physical support. Prediction of the changes is challenging because, in addition to the growth conditions, wood structure and chemical composition vary with tree age, among trees and among genotypes.

According to previous findings, elevated [O_3] as a single factor reduced annual xylem increment, size of xylem cells (Matyssek et al. 2002), vessel percentage and nitrogen concentration, and increased cell wall percentage (Kostiainen et al. 2006) in silver birch. Elevated [O_3] also increased lignin concentration in trembling aspen and paper birch (*Betula papyrifera* Marsh.), and caused an increase in cell wall thickness and a decrease in vessel lumen diameter in aspen (Kaakinen et al. 2004). Effects of elevated [CO_2] on deciduous tree species have been found to vary. In some species, elevated [CO_2] increases wood density (*Liquidambar styraciflua* L., Rogers et al. 1983; *Populus deltoides* Bartr. ex Marsh., Druart et al. 2006), whereas in other species wood density (*Fagus sylvatica* L., Beismann et al. 2002; *Quercus ilex* L., Gartner et al. 2003) shows no response to [CO_2]. Changes in xylem structure in response to elevated [CO_2] include decreased cell wall thickness (*Populus* × *euramericana* and *P. alba* L., Luo et al. 2005), increased number and total cross-sectional area of vessels (*Quercus robur* L., Atkinson and Taylor 1996), as well as increased vessel lumen area (*Quercus ilex*, Gartner et al. 2003) and diameter (*Populus nigra* L. and *P.* × *euramericana*, Luo et al. 2005). Contrasting results include no responses in vessel properties of *Prunus* sp. (Atkinson and Taylor 1996). Observed changes in stem wood chemistry in response to elevated [CO_2] include decreased concentrations of lignin (Cotrufo and Ineson 2000, Blaschke et al. 2002, Kostiainen et al. 2006) and cellulose (Kostiainen et al. 2006), and increased concentrations of extractives (Kostiainen et al. 2006), starch (Kaakinen et al. 2004, Kostiainen et al. 2006) and soluble sugars (Kaakinen et al. 2004). Interactive effects of elevated [CO_2] and [O_3] on wood properties have been reported in two studies (Kaakinen et al. 2004, Kostiainen et al. 2006), where elevated [CO_2] ameliorated some adverse effects of elevated [O_3].

We used wood materials from the Aspen FACE experiment (www.aspenface.mtu.edu) to investigate the effects of 5 years of exposure to elevated [CO_2] and [O_3] alone and in combination on radial growth, wood chemistry and structure of trembling aspen clones and the wood chemistry of paper birch. Our primary objective was to assess whether the responses of wood properties to 3 years of exposure (Kaakinen et al. 2004) were maintained as the canopies closed at the age of 5 years. Accordingly, we examined the annual rings from years 2001–2002, formed after the first assessment to test three hypotheses: (1) the responses of the species to elevated [CO_2] and [O_3] are the same after 5 years of exposure as after 3 years of exposure or have become stronger; (2) because of canopy closure, the slow-growing aspen genotypes have become more suppressed by the dominant genotypes in different treatments; and (3) the positive effects of elevated [CO_2] are largely counteracted by the negative effects of elevated [O_3].

Materials and methods

Materials

Material for this study was collected from the Aspen FACE (free-air CO_2 enrichment) experiment at Rhinelander, WI (45°6′ N, 89°5′ W). The FACE facility was constructed in 1997 and contains three replicate FACE rings (30 m in diameter) for each of the four treatments (ambient air, elevated [CO_2], elevated [O_3], elevated [CO_2] + elevated [O_3]). Exposure to the treatments started in 1998. The replicates were blocked to provide adequate coverage of the 32-ha site. Details of the experimental site, design and treatments of the experiment are provided by Dickson et al. (2000).

Trees were exposed to elevated [CO_2] and [O_3] during daylight hours (0700–1900 h) of each growing season (Dickson et al. 2000). Each fumigation unit included a series of 32 vertical vent pipes forming a ring around the perimeter of each plot. A high-volume fan mixed ambient air with pure CO_2 or O_3 gas and dispensed it from the perimeter vent pipes on the upwind side of each ring at canopy height. Atmospheric [CO_2] and wind speed and direction were monitored (LI-6252 infrared gas analyser, Li-Cor) continuously. The target value of the elevated [CO_2] treatment was ambient air enriched with 200 μl l^{-1} of CO_2 (the projected atmospheric [CO_2] for about year 2050) and the target value for the elevated [O_3] treatment was 1.5 times ambient [O_3]. During growing seasons 2001 and 2002, [CO_2] in the ambient treatments was 356 and 361 μl l^{-1} and in the elevated treatments it was 528 and 537 μl l^{-1}, respectively (King et al. 2005). The [O_3] in the ambient treatments was 38.8 and 33.1 nl l^{-1} and in the elevated treatments it was 52.6 and 49.5 nl l^{-1} during growing seasons 2001 and 2002, respectively (King et al. 2005). Fumigation started at bud break in the spring and ended at leaf senescence in the fall; the duration of exposure was 143 days in growing season 2001 and 138 days in 2002 (King et al. 2005). No supplemental fertilizer or water was applied to the plots.

The plots were planted at 1 × 1 m spacing with five vegetatively propagated trembling aspen (*Populus tremuloides*) clones in one half of each ring, a trembling aspen clone (Clone 216) interplanted with paper birch (*Betula papyrifera*) seedlings in a quarter of each plot, and aspen Clone 216 interplanted with sugar maple (*Acer saccharum* Marsh.) in the remaining quarter. In this study, wood properties of sugar maple were not analyzed. Three aspen clones were included in the experiment according to their differing sensitivity to O_3: Clones 216 and 271 were relatively O_3 tolerant, and Clone 259 was relatively O_3 sensitive (Karnosky et al. 1996). The other two clones were included according to their leaf phenology and differing response to elevated [CO_2]. Early leaf-fall genotype (42E) had higher photosynthetic rates in elevated [CO_2] than late leaf-fall genotype (8L) (Kubiske et al. 1998).

In July 2002, after 5 years of fumigation treatments, one tree of each aspen clone from the pure aspen half and one birch from the aspen-birch quadrant were harvested from each of the 12 FACE rings. Mean values of all aspen clones per treatment are presented because of the small number of sample trees. Trees harvested were selected to represent the full range of

heights of each clone and species, consisting of those within 20% of the minimum height, within 20% of the mean and within 20% of the maximum height. Trees were hand cut about 3 cm above the soil surface. Stem dry mass was determined for each harvested tree. Stem wood samples were collected from the stem base and from 40% of stem total height to ensure representative sampling for the whole tree. Stem sections were transported in dry ice (−80 °C) to the Suonenjoki Research Unit of the Finnish Forest Research Institute and stored at −20 °C until processed.

Chemical analysis

Wood chemistry of aspen and birch was analyzed at both stem sampling heights. In preparation for chemical analyses, the bark, phloem and cambium were first removed from the wood discs. Then the annual rings formed during the treatment years 2001–2002 were separated from the older wood. Sections of these annual rings were further cut into picks (about 2–3 mm in thickness) that were freeze-dried to constant mass, then milled (Polymix A10, Kinematica AG, Switzerland) to powder at −25 °C and stored at −20 °C.

Milled stem wood (2 g) was extracted in acetone (150 ml) by the Soxhlet method according to the SCAN–CM (1994) standard for the measurement of extractives and to yield extractive-free samples. Alpha-cellulose, uronic acids, gravimetric lignin and acid-soluble lignin were analyzed in the extractive-free samples as described by Anttonen et al. (2002). Soluble sugars were extracted from freeze-dried and milled wood (100 mg) with 15 ml of 80% aqueous ethanol. Starch was extracted from the residue with 20 ml of 30% perchloric acid. Soluble sugars and starch were measured by the anthrone method (Hansen and Møller 1975). Total nitrogen (N) concentration and C/N ratio of the stem wood were measured with a CHN–1000 analyzer (Leco Co., St. Joseph, MI).

Microscopic analysis

The structure of aspen wood was analyzed only at the stem base. The widths of annual rings were measured with a digital caliper on stem discs in four directions, and the mean stem diameter was calculated from the measurements. Preparation of cross sections and image analysis of wood anatomical properties were performed as described by Kaakinen et al. (2004). Because of the early harvesting time, lignification of the last-formed annual ring was first tested and confirmed by alcian blue–safranin staining, and the structural analyses were made in wood produced at the beginning of the growing season. Safranin stains lignified and alcian blue stains nonlignified cellulosic cell walls. Microscopic measurements of cell wall and vessel percentage, and fiber and vessel lumen diameter were made on 16-µm-thick safranin-stained cross sections—prepared from the annual ring formed in year 2002—with an Olympus BX60 (Olympus Optical) microscope connected to a Spot insight B/W video camera (Diagnostic Instruments, Sterling Heigths, MI) and Image-Pro plus 4.1.1.2 for Windows (Media Cybernetics, Silver Spring, MD) software. The image area was 1600 × 1200 pixels (1.18 × 0.89 mm).

In the analyses, three images of cross sections per annual ring were captured at 10× magnification about 0.05 mm from the border of the annual ring. The resolution of the images was 0.74 µm pixel^{-1}. In the analyses of fiber and vessel lumen diameters, cells that were partly out of the image or whose cell walls were damaged during section preparation were excluded. Cell lumina larger than 625 µm^2 (selected as the threshold value for vessels) were considered vessels. The selected threshold differs from the value in our previous study (Kaakinen et al. 2004), because the sampling procedures were different. In the previous study, sample trees represented the mean size within treatments/clones, whereas in the present study, trees were selected from three stem height classes. As a result, some trees in the present study were smaller than in the previous study, and so a lower threshold area for vessels was used. The lumen diameter of fibers was measured for cell lumina equal to 15–500 µm^2. Cell wall percentage was determined as the proportion of cell walls in the total image area. Vessel percentage was determined as the area occupied by vessel lumina in the total image area.

Statistical analysis

The main effects of elevated [CO_2] and [O_3] on wood chemistry were analyzed by mixed-model analysis of variance (ANOVA) with CO_2, O_3 and clone (in aspen) as fixed factors and treatment replicate (block) as a random factor. For the wood chemistry data, the mean of the two stem sampling heights was analyzed. The distance from the pith to the measured annual ring correlated with the measured structural parameters. Therefore, in the statistical analyses of wood structure, distance from the pith was used in the analysis of covariance (ANCOVA) model as a covariate to reduce the error associated with measuring trees of different diameters. Treatment effects were considered significant at $P < 0.05$.

Results

Growth and stem wood structure of aspen

Elevated [CO_2] in the presence of ambient [O_3] tended to increase, and elevated [O_3] in the presence of ambient [CO_2] tended to decrease, stem radial growth in aspen after a 5-year exposure, whereas stem radial growth of trees in the combined elevated [CO_2] + [O_3] treatment did not differ from controls (Figure 1A). The main effect of elevated [CO_2] on stem diameter was near significant ($P = 0.093$), and no significant effect of elevated [O_3] was observed (Table 1), partly because of large variation in sizes of the sample trees. Annual ring increment and differences between treatments showed a decreasing trend during the exposure period (Figure 1B), partly because of continuously increasing stem circumference (Figure 1A). Elevated [O_3] tended to decrease vessel lumen diameter ($P < 0.1$; Table 1). There was an interaction between elevated [O_3] and clone on fiber lumen diameter ($P = 0.004$), because the diameter increased in Clone 8L in elevated [O_3]. There was a near significant interaction between elevated [CO_2] and elevated [O_3] on fiber lumen diameter (Table 1). Elevated [CO_2] and el-

evated [O_3] as single factors tended to increase fiber lumen diameter compared with control trees ($P < 0.1$), whereas the combined elevated [CO_2] + [O_3] treatment had no effect on fiber lumen diameter ($P > 0.1$).

Stem wood chemistry of aspen

Stem wood chemistry of trembling aspen was affected by both elevated [CO_2] and elevated [O_3]. Uronic acids, which are constituents of hemicellulose (Pereira et al. 2003), were decreased by elevated [CO_2], irrespective of aspen clone (Table 2); however, the decrease in concentration of uronic acids may not lead to an overall decrease in hemicellulose. Interactions between elevated [CO_2] and clone were observed on the concentrations of acetone-soluble extractives ($P = 0.027$) and soluble sugars ($P = 0.027$). Elevated [CO_2] increased the concentrations of extractives and soluble sugars in Clone 42E and decreased the concentration of soluble sugars in Clone 8L.

Elevated [O_3] decreased the concentration of acid-soluble lignin and tended to decrease the concentrations of extractives and soluble sugars ($P < 0.1$; Table 2). However, these changes in concentrations did not result in changes in absolute contents of the components in the tree stems (and by extension, per unit land area) because of the accompanying increase in stem biomass in response to elevated [CO_2] and decrease in stem biomass in response to elevated [O_3] (Figure 2A). For example, although elevated [CO_2] decreased the concentration of uronic acids, the total stem content of uronic acids increased in trees fumigated with elevated [CO_2]. The major components of the cell wall, gravimetric lignin and α-cellulose, were unaffected by the treatments, and no interactions between elevated [CO_2] and elevated [O_3] on stem wood chemistry of aspen were observed (Table 2).

Stem wood chemistry of paper birch

In paper birch, the concentrations of acetone-soluble extractives and starch were affected by both elevated [CO_2] and [O_3] (Table 2). Elevated [CO_2] increased the concentration of extractives but decreased that of starch, whereas elevated [O_3] increased the concentration of both extractives and starch. As with aspen, these changes in concentrations in paper birch were diluted by the larger differences among treatments in stem biomass production (Figure 2B). There was no interaction between elevated [CO_2] and elevated [O_3] on stem wood chemistry of paper birch (Table 2).

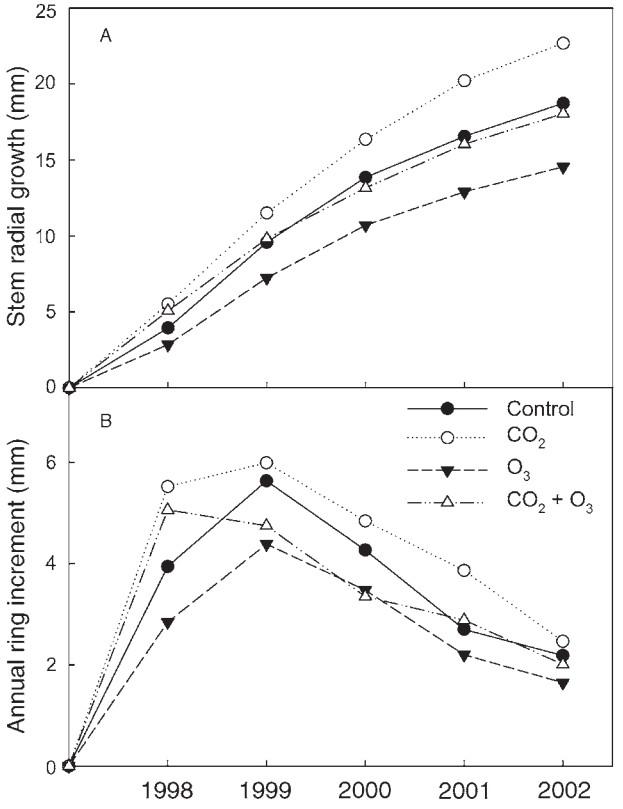

Figure 1. (A) Stem radial growth and (B) annual ring increment measured at the base of aspen stems after 5 years (1998–2002) of exposure to elevated [CO_2] and elevated [O_3] alone or in combination. Values are treatment means of five clones.

Discussion

Growth and wood properties in the Aspen FACE experiment

The long-term Aspen FACE experiment provides an opportunity to monitor changes in tree performance among treatments

Table 1. Effects of 5 years (1998–2002) of exposure to elevated [CO_2] and elevated [O_3] alone or in combination on aspen stem diameter and wood structure. Data shown are treatment means with the five clones pooled together, standard deviation in parentheses. Values of $P > 0.1$ are indicated as non-significant (ns). Abbreviations and symbols: ↑ = increase; ↓ = decrease; ns = not significant; and $n = 13–15$.

Treatment	Vessel %	Vessel lumen diameter (μm)	Fiber lumen diameter (μm)	Cell wall %	Stem diameter (mm)
Control	29.6 (5.6)	51.5 (9.3)	10.6 (1.0)	44.5 (4.6)	36.3 (16.2)
CO_2	29.6 (5.8)	56.2 (10.8)	11.3 (1.3)	41.9 (4.8)	45.4 (22.6)
O_3	29.3 (4.4)	49.4 (8.2)	10.8 (0.7)	42.6 (3.3)	29.1 (14.4)
$CO_2 + O_3$	29.0 (5.5)	53.8 (8.9)	10.9 (0.8)	42.9 (5.0)	36.1 (15.8)
P values					
CO_2	ns	ns	ns	ns	0.093↑
O_3	ns	0.058↓	ns	ns	ns
$CO_2 \times O_3$	ns	ns	0.084	ns	ns

Table 2. Effects of 5 years (1998–2002) of exposure to elevated [CO_2] and elevated [O_3] alone or in combination on the chemical composition of aspen and paper birch wood (% of dry mass), with data for the five aspen clones pooled. Data shown are the treatment means with standard deviation in parentheses. Values of $P > 0.1$ are considered non-significant (ns). Values of $P < 0.1$ but > 0.05 are italicized. Abbreviations and symbols: ↑ = increase; ↓ = decrease; and ns = not significant. The number of independent replicates is 12–15 for aspen and 2–3 for birch.

Treatment	Cellulose	Gravimetric lignin	Acid-soluble lignin	Uronic acids	Extractives	Starch	Soluble sugars	C	N	C/N
Aspen										
Control	48.2 (1.5)	20.5 (1.2)	2.0 (0.2)	5.2 (0.3)	3.4 (0.6)	4.5 (0.5)	1.3 (0.3)	48.7 (0.2)	0.17 (0.05)	297 (59)
CO_2	49.1 (1.6)	20.5 (0.9)	1.9 (0.2)	5.1 (0.2)	3.3 (0.9)	4.3 (0.3)	1.4 (0.5)	48.6 (0.3)	0.17 (0.02)	289 (45)
O_3	48.8 (1.6)	20.8 (1.2)	1.9 (0.2)	5.2 (0.2)	3.1 (0.6)	4.5 (0.6)	1.2 (0.3)	48.5 (0.5)	0.17 (0.02)	285 (31)
$CO_2 + O_3$	48.8 (1.4)	20.5 (1.0)	1.9 (0.2)	5.2 (0.2)	3.1 (0.6)	4.5 (0.7)	1.2 (0.3)	48.6 (0.3)	0.16 (0.03)	313 (47)
P values										
CO_2	ns	ns	ns	0.042↓	ns	ns	ns	ns	ns	ns
O_3	ns	ns	0.040↓	ns	*0.082*↓	ns	*0.086*↓	ns	ns	ns
$CO_2 \times O_3$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Birch										
Control	40.5 (2.8)	20.2 (1.0)	2.2 (0.1)	4.8 (0.3)	4.0 (0.3)	6.9 (0.5)	1.0 (0.0)	48.9 (0.2)	0.19 (0.02)	260 (29)
CO_2	39.9 (0.8)	20.5 (0.5)	2.1 (0.1)	5.0 (0.3)	4.6 (0.2)	6.7 (0.5)	1.2 (0.2)	49.0 (0.2)	0.21 (0.03)	237 (37)
O_3	39.5 (0.7)	20.0 (0.7)	2.1 (0.2)	4.8 (0.4)	4.5 (0.2)	8.4 (0.2)	1.3 (0.2)	48.9 (0.1)	0.21 (0.03)	235 (34)
$CO_2 + O_3$	38.6 (0.7)	19.7 (0.6)	2.1 (0.0)	4.7 (0.0)	4.8 (0.1)	7.4 (0.2)	1.1 (0.1)	49.0 (0.0)	0.20 (0.01)	249 (22)
P values										
CO_2	ns	ns	ns	ns	0.004↑	0.047↓	ns	ns	ns	ns
O_3	ns	ns	ns	ns	0.007↑	0.012↑	ns	ns	ns	ns
$CO_2 \times O_3$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

and species over time. Although the trees entered canopy closure during 2002, the 5-year-old aspen and paper birch trees were still producing juvenile wood. However, because foliage biomass becomes uncoupled from stem growth after canopy closure in young stands (Miller 1995), stem growth may not be accompanied by proportional increments in foliage biomass (King et al. 2005). In our study, elevated [CO_2] tended to increase stem diameter growth in trembling aspen, which accords with previous data from the Aspen FACE experiment showing a significant increase in stem diameter (Isebrands et al. 2001, Karnosky et al. 2003, 2005, King et al. 2005). A recent study by Kubiske et al. (2006), however, suggested that, although elevated [CO_2] enhanced aspen growth each year during a 7-year follow-up, the enhancement of growth diminished year by year. The trend of declining growth response to elevated [CO_2] was influenced by interannual variability in climate, but there was no evidence that the growth responses declined systematically with increasing limitations in N or water (Kubiske et al. 2006).

Sapling responses to the 5-year exposure to elevated [CO_2] and [O_3] differed from those observed in saplings exposed for 3 years to the same treatments at the same site (for results of the 3-year exposure see Kaakinen et al. 2004) (Table 3). Possible reasons for the differences include ontogenetic changes, increased competition for resources following canopy closure and interannual climatic variability. In a 7-year survey at the Aspen FACE site, Kubiske et al. (2006) found that relative growth rate of aspen varied considerably from year to year, with growth responses to elevated [CO_2] and [O_3] interacting strongly with interannual variability in climatic conditions. Thus, the amount of photosynthetically active radiation during the current year and temperature at the end of the previous year explained 20–63% of the annual variation (Kubiske et al. 2006). The differential wood property response between the saplings at the end of the 3-year and 5-year exposures, covering annual rings 1997–2000 and 2001–2002, may indicate that the growth response to elevated [CO_2] and [O_3] paralleled the decreasing July photosynthetic photon flux (PPF) and the decreasing previous October temperature in 2001–2002, but not in 1999–2000 (Kubiske et al. 2006). July PPF influences the amount of photosynthates available for stem growth, whereas October temperature affects the amount of stored carbohydrate available to support early growth the following year (Kubiske et al. 2006).

We observed ontogeny-dependent changes in wood chemical composition during the experiment. The 5-year-old aspen trees tended to have lower concentrations of cellulose and carbon and higher concentrations of gravimetric lignin and extractives than the 3-year-old trees. The 3-year-old aspen stems were more flexible and thus may have formed more tension wood than the more rigid 5-year-old stems, resulting in comparatively lower lignin and higher cellulose concentrations because of reduced lignification and the presence of a cellulose-rich gelatinous layer in the tension wood fibers (Andersson-Gunnerås et al. 2006). However, this reasoning does not apply to paper birch, because the concentrations of cellulose, carbon and nitrogen were higher in the 5-year-old trees than in the 3-year-old trees.

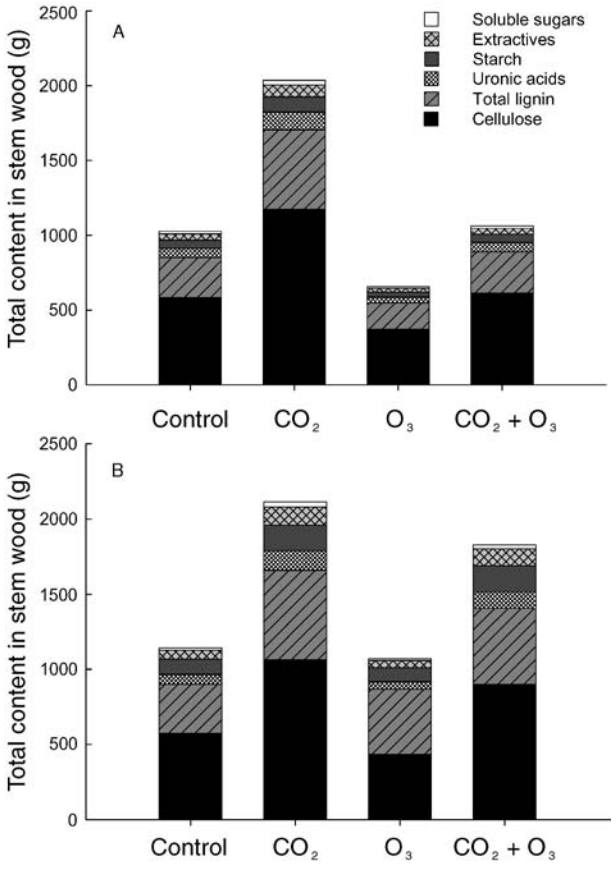

Figure 2. Effects of 5 years (1998–2002) of exposure to elevated [CO_2] and elevated [O_3] alone or in combination on dry mass and absolute contents (g $stem^{-1}$) of chemical compounds in (A) aspen and (B) paper birch stem wood. Values are treatment means with the five aspen clones pooled.

Table 3. Comparison of treatment effects on the radial growth and wood properties of aspen and paper birch sampled after 3 years of exposure in 2000 (Kaakinen et al. 2004) and after 5 years of exposure in 2002. Chemical analyses were done on pooled material from annual rings 1997–2000 (Kaakinen et al. 2004) and 2001–2002 (this study) and the structural analyses were examined in annual rings 2000 and 2002, respectively. Significant effects ($P < 0.05$) are indicated. Abbreviations and symbols: ↓ = decrease; ↑ = increase; ↓↑ = decrease or increase depending on clone; ns = not significant; * = interaction with clone. Italicized parameters show a trend ($0.05 < P < 0.1$).

Treatment	Three-year exposure	Five-year exposure
Aspen		
CO_2	Stem diameter↑*	*Stem diameter*↑
	Soluble sugars↑*	Soluble sugars↓↑*
	Starch↑*	Extractives↑*
O_3	Fiber lumen diameter↓*	Fiber lumen diameter↑*
	Vessel lumen diameter↓	*Vessel lumen diameter*↓
	Cell wall %↑	Acid-soluble lignin↓
	Stem diameter↓	*Extractives*↓
	Gravimetric lignin↑*	*Soluble sugars*↓
	Total lignin↑*	
	Nitrogen↓*	
$CO_2 + O_3$	*Cellulose*	*Fiber lumen diameter*
	Total lignin	
	Nitrogen	
Birch		
CO_2	ns	Extractives↑
		Starch↓
O_3	*Total lignin*↑	Extractives↑
		Starch↑
$CO_2 + O_3$	*Gravimetric lignin*	ns
	Total lignin	

Effects of elevated [CO_2] on wood structure and wood chemistry

None of the structural variables measured were affected by the elevated [CO_2] treatment. This means that the tendency for increased diameter growth in elevated [CO_2] was the result of an increase in number of vessels and fibers rather than an increase in size of individual cells. It also indicates that the production of vascular and stem tissue relative to tree biomass was unchanged.

It was expected that the previously reported increase in photosynthesis of aspen clones in response to elevated [CO_2] (Karnosky et al. 2005) would result in increased concentrations of soluble carbon compounds in the stem wood, because the presence of nonstructural carbohydrates (starch and soluble sugars) in tree tissues is considered a measure of carbon available for growth (Körner 2003). We found changes in concentrations of acetone-soluble extractives and soluble sugars in two clones that had been selected for study because of their differing response to elevated [CO_2] (Kubiske et al. 1998). The concentrations of extractives and soluble sugars increased in Clone 42E, which Kubiske et al. (2007) identified as the most productive clone after 7 years of exposure to elevated [CO_2], indicating an enhanced capacity for carbohydrate production. An accumulation of extractives in response to elevated [CO_2] was also observed in 10-year-old silver birch (Kostiainen et al. 2006). In contrast to our previous study (Kaakinen et al. 2004), the concentration of soluble sugars decreased in Clone 8L, which has shown reduced stem volume growth (Karnosky et al. 2005) and photosynthesis (Kubiske et al. 1998) in response to elevated [CO_2].

In paper birch, elevated [CO_2] caused changes in nonstructural compounds: an increase in extractives and a decrease in starch, neither of which was affected after 3 years of exposure. Photosynthesis provides carbon compounds for several processes that can roughly be grouped as growth, storage and defense. Extractives include mostly secondary metabolites, which have important roles in defense against pathogens and other biotic attacks (Pereira et al. 2003), and are composed of fats, waxes, triterpenoids and steroids (Sjöström and Westermark 1999). In paper birch wood, the opposite responses of extractives and starch to elevated [CO_2] (increase in extractive and decrease in starch) suggest that carbon partitioning favors the production of high-energy-demanding sec-

ondary compounds instead of starch. The reasons for the increased synthesis of secondary metabolites in the elevated [CO_2] treatment are not known. The decreased starch concentration in wood may be associated with the end of July sampling time, which was before the onset of leaf senescence. Starch accumulation often occurs in green foliage in response to elevated [CO_2] (e.g., Riikonen et al. 2005), as was also found in birch leaves at our study site during the second exposure year (Lindroth et al. 2001). Thus, most of the carbohydrate reserves may still have been in leaves or were being used for growth or for protection of the trees at the July sampling time.

Effects of elevated [O_3] on wood structure and wood chemistry

We found no significant effect of elevated [O_3] on stem diameter, and among the structural variables examined, only vessel lumen diameter was affected. In a previous study with the aspen clones, we observed decreased stem diameter growth in response to a 3-year exposure to elevated [O_3], which is in line with other data from the Aspen FACE site (Isebrands et al. 2001, Karnosky et al. 2005, King et al. 2005), and parallels changes in xylem cell structure such as decreased vessel lumen diameter. Vessel size has been shown to correlate with circumferential stem growth, and also with climatic conditions such as precipitation (Schume et al. 2004). Narrow vessels are hydraulically less efficient in water transport, but have a lower risk of embolism and cavitation than wide vessels (Schume et al. 2004). Karnosky et al. (2005) reported that elevated [O_3] had a negative effect on leaf area index and leaf display duration. Thus, the decrease in vessel lumen diameter in response to elevated [O_3] may be linked to lower transpirational leaf area and lower demand for water transport (Joyce and Steiner 1995). It is known that the morphogenesis of leaves and secondary vasculature in the stem of woody plants are closely coordinated (Isebrands 1972), and the decrease in vessel lumen diameter may be another example of coordinated development. We observed that elevated [O_3] increased fiber lumen diameter in Clone 8L, which contrasts with our earlier finding that a 3-year exposure to elevated [O_3] decreased fiber lumen diameter. The observed change in fiber lumen diameter response may be associated with the competitive status of the genotypes. Based on a 7-year follow-up study, Clone 8L showed no decrease in volume growth in response to elevated [O_3] (Kubiske et al. 2007).

The wood chemistry responses to elevated [O_3] differed between the 3-year and 5-year exposure times in both aspen and paper birch. The previously observed increase in total lignin concentration in response to a 3-year fumigation with elevated [O_3] did not occur in either aspen or paper birch following a 5-year fumigation period, although a minor component of total lignin concentration i.e., acid-soluble lignin, decreased. Increased lignin concentration after the 3-year fumigation was hypothesized to be linked to the observed up-regulation of the phenylpropanoid pathway in O_3-exposed trees (Wustman et al. 2001). There is no recent study from the Aspen FACE site on the activation of the phenylpropanoid pathway, and thus it is unclear whether the lack of lignin response was associated with a down-regulation of protective metabolism against oxidative stress under elevated [O_3] conditions. The concentrations of nonstructural compounds in both aspen and paper birch were altered by elevated [O_3]. In aspen, a lower concentration of extractives can be a sign of decreased photosynthesis in response to elevated [O_3] (Karnosky et al. 2003) and of reduced carbon availability for chemical defense. A different allocation pattern was seen in paper birch, with increased concentrations of extractives and starch perhaps indicating greater allocation of carbon to storage and chemical defense in response to both elevated [CO_2] and [O_3].

The only $CO_2 \times O_3$ interaction we found was on aspen wood structure: the increases in fiber lumen diameter in response to elevated [CO_2] and [O_3] alone were counteracted by the combined treatment. After the 3-year exposure, there were significant interactions between elevated [CO_2] and [O_3] on the wood chemistry of the saplings. An increase in lignin concentration in response to elevated [O_3] in aspen and birch was ameliorated by elevated [CO_2], whereas the combined treatment counteracted the CO_2-induced decrease in cellulose and decreased nitrogen concentration in aspen. None of these interactions were maintained over two more years of exposure. An interaction between elevated [CO_2] and [O_3] on hardwood xylem anatomy has been reported previously by Kostiainen et al. (2006), with elevated [CO_2] ameliorating the O_3-induced decrease in vessel percentage in silver birch. Based on these two studies, it seems that elevated [CO_2] and [O_3] can counteract each other's effects on wood properties.

In conclusion, the wood chemical composition of aspen and birch differed: after 5 years of fumigation, aspen had a higher concentration of structural compounds (cellulose and uronic acids), whereas birch had a higher concentration of soluble carbon compounds (acetone -soluble extractives and starch). The same trend was seen in trees fumigated for 3 years. However, the difference in cellulose concentration between aspen and birch decreased and the difference in total lignin concentration disappeared after 5 years of exposure, indicating an effect of tree age on wood properties. Differences in carbon partitioning between the species were also apparent when calculated on a stem mass basis.

The responses of wood properties of aspen and paper birch to 5 years of fumigation with elevated [CO_2] or elevated [O_3] or both, during which crown closure occurred, differed from the results obtained after 3 years of fumigation before crown closure (Kaakinen et al. 2004). In aspen and paper birch, a tendency for increased stem diameter in elevated [CO_2] and decreased vessel lumen diameter in elevated [O_3] were the only consistent treatment effects observed in both datasets. Thus our hypothesis that the responses to elevated [CO_2] and [O_3] would be the same after 5 years of exposure as after 3 years of exposure was only partially confirmed. The observed changes in tree responses to elevated [CO_2] and [O_3] over time may have implications for both the ecology and utility of these species. Increased growth in response to elevated [CO_2] can be foreseen to shorten rotation lengths, with only moderate

changes in wood properties. In response to elevated [O_3], stem wood production decreased and was accompanied by changes in vessel properties, which may indicate decreasing efficiency of water and nutrient transport. The different responses of wood properties to the 5-year fumigation compared with the 3-year fumigation highlight the importance of long-term experiments. Responses may change over the years because of aging: as cambium ages, gene expression changes in relation to diameter growth producing differences in wood chemistry and structure (Savidge 2000). Other factors possibly contributing to the variable responses of wood properties include increased competition for resources as a result of canopy closure and acclimation to the treatments during exposure. Less predictable factors may also influence tree responses to elevated [CO_2] and [O_3], such as year-to-year variability in fumigation dose, nutrient relations, pathogens and weather.

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