

Tropospheric O₃ moderates responses of temperate hardwood forests to elevated CO₂: a synthesis of molecular to ecosystem results from the Aspen FACE project

D. F. KARNOSKY,^{1†} D. R. ZAK,² K. S. PREGITZER,^{1,5} C. S. AWMACK,³
J. G. BOCKHEIM,⁴ R. E. DICKSON,⁵ G. R. HENDREY,⁶ G. E. HOST,⁷
J. S. KING,¹ B. J. KOPPER,³ E. L. KRUGER,³ M. E. KUBISKE,⁵
R. L. LINDROTH,³ W. J. MATTSON,⁵ E. P. MCDONALD,⁵ A. NOORMETS,⁸
E. OKSANEN,⁹ W. F. J. PARSONS,³ K. E. PERCY,¹⁰ G. K. PODILA,¹¹
D. E. RIEMENSCHNEIDER,⁵ P. SHARMA,¹ R. THAKUR,¹ A. SÔBER,¹²
J. SÔBER,¹ W. S. JONES,¹ S. ANTONEN,¹³ E. VAPAAVUORI,¹³
B. MANKOVSKA,¹⁴ W. HEILMAN¹⁵ and J. G. ISEBRANDS¹⁶

¹School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, Michigan 49931, USA; ²University of Michigan, 430 E. University, Ann Arbor, Michigan 48109, USA; ³University of Wisconsin, 1630 Linden Drive, Madison, Wisconsin 53706, USA; ⁴University of Wisconsin, 1525 Observatory Drive, Madison, Wisconsin 53706, USA; ⁵USDA Forest Service, North Central Research Station, Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, Wisconsin 54501, USA; ⁶Brookhaven National Laboratory, 1 South Technology Street, Upton, New York 11973, USA; ⁷University of Minnesota-Duluth, Natural Resources Research Institute, 5013 Miller Trunk Highway, Duluth, MN 55811, USA; ⁸University of Toledo, Department EEES, LEES Laboratory, Mail Stop 604, Toledo, Ohio 43606, USA; ⁹University of Kuopio, PO Box 1627, 70211 Kuopio, Finland; ¹⁰Natural Resources Canada, Canadian Forest Service, PO Box 4000, Fredericton, New Brunswick, Canada E3B 5P7; ¹¹Biological Sciences Department, University of Alabama-Huntsville, 301 Sparkman Drive, WH14Z, Huntsville, Alabama 35899, USA; ¹²Estonian Institute of Ecology, 181 Raa Str, EE2400 Tartu, Estonia; ¹³Suonenjoki Research Station, FIN-77600 Suonenjoki, Finland; ¹⁴Forest Research Institute, T.G. Masarykova Street 2195, 960 92 Zvolen, Slovakia; ¹⁵USDA Forest Service, 1407 S. Harrison Road, East Lansing, Michigan 48823, USA; ¹⁶Environmental Forestry Consultants, LLC, PO Box 54, E7323 Hwy 54, New London, Wisconsin 54961, USA

Summary

1. The impacts of elevated atmospheric CO₂ and/or O₃ have been examined over 4 years using an open-air exposure system in an aggrading northern temperate forest containing two different functional groups (the indeterminate, pioneer, O₃-sensitive species Trembling Aspen, *Populus tremuloides* and Paper Birch, *Betula papyrifera*, and the determinate, late successional, O₃-tolerant species Sugar Maple, *Acer saccharum*).
2. The responses to these interacting greenhouse gases have been remarkably consistent in pure Aspen stands and in mixed Aspen/Birch and Aspen/Maple stands, from leaf to ecosystem level, for O₃-tolerant as well as O₃-sensitive genotypes and across various trophic levels. These two gases act in opposing ways, and even at low concentrations (1.5 × ambient, with ambient averaging 34–36 nL L⁻¹ during the summer daylight hours), O₃ offsets or moderates the responses induced by elevated CO₂.
3. After 3 years of exposure to 560 μmol mol⁻¹ CO₂, the above-ground volume of Aspen stands was 40% above those grown at ambient CO₂, and there was no indication of a diminishing growth trend. In contrast, O₃ at 1.5 × ambient completely offset the growth enhancement by CO₂, both for O₃-sensitive and O₃-tolerant clones. Implications of this finding for carbon sequestration, plantations to reduce excess CO₂, and global models of forest productivity and climate change are presented.

Key-words: Aggrading aspen forest, carbon budgets, carbon sequestration, interacting pollutants

Functional Ecology (2003) **17**, 289–304

Introduction

Global atmospheric CO₂ concentrations have risen by nearly 30% since pre-industrial times (Barnola *et al.* 1995; Stott *et al.* 2000; IPCC 2001). These increases are primarily due to fossil fuel emissions (Keeling *et al.* 1995). Similarly, emissions of oxidized nitrogen (NO_x) and volatile organic compounds from fossil fuel combustion have increased background concentrations of O₃ (Finlayson-Pitts & Pitts 1997; Fowler *et al.* 1998; Stevenson *et al.* 1998; Ryerson *et al.* 2001), which have risen some 36% over the same period (IPCC 2001). Fowler *et al.* (1999a, 1999b) suggest that nearly a quarter of the Earth's forests are currently at risk from tropospheric O₃ where peak concentrations exceed 60 nL L⁻¹. They further predict that half of the Earth's forests will be subjected to peak concentrations exceeding 60 nL L⁻¹.

Thousands of studies have been conducted to examine the impacts of elevated CO₂ (Ceulemans & Mousseau 1994; Saxe, Ellsworth & Heath 1998; Norby *et al.* 1999; Körner 2000) and O₃ (Chappelka & Samuelson 1998; Matyssek & Innes 1999; Bortier, Ceulemans & Temmerman 2000) on plant growth and biomass accrual. Many of these studies have been confounded by the artificial greenhouse conditions inside the exposure chambers (Olszyk, Tibbitts & Hertzberg 1980; McLeod, Fackrell & Alexander 1985). They have been limited by the available space to include only single trees or a few young, immature trees. Thus there is a need for larger-scale and longer-term studies to examine the impact of these gases on ecosystem structure and function (Heck *et al.* 1998).

Elevated CO₂ and O₃ affect trees in opposite ways. Elevated CO₂ stimulates photosynthesis (Tjoelker, Oleksyn & Reich 1998; Noormets *et al.* 2001a, 2001b) and growth above ground (Norby *et al.* 1999) and below ground (King *et al.* 2001; Kubiske & Godbold 2001), and delays autumnal foliar senescence (J.G. Isebrands, unpublished results). Trees grown under elevated CO₂ generally have lower nitrogen concentrations in their foliage (Cotrufo, Ineson & Scott 1998), lower Rubisco concentrations (Moore *et al.* 1999), and altered concentrations of defence compounds (Lindroth, Kinney & Platz 1993; Lindroth *et al.* 1997) and of antioxidants and other secondary metabolites (Norby *et al.* 2001a; Wustman *et al.* 2001).

In contrast to the largely beneficial effects of CO₂, O₃ is generally detrimental to tree growth and forest productivity. Ozone induces foliar injury (Karnosky 1976), decreases foliar chlorophyll content (Gagnon *et al.* 1992), accelerates leaf senescence (Karnosky *et al.* 1996), decreases photosynthesis (Coleman *et al.* 1995a), alters carbon allocation (Coleman *et al.* 1995b) and epicuticular wax composition (Mankovska, Percy & Karnosky 1998; Karnosky *et al.* 1999, 2002a), predisposes trees to attack by pests (Stark *et al.* 1968; Karnosky *et al.* 2002a) and decreases growth (Wang, Karnosky & Borman 1986; Karnosky *et al.* 1992,

1996, 1998). Extrapolation of open-top chamber O₃ exposures of Aspen to native Aspen stands suggests that 14–33% growth decreases could occur over 50% of its range in the eastern USA (Hogsett *et al.* 1997).

Current climate change scenarios predict further increases in atmospheric CO₂ (Stott *et al.* 2000) and O₃ concentrations (Stevenson *et al.* 1998; Fowler *et al.* 1999a, 1999b) over the next century. Little research has been done on the interactive impacts of these pollutants. Furthermore, conflicting results have been reported, even for a given species. For example, Volin & Reich (1996) and Volin, Reich & Givnish (1998) suggest that CO₂ ameliorates the effects of O₃ on trembling Aspen (*Populus tremuloides* Michx.) while Kull *et al.* (1996), McDonald *et al.* (2000, 2002), Sôber *et al.* (2002), Isebrands *et al.* (2001) and Wustman *et al.* (2001) suggest that CO₂ does not ameliorate, but sometimes exacerbates the negative impacts of O₃. Thus we do not fully understand how forest tree growth, or the composition and functioning of forests, will be influenced by interacting CO₂ and O₃.

The FACTS II (Aspen FACE) project was established in 1997 as the first open-air facility to examine the responses of forest trees to interacting CO₂ and O₃ (Dickson *et al.* 2000). Our objective was to examine how elevated atmospheric CO₂ and O₃ will affect the carbon and nitrogen cycles and ecological interactions of forests. Specifically, we are studying the impacts of these co-occurring greenhouse gases on aggrading northern forests in terms of carbon sequestration, physiological processes, growth and productivity, competitive interactions and stand dynamics, interactions with pests, and ecosystem processes such as foliar decomposition, mineral weathering and nutrient cycling.

This review (1) summarizes early results from Aspen FACE which show remarkable consistency from molecular through to ecosystem levels, in that relatively low O₃ concentrations offset the responses of Aspen and Birch to elevated CO₂; (2) places our findings in regard to forest productivity in the context of other CO₂/O₃ interaction studies; (3) draws implications in terms of global modelling; (4) summarizes effects on higher trophic levels; and (5) addresses research gaps and opportunities to better understand ecosystem responses to long-term exposures to interacting CO₂ and O₃.

SUMMARY OF EXPOSURE METHODS AND PLANT MATERIALS

The Aspen FACE project is a full factorial experiment with three replicate 30 m diameter rings of four treatments: control (ambient CO₂, ambient O₃); elevated CO₂ (560 µmol mol⁻¹ CO₂ vs. ambient CO₂ of ≈360 µmol mol⁻¹; elevated O₃ (1.5 × ambient); and elevated CO₂ plus O₃ (Fig. 1). The experiment was planted in July 1997. The two gases have been administered during daylight hours from budbreak to budset during 1998–2001 for total growing seasons of 166,

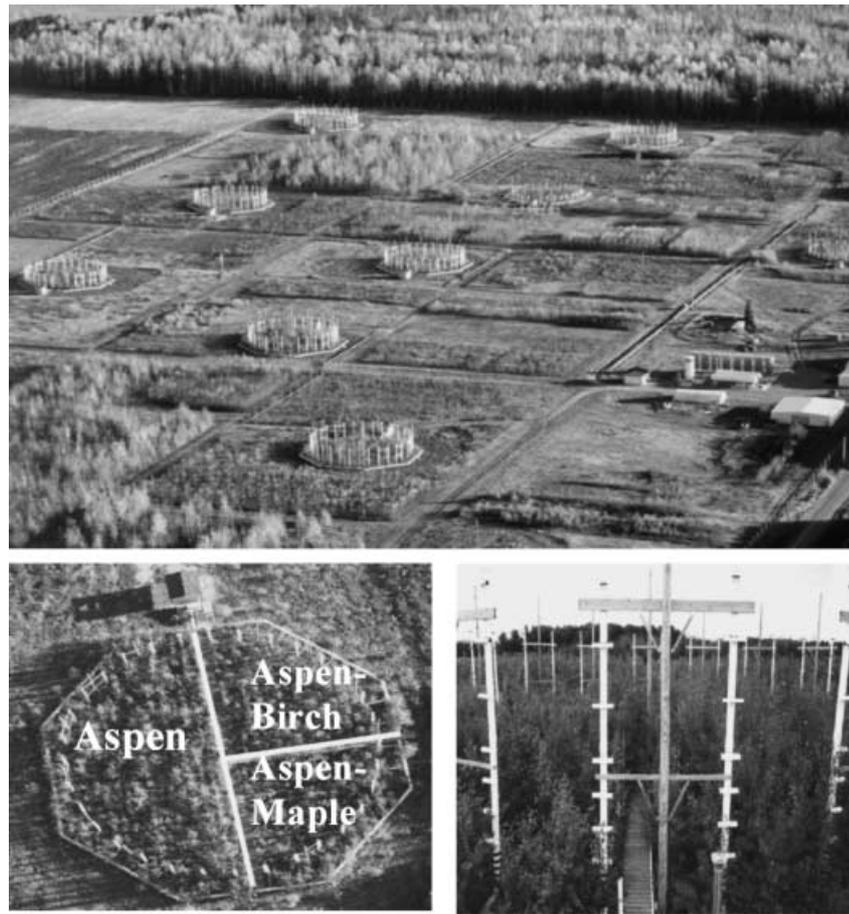


Fig. 1. Aerial view of the Aspen FACE project showing eight of 12 30 m diameter exposure rings (top). Each ring is divided into three sections as shown (bottom left). The gases are released through slots in the vertical vent pipes as shown in the bottom right photo.

143, 139 and 143 days, respectively. Daytime CO_2 concentrations in elevated CO_2 treatments averaged 530, 548, 548 and 541 $\mu\text{mol mol}^{-1}$ for the four growing seasons. The respective 1 min average CO_2 concentrations were within 10% of the target for 78.5, 74.0, 67.3 and 71.2% of the time, and within 20% of the target for 94.0, 93.0, 91.9 and 92.7% of the time. O_3 exposures, which are summarized in Figs 2 and 3, averaged 54.5, 51.1, 48.9 and 52.8 nL L^{-1} (12 h daytime mean during the growing season) compared to control ring O_3 , which averaged 34.6, 36.9, 36.0 and 36.6 nL L^{-1} for the same period. The growing season doses (SUM 00) for daylight hours were 97 900, 87 900, 78 800 and 90 700 $\text{nL L}^{-1}\text{h}$ for O_3 treatments, compared with control values at 59 100, 62 800, 58 200 and 66 100 $\text{nL L}^{-1}\text{h}$. The CO_2 and O_3 concentrations were chosen to represent the predicted atmospheric concentrations of these gases in the northern Great Lakes Region in the year 2050.

The rings were planted using 3–6-month-old potted plants in midsummer 1997. The eastern half of each ring was randomly planted at 1×1 m spacing in two tree plots of five Aspen clones differing in O_3 tolerance (8L, 216 and 271 = relatively tolerant; 42E and 259 = relatively sensitive). The remaining half of each ring was further subdivided with a quarter ring being

planted with alternating Aspen clone 216 and Birch (*Betula papyrifera*) and a quarter ring being planted with alternating Aspen clone 216 and Sugar Maple (*Acer saccharum*). Trembling Aspen is the most widely distributed tree species in North America. Aspen and Birch are the most important pulpwood species of the Great Lakes region, comprising over 70% of the round wood harvest (Piva 1996). According to the International Poplar Commission, the Aspen forest types make up more than 8.8 million ha in the USA and 17.8 million ha in Canada (Isebrands *et al.* 2001). Aspen–Birch–Maple stands are also important aesthetic components of northern forests. More details regarding the plant material, planting design, and the generation, dispensing and monitoring of CO_2 and O_3 are presented by Karnosky *et al.* (1999) and Dickson *et al.* (2000).

Synthesis of results

A summary of key results from the Aspen FACE project's establishment years, from time of plantation establishment in 1997 to crown closure in 2000, is shown in Table 1. The results are consistent across functional groups, from leaf biochemistry, gene

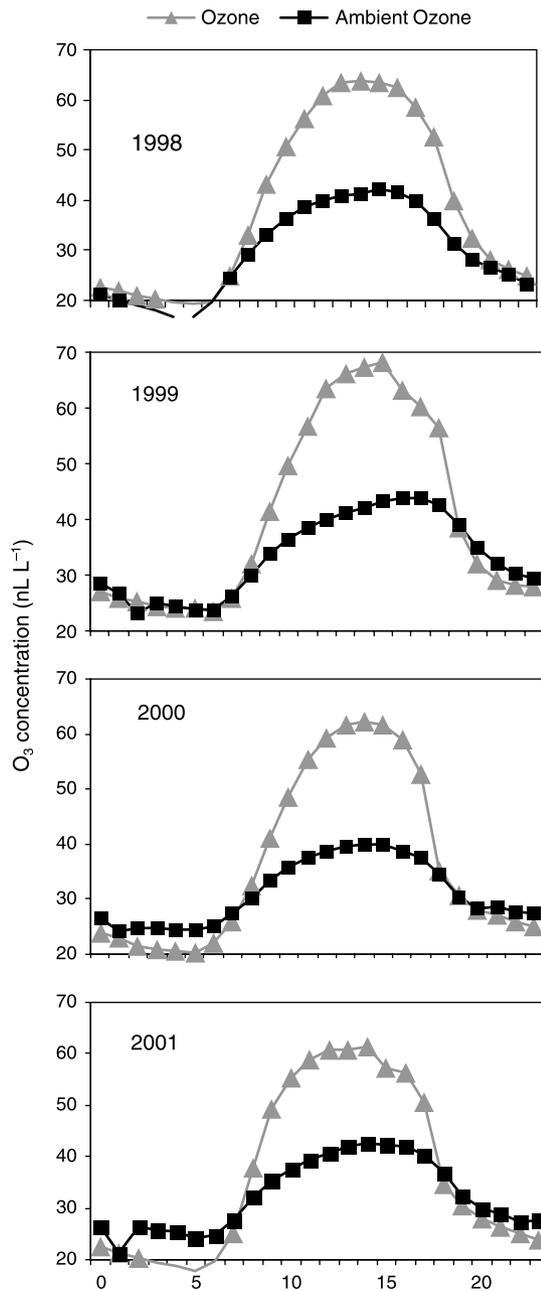


Fig. 2. Seasonal mean O₃ concentrations for ambient air (▲) and one elevated O₃ ring (■) in the Aspen FACE project.

expression and gas exchange through to ecosystem level, and across trophic levels in that elevated CO₂ and O₃ frequently exert opposite effects. When the two gases co-occur, low concentrations of ambient O₃ offset or substantially moderate the responses attributable to elevated CO₂.

GENE EXPRESSION AND BIOCHEMISTRY

Plants largely respond to stress by changes in assimilation of carbon and in the repartitioning of other resources. Elevated CO₂ and O₃ are sensed primarily by leaves (Dickson & Isebrands 1991) and result in

dynamic and rapid changes in gene expression (Noormets, Podila & Karnosky 2000) and gas exchange (Hendrey *et al.* 1997). We have documented O₃-induced stimulations of transcript production of several antioxidants, including ascorbate peroxidase, catalase and glutathione reductase (Wustman *et al.* 2001). Interestingly, these same antioxidants appear to be downregulated under elevated CO₂, regardless of O₃ exposure, as was phenylalanine ammonia-lyase (PAL), a key enzyme in the shikimic acid pathway. CO₂- and O₃-induced decreases in transcripts of the small subunit of Rubisco were closely linked to independently measured decreases in Rubisco concentrations (Noormets *et al.* 2001a). Decreases in chlorophyll content, as measured by Wustman *et al.* (2001), were consistent with the degradation of chloroplasts (Oksanen, Sober & Karnosky 2001) under elevated O₃.

GAS EXCHANGE

The three tree species that we examined have differing photosynthetic responses to CO₂, O₃ and CO₂ + O₃. In the response of the upper crown to elevated CO₂, the rapid-growing, early successional species showed significant increases in light-saturated CO₂ assimilation rate (A_{max}): 20–33% for Aspen (Noormets *et al.* 2001a, 2001b; Sôber *et al.* 2003), and 50–72% for Birch (Takeuchi *et al.* 2001). These values were seen consistently from year 1, and no acclimation to CO₂ has yet been seen in sun leaves of Aspen and Birch. The relative limitation imposed by stomatal conductance on A_{max} in Aspen declined under elevated CO₂, indicating that upper canopy leaves operated closer to their CO₂-saturated rate (Noormets *et al.* 2001a). Improvements in shade photosynthesis of Aspen and Birch under elevated CO₂ were small, so that net gains in daily canopy C fixation were largely realized at the top of the canopy and were driven by increases in A_{max} (Takeuchi *et al.* 2001). We found minimal effects of CO₂ or O₃ on leaf dark respiration, with significant late-season increases in respiration only under elevated O₃ (Noormets 2001). Thus elevated CO₂ increased upper canopy A_{max} in Aspen by 33% and in Birch by 64% (Fig. 4), but not in Sugar Maple. Moreover, we found that O₃ and CO₂ + O₃ reduced the carboxylation efficiency of Aspen and Birch, but did not reduce A_{max} (Sôber *et al.* 2003). The declines in capacity were sufficient to eliminate any increase in A_{max} due to elevated CO₂ in Aspen and Birch (compare CO₂ and CO₂ + O₃ treatments in Fig. 4). Elevated O₃ did not reduce photosynthetic capacity or A_{max} in Sugar Maple.

In addition to species-specific responses, we also observed substantial variation in photosynthetic assimilation among Aspen genotypes. Elevated CO₂ stimulated A_{max} to a similar degree in two Aspen genotypes of contrasting sensitivity to O₃. However, elevated O₃ reduced A_{max} slightly in an O₃-tolerant genotype (clone 216) and more so in an O₃-sensitive genotype (clone 259). Elevated CO₂ counteracted this reduction

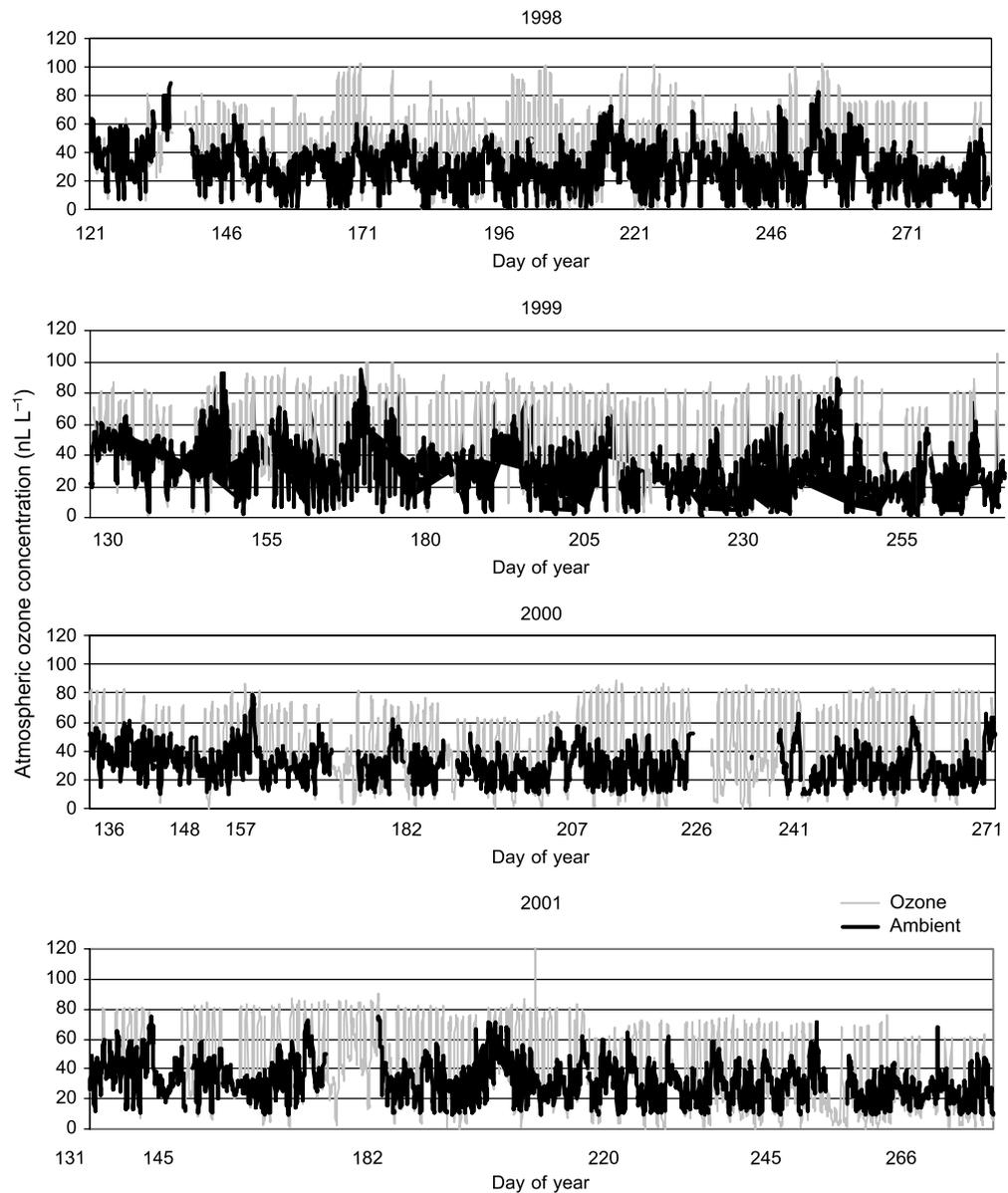


Fig. 3. Hourly O_3 concentrations from ambient air (black) and one elevated O_3 ring (grey) in the Aspen FACE project during 1998–2001.

in the O_3 -tolerant genotype, in that A_{max} was 35% greater than that of clone 216 plants in the controls (Noormets *et al.* 2001a). In the O_3 -sensitive genotype, however, A_{max} in the $CO_2 + O_3$ treatment was equivalent to that of clone 259 individuals from the controls. These responses occurred across leaves of different developmental stages and throughout the growing season.

Significant effects of elevated CO_2 and O_3 were also found for stomatal conductance (g_s). Generally, the stomata of Maple were much more responsive than those of Aspen and Birch to changing environmental conditions (light, CO_2 and relative humidity) across all treatments. Elevated CO_2 tended to reduce g_s for all three species, as expected (Noormets *et al.* 2001a; A. Söber & P. Sharma, unpublished results). The largest decreases in g_s for Maple were under the combined $CO_2 + O_3$ treatment (A. Söber & P. Sharma, unpublished results).

Increased leaf A_{max} of Aspen and Birch under elevated CO_2 was accompanied by significantly greater canopy leaf-area production (Fig. 5), a finding we believe is simply linked to the trees being larger in the elevated CO_2 rings, as we have not found changes in allometry associated with treatments. The O_3 -induced decline in A_{max} of Aspen (Fig. 4) was also reflected in the decreased LAI of the Aspen canopies. Interestingly, Paper Birch had no O_3 -induced decline in A_{max} , and the Aspen–Birch canopies exhibited no significant decrease in LAI under elevated O_3 , reflecting the contribution of Birch to total canopy leaf area. The combination of elevated $CO_2 + O_3$ did not affect canopy LAI of either pure Aspen or Aspen–Birch.

The differences in A_{max} and LAI that we observed indicate that CO_2 and O_3 will alter carbon assimilation into terrestrial ecosystems in a manner consistent

Table 1. Summary of responses of Trembling Aspen to elevated CO₂ (+200 µmol mol⁻¹), O₃ (1.5 × ambient), or CO₂ + O₃ compared with control during 3 years of treatments at the Aspen FACE project

	CO ₂	O ₃	CO ₂ + O ₃	Source
Foliar gene expression and biochemistry				
Rubisco	↓*	↓	↓↓	Wustman <i>et al.</i> (2001); Noormets <i>et al.</i> (2001a)
RbcS transcripts†	↓	↓	↓↓	Wustman <i>et al.</i> (2001)
Chalcone synthase transcripts	n.s.	n.s.	n.s.	Wustman <i>et al.</i> (2001)
PAL transcripts	↓	↑	↓	Wustman <i>et al.</i> (2001)
SOD	n.s.	n.s.	n.s.	Wustman <i>et al.</i> (2001)
ACC oxidase	↓	↑	↓	Wustman <i>et al.</i> (2001)
Ascorbate peroxidase	↓	n.s.	↓	Wustman <i>et al.</i> (2001)
Catalase	↓	↑	↓	Wustman <i>et al.</i> (2001)
Glutathione reductase	↓	↑	↓	Wustman <i>et al.</i> (2001)
Phenolic glycosides	↑	↓	n.s.	Lindroth <i>et al.</i> (2002); Kopper & Lindroth (2002)
Tannins	n.s.	↑	↑	Lindroth <i>et al.</i> (2001); Kopper & Lindroth (2002)
Foliar nitrogen	↓	n.s.	↓	Lindroth <i>et al.</i> (2001); Kopper & Lindroth (2002)
C : N ratio of foliage	↑	n.s.	↑↑	Lindroth <i>et al.</i> (2001)
Starch	↓	↓	n.s.	Wustman <i>et al.</i> (2001)
Gas exchange				
A _{max} lower canopy	n.s.	↓↓	↑(young leaves) ↓(older leaves)	Takeuchi <i>et al.</i> (2001); Noormets <i>et al.</i> (2001a)
A _{max} whole canopy	↑↑	↓↓	n.s.	Noormets <i>et al.</i> (2001b)
Carboxylation efficiency	n.s.	↓	↓↓	Söber <i>et al.</i> (2002)
Stomatal limitation	↓	n.s.	↓	Noormets <i>et al.</i> (2001a)
Stomatal conductance	↓	↓↑	↓	öber <i>et al.</i> (2000), Noormets <i>et al.</i> (2001a)
Foliar respiration	n.s.	↑	n.s.	Takeuchi <i>et al.</i> (2001), Noormets (2001)
Soil respiration	↑↑	↓	n.s.	King <i>et al.</i> (2001)
Microbial respiration	↑↑	n.s.	n.s.	Phillips <i>et al.</i> (2002)
Stomatal density	n.s.	n.s.	n.s.	Percy <i>et al.</i> (2002a)
Chlorophyll content	↓	↓	↓	Wustman <i>et al.</i> (2001)
Chloroplast structure	↑	↓	↓	Oksanen <i>et al.</i> (2001), Takeuchi <i>et al.</i> (2001), Wustman <i>et al.</i> (2001)
O ₃ flux	↓	↑↑	↑	Noormets <i>et al.</i> (2001a)
Growth and productivity				
Leaf thickness	↑	n.s.	n.s.	Oksanen <i>et al.</i> (2001)
Leaf size	↑	↓	↓	Wustman <i>et al.</i> (2001)
Leaf area	↑	↓	n.s.	Noormets <i>et al.</i> (2001b)
LAI	↑	↓	n.s.	Noormets (2001)
Height growth	↑	↓	n.s.	Isebrands <i>et al.</i> (2001)
Diameter growth	↑	↓	n.s.	Isebrands <i>et al.</i> (2001)
Volume growth	↑	↓	n.s.	Isebrands <i>et al.</i> (2001)
Leaf biomass	↑	↓	n.s.	McDonald & Isebrands, unpublished
Stem biomass	↑	↓	↓	McDonald & Isebrands, unpublished
Coarse root biomass	↑	↓	n.s.	King & Pregitzer, unpublished
Fine root biomass	↑	↓	n.s.	King <i>et al.</i> (2001)
Fine root turnover	↑	n.s.	n.s.	King <i>et al.</i> (2001)
Spring budbreak	n.s.	Delayed	n.s.	Isebrands & Karnosky, unpublished
Autumn budset	Delayed	Early	n.s.	Isebrands & Karnosky, unpublished
Foliar retention (autumn)	↑↑	↓↓	n.s.	Isebrands & Karnosky, unpublished
Wood chemical composition				
Lignin	n.s.	↑	n.s.	Anttonen <i>et al.</i> (2001)
Cellulose	n.s.	n.s.	n.s.	Anttonen <i>et al.</i> (2001)
Hemicellulose	n.s.	n.s.	n.s.	Anttonen <i>et al.</i> (2001)
Extractives	n.s.	n.s.	n.s.	Anttonen <i>et al.</i> (2001)
Leaf surfaces				
Crystalline wax structure	↓	↓	↓↓	Karnosky <i>et al.</i> (1999); Karnosky <i>et al.</i> (2002a)
Stomatal occlusion	↑	↑	↑↑	Karnosky <i>et al.</i> (1999)
Wax amount	↑	↑	n.s.	Karnosky <i>et al.</i> (2002a); Percy <i>et al.</i> (2002a)
Wax chemical composition	n.s.	Change	n.s.	Karnosky <i>et al.</i> (2002a)
Wax fatty acid <i>de novo</i> synthesis	↑↑	↑↑	↑	Percy <i>et al.</i> (2002a)
Wax hydrocarbon biosynthesis	↑↑	↑↑	n.s.	Percy <i>et al.</i> (2002a)
Wax carbon-chain length	↑↑	↑	↓	Karnosky <i>et al.</i> (2002a)
Wettability	n.s.	↑↑	↑	Karnosky <i>et al.</i> (2002a)

Table 1. Continued

	CO ₂	O ₃	CO ₂ + O ₃	Source
Trophic interactions				
<i>Melampsora</i> leaf rust	n.s.	↑↑	↑↑	Karnosky <i>et al.</i> (2002a); Percy <i>et al.</i> (2002b)
Aspen aphids	n.s.	n.s.	n.s.	Percy <i>et al.</i> (2002b)
Birch aphids	n.s.	n.s.	n.s.	Awmack & Lindroth, unpublished
Aspen Blotch Leafminer	↓	↓	↓	Kopper & Lindroth (2002)
Forest Tent Caterpillar	n.s.	↑	n.s.	Kopper (2001), Kopper & Lindroth (2002)
<i>Oberea</i> woodborer	↑	n.s.	↑	Mattson, unpublished
Ecosystem level				
NPP	↑	↓	n.s.	Kruger & McDonald, unpublished
Litter decomposition (<i>k</i> value)	↓	n.s.	↓	Parsons <i>et al.</i> (2000)
Nutrient mobilization	↑↑	n.s.	↑	King, unpublished
Water-use efficiency	↑	↓	↑	Sober <i>et al.</i> (2000)
Soil moisture	↑	↑	↑	King, unpublished
Competitive indices	↑	↓	↓↓	McDonald <i>et al.</i> (2000); McDonald <i>et al.</i> (2002)
Soil invertebrate diversity	n.s.	↓	↓	Loranger & Pregitzer, unpublished
Microbial enzymes	↑	n.s.	n.s.	Phillips <i>et al.</i> (2002); Larson <i>et al.</i> (2002)
Microbial biomass	↑	n.s.	n.s.	Phillips <i>et al.</i> (2002); Larson <i>et al.</i> (2002)

*Responses are shown as small but significant increases (↑), large and significant increases (↑↑), small but significant decreases (↓), large and significant decreases (↓↓), non-significant effects (n.s.) compared to trees grown in control rings with ambient CO₂ and O₃. Foliar analyses and leaf surface properties were largely determined from recently mature leaves of all three species during mid-season. Gas-exchange data were taken from all leaf ages and throughout the growing season.

†RbcS = small subunit of Rubisco; PAL = phenylalanine ammonia-lyase; SOD = super oxide dismutase; ACC = 1-aminocyclopropane-1-carboxylic acid; C = carbon; N = nitrogen; A_{max} = maximum photosynthesis rate; LAI = leaf area index; NPP = net primary productivity.

with the physiological response of the dominant vegetation. This key observation supports our hypothesis that ecosystem C assimilation, allocation and cycling are strongly influenced by the life-history traits of the dominant plant taxa, coupled with the manner in which plants increase or decrease amounts of C and N allocated to plant growth, storage and defence.

GROWTH AND PRODUCTIVITY

The photosynthetic responses of the species and genotypes in our experiment have influenced growth and litter production above and below ground (Parsons, Bockheim & Lindroth 2000; Isebrands *et al.* 2001; King *et al.* 2001). Enhanced rates of photosynthesis under

elevated CO₂ contributed to increased above-ground growth of Aspen and Birch (Fig. 6). In contrast, decreases in photosynthesis by O₃ depressed above-ground growth in Aspen, but not Birch. Interacting CO₂ and O₃ resulted in intermediate responses in Aspen and Birch, such that these treatments did not generally differ significantly from controls. Although Maple photosynthesis exhibited little response to any of the treatments, we did observe an overall negative above-ground growth response to all treatments. Nevertheless, variability in above-ground growth was extremely high in the smaller Maple saplings (CV = 250%), and it is premature to conclude that the overall growth of Maple will decline as CO₂ and O₃ accumulate in the atmosphere.

As with photosynthesis, above-ground growth responses varied widely in magnitude among Aspen genotypes. For example, the response to elevated CO₂ was least in genotype 42E and greatest in genotype 271 (Isebrands *et al.* 2001). Similarly, O₃-sensitive genotype 259 had the greatest reduction in above-ground biomass under elevated O₃, consistent with its reduced A_{max} . In contrast, mean above-ground biomass of genotype 8L was not influenced by elevated O₃ to any extent. Given the photosynthetic and above-ground growth responses of these genotypes, we expect those genotypes that are most responsive to CO₂ and least responsive to O₃ will eventually dominate our aggrading stands. By observing the growth and allometry of individual genotypes over the next several years, our intensive measurements of each plant in the experiment will enable us to document mortality and dominance at the genotype level over time, as well as to study interspecific interactions (McDonald *et al.* 2002).

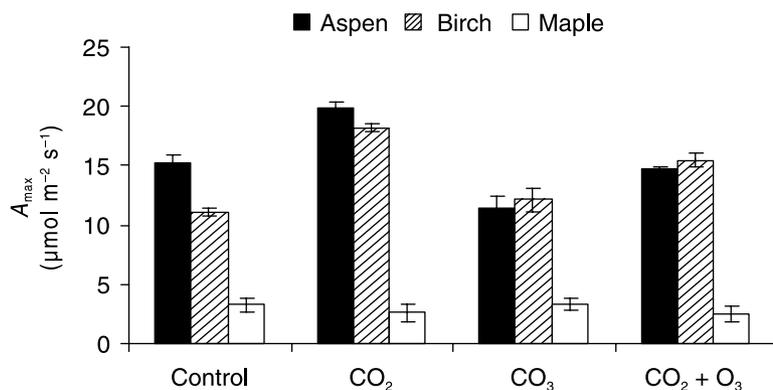


Fig. 4. Light-saturated CO₂ assimilation rates of Aspen, Birch and Maple growing under experimental atmospheric CO₂ and O₃ treatments. Data represent the mean and SE of three trees from each of three replicates for three to five measurement times over the 1999 and 2000 growing seasons.

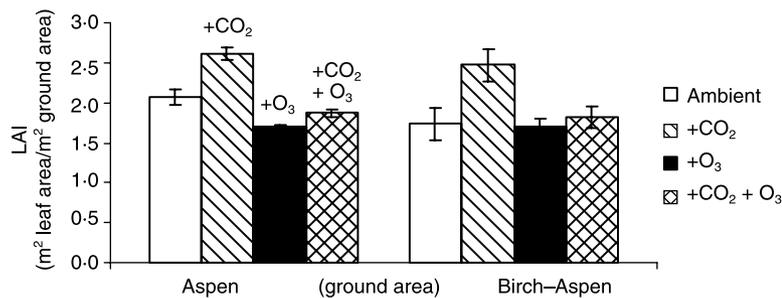


Fig. 5. Estimates of leaf area index (LAI, m² leaf area per m² ground area) for Aspen and Aspen–Birch stands in mid-season 2000. A destructive subsample harvest in August 2000 was used to calibrate the relationship between total leaf area and stem basal diameter. Significant linear regressions of log–log-transformed leaf area and diameter data were developed ($R^2 = 0.80$, $P < 0.0001$), with specific intercepts for each species. Whole-stand estimates of LAI were obtained by estimating individual tree leaf area from mid-season basal diameter measurements using the regression equations, summing the total leaf area of all trees in each stand, and dividing by the total ground area in each stand type (means ± 1 SE).

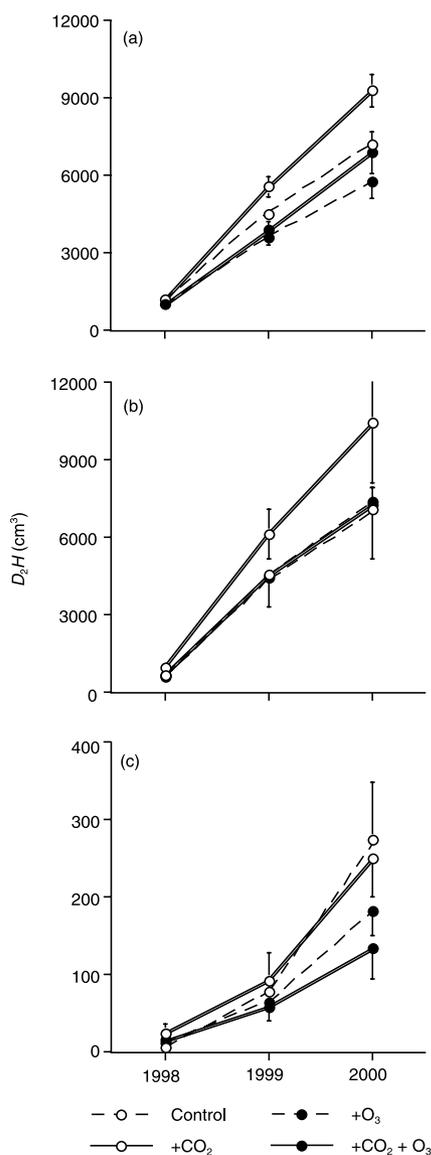


Fig. 6. Volume growth index (diameter² × height) of Aspen (a), Birch (b) and Maple (c) grown for 3 years in full factorial combinations of elevated atmospheric CO₂ (solid lines) and elevated O₃ (solid symbols).

WOOD CHEMISTRY

Concentration of total lignin (= gravimetric + acid-soluble lignin) in stem wood of Aspen increased by 2.5% under elevated O₃ as compared to the control, while elevated CO₂ had no effect. This result agrees with a previous study where no changes in lignin concentration caused by elevated CO₂ were found (Hättenschwiler, Schweingruber & Körner 1996). Increases in total lignin concentration under elevated O₃ are interesting, as there was previously no evidence for O₃ effects on wood chemical composition. Increase in lignin may indicate changes in carbon allocation leading to enhanced activity of the phenylpropanoid biosynthetic pathway, which is consistent with the finding that PAL transcripts increased under O₃ exposure (Wustman *et al.* 2001). The concentrations of the other main structural components of cell wall, α -cellulose and hemicellulose, were not affected by any treatment, nor were the minor nonstructural components (acetone-soluble extractives). A CO₂ effect on N dilution was also evident in Aspen branch wood, although there was a significant CO₂–O₃ interaction, with low-CO₂/high-O₃ branches containing 30% more N per unit dry mass (W.J. Mattson & R. Julkunen-Tiitto, unpublished results). In the case of Aspen branch wood, the sum total of phenolics (total per g dry weight) tended to increase under high CO₂, but the differences were not statistically significant. Out of 14 molecular species of phenolics, the concentrations of only four increased significantly, whereas the others did not. Likewise, neither starch nor fibre content of branch wood changed in response to CO₂ and O₃ (W.J. Mattson & R. Julkunen-Tiitto, unpublished results).

Apart from the environmental control, genetic factors also have a strong impact on wood properties (Costa e Silva *et al.* 1998; King *et al.* 1998; Denne, Calahan & Aebischer 1999; Hylén 1999). In Aspen, genotype significantly affected total lignin and acetone-soluble extractives (Anttonen *et al.* 2001). The O₃-sensitive clone 259 had 3% higher total lignin concentration than the O₃-tolerant clone 216. The O₃-sensitive clone 259 also differed from the other clones in having 26% lower concentration of acetone-soluble extractives. Genotype did not have an effect on α -cellulose and hemicellulose. Wood chemical composition and fibre properties may be different in the juvenile phase from that in mature trees (Zobel & van Buijtenen 1989; Hatton & Hunt 1992); further studies are needed as these trees reach maturity to predict the effects of future climate on wood chemical composition.

FOLIAR CHEMISTRY, SURFACE PROPERTIES AND HETEROTROPHIC INTERACTIONS

Our hypothesis is that changes in the quantity and chemistry of plant tissues, elicited by CO₂ and O₃, cascade through terrestrial ecosystems and alter the performance of heterotrophic organisms, and thus

potentially the entire community structure. Elevated CO₂ and O₃ can alter foliar chemistry and surface properties. Foliar N concentrations in Aspen and Birch declined by 16–21% in response to enriched CO₂, but declined only marginally in response to elevated O₃ (Lindroth *et al.* 2001). Decreased N concentrations in foliage under elevated CO₂, as has been commonly reported in CO₂ studies with other tree species (Cotrufo, Ineson & Scott 1998; Norby *et al.* 2000), increased C : N ratios in Aspen and Birch foliage, particularly under the combination of elevated CO₂ and O₃ (Lindroth *et al.* 2001). For example, as N declined in late-season Birch leaves, starch concentrations increased threefold under elevated CO₂ (W.J. Mattson & R. Julkunen-Tiitto, unpublished results). Differences in C : N ratios among the treatments were maintained through leaf senescence and litterfall (Lindroth *et al.* 2001; Parsons, Bockheim & Lindroth 2000). CO₂ enrichment, regardless of whether it was combined with O₃ or not, increased litter C : N by 39% in Aspen, and by 24% in Birch, relative to the controls.

Elevated CO₂ and O₃ altered concentrations of C-based secondary metabolites in Aspen and Birch (Lindroth *et al.* 2001; Lindroth, Wood & Kopper 2002); but the direction and magnitude of response differed among particular metabolites and between Aspen clones. Common secondary compounds, such as tannins and the phenolic glycosides of Aspen, were responsive to these two gases (Kopper & Lindroth 2002; Lindroth, Wood & Kopper 2002). Eight of 11 Birch leaf phenolic compounds increased 15–30% under high CO₂ (W.J. Mattson & R. Julkunen-Tiitto, unpublished). As was the case with C : N ratios, secondary metabolite concentrations were highest in litter originating in the CO₂-enriched rings and lowest in the rings exposed to high O₃ (W.F.J. Parsons, R.L. Lindroth & J.G. Bockheim, unpublished results).

Elevated CO₂ and O₃ also altered rates of leaf epicuticular wax biosynthesis with increases or decreases depending on clone and treatment, modified amounts of carbon allocated to various wax forms, and changed chemical composition, with O₃ increasing the ratio of long-chain alkane compounds (Karnosky *et al.* 1999, 2002a). O₃ modified wax structure from crystalline to amorphous masses (Mankovska *et al.* 1998) in Aspen and Birch. These changes in leaf surface properties may have contributed to the threefold to fivefold increased incidence of the Aspen leaf rust *Melampsora medusa* Thuem. f.sp. *Tremuloidae* in the O₃ and CO₂ + O₃ treatments (Karnosky *et al.* 2002a; Percy *et al.* 2002b) by altering the wettability of the leaf surface and providing a leaf surface more conducive to spore germination.

Elevated CO₂ and O₃ may alter the performance of insects through changes in bottom-up (plant) and top-down (natural enemy) factors. Colonization of Aspen trees by the Aspen Blotch Leafminer (*Phyllonorycter tremuloidiella*) declined under enriched CO₂ and O₃, a response probably caused by changes in epicuticular

waxes easing insect attachment and leaf surface penetration (Kopper & Lindroth 2002). Tent Caterpillar (*Malacosoma disstria*) pupal weights improved under elevated O₃, but this was negated under enriched CO₂, responses consistent with changes in concentrations of foliar phenolic glycosides. Such effects were not always consistent, however, across Aspen clones. In contrast to well established findings with potted Aspen trees in greenhouses (Lindroth, Kinney & Platz 1993; Bezemer & Jones 1998), CO₂ fumigation did not increase leaf foliar consumption by insects. On the other hand, first and late-instar Gypsy Moth larvae consistently increased consumption (>15%) of CO₂-fumigated Birch leaves, though growing no better than control larvae (W.J. Mattson & T. Trier, unpublished results). The beetle *Oberea schaumii*, which bores into the stems and young branches of Aspen trees, responded to CO₂ and O₃ by being least abundant on trees under O₃ fumigation, but most abundant on trees under the CO₂ + O₃ fumigation. Its populations were intermediate in abundance under elevated CO₂, but still exceeded the controls. The Bark Scale, *Chionaspis*, and the Fly Gall-maker, *Hexomyza*, were most abundant on trees growing under CO₂ fumigation, showing no other treatment responses (W.J. Mattson, unpublished results).

Elevated CO₂ and O₃ also have the potential to alter insect community composition. Population censuses of aphids and natural enemies on Aspen revealed that while aphid abundance was unaffected by CO₂ or O₃, natural enemy abundance increased at elevated CO₂ and declined at elevated O₃ (Percy *et al.* 2002b). Awmack & Harrington (2000) showed that the damage caused by aphids increased at elevated CO₂ and negated the large growth stimulations otherwise expected of Bean plants (*Vicia faba*) under elevated CO₂. Therefore increases in pest numbers may have a considerable impact on forest health and productivity in the future. The importance of insect pest and disease eruptions in altering carbon fluxes from ecosystems has been highlighted by Kurz & Apps (1999), who detected a decade-long shift from carbon sink to source in the boreal forests of Canada, attributable to an increase in disturbances by pests and fire.

ECOSYSTEM RESPONSES

Most studies dealing with forest trees and elevated CO₂ and O₃ have been conducted in chambers in which it is not possible to address long-term, large-scale, ecosystem-level questions (Heck *et al.* 1998; Hendrey *et al.* 1999; McLeod & Long 1999; Karnosky *et al.* 2001). After 4 years' research at Aspen FACE, we are beginning to see indications that the physiological and genetic responses, which we detected early in this experiment, are cascading through the ecosystem and resulting in significant ecosystem-level responses to elevated CO₂ and O₃. It should be noted that we have found slight differences in soil fertility across our site, but we accommodated these differences in our experimental

design by using a randomized complete block design with the blocking being done by maintaining rings of common fertility within each replicate (Dickson *et al.* 2000). Our soil fertility levels are in the range of natural Aspen forest soil fertility, but sufficient to ensure that our tree growth responses to elevated CO₂ have been unconstrained by nutrient limitations.

LITTER PRODUCTION, CHEMISTRY AND DECOMPOSITION

Several pieces of evidence collectively suggest that greater rates of carbon assimilation and growth under elevated CO₂ directly influence the amount of organic substrate entering the soil for microbial metabolism, and that this response has been dampened by elevated O₃. Elevated atmospheric CO₂ increased leaf litter production by 36% in Aspen, and Birch leaf litter production doubled in the Aspen–Birch community. Elevated O₃ did not substantially alter leaf litter production relative to the control, but elevated O₃ decreased the CO₂-induced production of litter in Aspen and Birch. Similarly, elevated CO₂ significantly increased the mass of dead fine roots by 140% beneath Aspen, and by 340% beneath Aspen–Birch (King *et al.* 2001). Although elevated O₃ did not influence dead fine-root mass (relative to the control), it did nullify the increase in fine-root mass caused by elevated CO₂. We also found that elevated CO₂ increased live fine root biomass by 113% beneath Aspen and by 83% beneath Aspen–Birch; elevated O₃ did not significantly alter live fine root biomass (King *et al.* 2001). We also observed no change in the lignin content of live and dead fine roots (J.S. King, unpublished results), a result supported by greenhouse studies (W.F.J. Parsons, B.J. Kopper & R.L. Lindroth, unpublished results). Taken together, our results suggest that CO₂ substantially increased above-ground and below-ground litter production beneath Aspen and Aspen–Birch, but this response was almost eliminated by elevated O₃.

The 1 year decay rate (*k* value) of Birch litter was significantly reduced by elevated CO₂ regardless of O₃ (Parsons, Bockheim & Lindroth 2000). Aspen decay showed similar trends, although differences among treatments were not significant. Initial differences in foliar quality among the treatments were sustained throughout Aspen and Birch decomposition, and these distinctive chemical signatures probably contributed to controlling mass loss from the decomposing litter, whether it was returned to its ring of origin or transplanted into another treatment ring. From reciprocal litter transplant experiments we observed slight moderation of litter quality effects: a weak substrate–environment interaction (Parsons, Bockheim & Lindroth 2000).

SOIL AND MICROBIAL RESPIRATION

Elevated CO₂ substantially increased soil respiration rates beneath Aspen (by 30%) and Aspen–Birch (by

60%); however, soil respiration increased much less (10%) beneath Aspen–Maple. Elevated O₃ had a relatively minor influence on mean soil respiration at both atmospheric CO₂ concentrations except for late in each growing season, possibly due to increased fine root senescence caused by O₃. This pattern of soil respiration was reflected in differences in soil pCO₂ among our experimental treatments (King *et al.* 2001), wherein elevated CO₂ increased soil pCO₂ by 27% (averaged over three depths from 15 to 125 cm and two growing seasons), and O₃ had little effect. We believe this result is important because higher soil pCO₂ could lead to more carbonic and organic acids in the soil, leading to more rapid mineral weathering, nutrient leaching, and the export of dissolved inorganic C. Like soil respiration, microbial respiration under elevated CO₂ was significantly increased beneath Aspen (33%) and Aspen–Birch (55%), but the increase beneath Aspen–Maple was small (1%) and not significant (Phillips *et al.* 2002). We also observed that elevated O₃ did not significantly decrease microbial respiration relative to the control, but elevated O₃ did reduce rates of microbial respiration under elevated CO₂ (in the CO₂ + O₃ treatment). Greater rates of microbial respiration indicate that increased litter inputs under elevated CO₂ are being metabolized by a soil microbial population that is larger, more active, or both. We observed a nonsignificant increase in soil microbial biomass under elevated CO₂ (28%) across two growing seasons (Larson *et al.* 2002), while microbial biomass in the O₃ and CO₂ + O₃ treatments were equivalent to that of the control (data not shown). We have not yet determined if microbial respiration changes are in any way related to changes in the biochemical composition of the plant litter.

MICROBIAL COMMUNITY FUNCTION

We believe that changes in the quantity of organic substrate entering the soil from our experimental treatments have altered the metabolism of soil microbial communities. Again, we do not yet know if litter quality changes are also having an effect. Elevated CO₂ increased the activity of enzymes involved in plant and fungal cell-wall degradation at O₃ concentrations. We detected CO₂-induced increases in cellobiohydrolase, an enzyme catalysing the release of cellobiose during cellulose degradation, and *N*-acetylglucosaminidase, which catalyses the release of *N*-acetylglucosamine during chitin degradation (we did not find significant interactions between CO₂ and species or O₃ treatments; Larson *et al.* 2002). These results suggest that greater inputs of plant and fungal cell-wall substrates (cellulose and chitin) under elevated CO₂ have altered the metabolism of these plant-derived compounds in soil and the transport of C through the soil food web. This response was confirmed by increased recovery of ¹³C₂O₂ that was respired from labelled cellobiose and *N*-acetylglucosamine added to soil in a preliminary soil

incubation experiment (Phillips *et al.* 2002). It is possible that these responses are driven largely by greater inputs of dead fine roots and associated mycorrhizal fungi, and we intend to explore this possibility.

SOIL N CYCLING

Results from a short-term ¹⁵N tracer experiment suggest that changes in microbial metabolism among experimental treatments have altered rates and patterns of soil N cycling. We followed the flow of NH₄⁺ and ¹⁵NO₃⁻ in soil collected from each FACE ring during the 1999 field season. O₃ had no effect on the recovery of ¹⁵N in microbial biomass or soil organic matter. In contrast, elevated CO₂ (main effect) significantly increased the amount of ¹⁵NO₃⁻ recovered in microbial biomass and soil organic matter. Recovery of ¹⁵NH₄⁺ in these pools was greater under elevated CO₂, but this increase was not significant. These results indicate that larger amounts of N are forming soil organic matter under elevated CO₂. Because the C : N ratio of soil organic matter is relatively constant, this finding suggests that greater amounts of C also are forming soil organic matter in our experiment. This indicates that more C may be stored in soil as the atmospheric CO₂ concentration increases, a finding not strongly supported in the Duke FACE study (Schlesinger & Lichter 2001).

WATER BALANCE

There has been much speculation that ecosystem water balances will be altered under elevated CO₂ (Curtis 1996; Curtis & Wang 1998). However, this type of response has been impossible to test in greenhouse chamber or open-top chamber studies. We have found two independent lines of evidence suggesting possible water balance changes attributable to elevated CO₂ and O₃. First, water-use efficiency calculations by Sôber *et al.* (2000) suggest that water-use efficiency was highest for Aspen clones exposed to elevated CO₂ and lowest in those exposed to O₃. Trees exposed to elevated CO₂ + O₃ were intermediate between treatments, but still greater than controls in water-use efficiency. Second, during a relatively dry year (1999) but not in relatively wet year (2000), changes in volumetric soil moisture, as determined by time domain reflectometry, were detectable throughout the growing season under elevated CO₂, O₃ and CO₂ + O₃ (J.S. King & K.S. Pregitzer, unpublished results).

BIODIVERSITY

While elevated CO₂ (Vasseur & Potvin 1998) and O₃ (Berrang *et al.* 1986; Barbo *et al.* 1998) have been implicated in altering plant communities and biodiversity, few studies have investigated the impacts of the interacting effects of CO₂ and O₃ on community composition. We have evidence that the composition of

forest communities may be altered under these two greenhouse gases. First, in a study of competitive interactions between Aspen clones differing in O₃ sensitivity, McDonald *et al.* (2000, 2002) found that elevated CO₂ exacerbated growth reductions as elevated O₃ decreased growth 35% in elevated CO₂ compared with 24% in ambient CO₂. Clone-competitive interactions were significant, suggesting that interacting CO₂ and O₃ could exacerbate clonal competition for fitness, as previously described under elevated O₃ by Karnosky *et al.* 1999, 2002b). We expect these competitive interactions to increase over the next few years as interactions among crowns and among root systems intensify, and as seed production and dispersal occur in the pioneer species.

IMPLICATIONS FOR CARBON SEQUESTRATION AND NET PRIMARY PRODUCTIVITY MODELS

Our results suggest that elevated O₃ at relatively low concentrations can significantly reduce the growth enhancement by elevated CO₂. Our results follow similar trends found for many agricultural crops, other hardwood trees and a few conifers (Fig. 7). Together, these studies on plants of different genetic backgrounds, growth characteristics and life histories suggest that O₃ can seriously alter the capacity of vegetation to grow under elevated CO₂ and to sequester carbon. For example, the projected co-occurrence of elevated O₃ (as predicted by Fowler *et al.* 1999a) over a large portion of the natural range of the circumpolar Leuce (Aspen) section of Poplar (Fig. 8) may mean that worldwide growth stimulations will not be as great as predicted from previous studies of elevated CO₂. It is important to bring an understanding of O₃ as a moderator of CO₂ responses to global models of terrestrial net primary productivity. It is also important to expose forests for their entire rotation or life cycle to understand the practicality of using forests and forest plantations to sequester carbon and to offset anthropogenic CO₂ emissions.

Conclusions and research needs

The suite of responses to elevated CO₂ and/or O₃ at the Aspen FACE project have been remarkably consistent across functional groups, species and genotypes differing in O₃ tolerance, and from molecular to ecosystem levels. However, it must be noted that these responses are being found in a young, aggrading forest. We have not yet detected a diminishing of CO₂ growth enhancement, as has been reported for Loblolly Pine (Oren *et al.* 2001) and Sweetgum (Norby *et al.* 2001b). However, our forest is in a much younger stage of development than these other two sites. Comparisons among FACE sites are difficult because several factors differ, including soils, climate, species and treatments. Several new areas of study at our site – such as the occurrence and abundance of mycorrhizal fungi; the diversity and

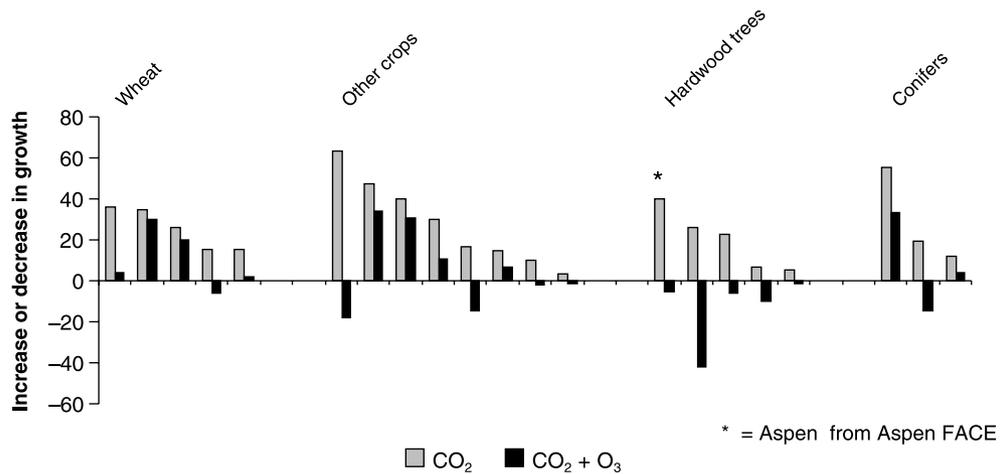


Fig. 7. Relative effects of controlled exposure to elevated CO₂ on normalized plant growth under CO₂ alone (striped bars, 500–713 μmol mol⁻¹ CO₂) and elevated CO₂ plus ozone (dotted bars). (Modified and expanded from Barnes & Wellburn 1998.) Data presented for wheat (*Triticum aestivum*) from Barnes, Ollerenshaw & Whitfield (1995); Rudorff *et al.* (1996); McKee, Bullimore & Long (1997); Bender, Herstein & Black (1999); Hudak *et al.* (1999); soybean (*Glycine max*) from Heagle, Miller & Pursley (1998) and Miller, Heagle & Pursley (1998); tomato (*Lycopersicon esculentum*) from Olszyk & Wise (1997) and Hao *et al.* (2000); rice (*Oryza sativa*) from Olszyk & Wise (1997); potato (*Solanum tuberosum*) from Donnelly *et al.* (2001) and Lawson *et al.* (2001); corn (*Zea mays*) from Rudorff *et al.* (1996); hardwood trees including hybrid poplars (*Populus* hybrids) from Dickson *et al.* (1998); Trembling Aspen (*Populus tremuloides*) from Volin & Reich (1996); Volin *et al.* (1998); Isebrands *et al.* (2001); oak (*Quercus petraea*) from Broadmeadow & Jackson (2000); conifers including Ponderosa Pine from David Olszyk (personal communication); Scots Pine (*Pinus sylvestris*) from Broadmeadow & Jackson (2000); Utriainen *et al.* (2000). Each pair of bars represents one species.

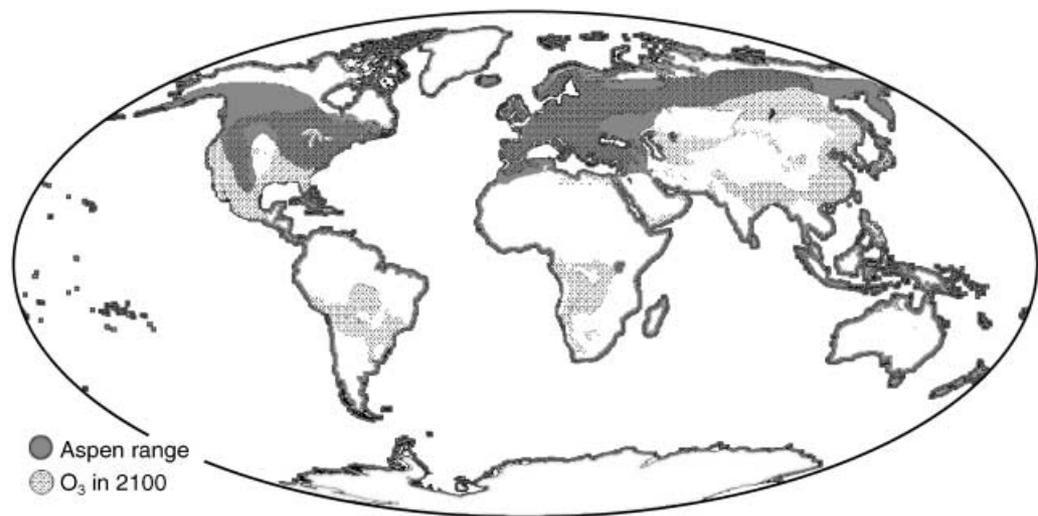


Fig. 8. Worldwide distribution of Aspen and projected areas with elevated O₃ in the year 2100. Ozone map from Fowler *et al.* (1999a).

quantity of understorey vegetation; and canopy-level sap flow – are still premature to discuss at this point, but should be an integral part of our project’s future research efforts. Finally, we continue to use our results to parameterize and test various growth models such as ECOPHYS (Martin *et al.* 2001) to broaden the inferences from our results.

Acknowledgements

This research was partially supported by the US Department of Energy’s Office of Biological and Envir-

onmental Research (BER) (DE-FG02-95ER62125; DE-FG02-93ER6166; DE-FG02-98ER62680), USDA Forest Service Northern Global Change Program, the National Science Foundation (DBI-9601942; IBN-9652675; DEB-9707263; DBI-972395), the USDA NRI Competitive Grants Programs (000-2982; 001-00796; 001-01193), the National Council of the Paper Industry for Air and Stream Improvement (NCASI), Michigan Technological University, the Slovakian Forest Research Institute, the Praxair Foundation, the University of Wisconsin Foundation, the McIntire–Stennis Program, the Brookhaven

National Laboratories/US Department of Energy (725-079), the Academy of Finland (Projects 39935; 42702; 43168), the Ministry of Agriculture and Forestry in Finland, Natural Resources Canada – Canadian Forest Service, the British Ecological Society, and the Natural Environmental Research Council of the United Kingdom.

References

- Anttonen, S., Vapaavuori, E., Kostiaainen, K., Isebrands, J.G., McDonald, E., Sober, J. & Karnosky, D.F. (2001) Effect of elevated CO₂ and O₃ on the chemical composition of wood in aspen clones: results after 3 years of exposure in the Aspen FACE project. *Forest Research: A Challenge for an Integrated European Approach*, International Conference Proceedings, Thessaloniki, Greece (Radoglou, K., ed.), pp. 239–242. ISBN: 960-869-47-3-6.
- Awmack, C.S. & Harrington, R. (2000) Elevated CO₂ affects the interactions between aphid pests and host plant flowering. *Agricultural and Forest Entomology* **2**, 257–261.
- Barbo, D.N., Chappelka, A.H., Somers, G.L., Miller-Goodman, M.S. & Stolte, K. (1998) Diversity of an early successional plant community as influenced by ozone. *New Phytologist* **138**, 653–662.
- Barnes, J.D. & Wellburn, A.R. (1998) Air pollutant combinations. *Responses of Plant Metabolism to Air Pollution and Global Change* (eds L.J. DeKok & I. Stulen), pp. 147–164. Backhuys, Leiden, the Netherlands.
- Barnes, J.D., Ollerenshaw, J.H. & Whitfield, C.P. (1995) Effects of elevated CO₂ and/or O₃ on growth, development and physiology of wheat (*Triticum aestivum* L.). *Global Change Biology* **1**, 129–142.
- Barnola, J.M., Anklin, M., Porheron, J., Raynaud, D., Schwander, J. & Stauffer, B.T.I. (1995) CO₂ evolution during the last millennium as recorded by Antarctic and Greenland ice. *Tellus B* **47**, 264–272.
- Bender, J., Herstein, U. & Black, C.R. (1999) Growth and yield responses of spring wheat to increasing carbon dioxide, ozone and physiological stresses: a statistical analysis of 'ESPACE – wheat' results. *European Journal of Agronomy* **10**, 185–195.
- Berrang, P.C., Karnosky, D.F., Mickler, R.A. & Bennett, J.P. (1986) Natural selection for ozone tolerance in *Populus tremuloides*. *Canadian Journal of Forest Research* **16**, 1214–1216.
- Bezemer, T.M. & Jones, T.H. (1998) Plant–insect herbivore interactions in elevated atmospheric CO₂: quantitative analyses and guild effects. *Oikos* **82**, 212–222.
- Bortier, K., Ceulemans, R. & Temmerman, L.D. (2000) Effects of tropospheric ozone on woody plants. *Environmental Pollution and Plant Responses* (eds S.B. Agrawal & M. Agrawal), pp. 153–182. Lewis Publishers, New York.
- Broadmeadow, M.S. & Jackson, S.B. (2000) Growth responses of *Quercus petraea*, *Fraxinus excelsior* and *Pinus sylvestris* to elevated carbon dioxide, ozone, and water supply. *New Phytologist* **146**, 437–451.
- Ceulemans, R. & Mousseau, M. (1994) Effects of elevated atmospheric CO₂ on woody plants. *New Phytologist* **127**, 425–446.
- Chappelka, A.H. & Samuelson, L.J. (1998) Ambient ozone effects on forest trees of the eastern United States: a review. *New Phytologist* **139**, 91–108.
- Coleman, M.D., Dickson, R.E., Isebrands, J.G. & Karnosky, D.F. (1995a) Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* **15**, 585–592.
- Coleman, M.D., Dickson, R.E., Isebrands, J.G. & Karnosky, D.F. (1995b) Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* **15**, 593–604.
- Costa e Silva, J., Wellendorf, H. & Pereira, H. (1998) Clonal variation in wood quality in young Sitka spruce (*Picea sitchensis* (Bong.) Carr.): estimation of quantitative genetic parameters and index selection for improved pulpwood. *Silvae Genetica* **47**, 20–33.
- Cotrufo, M.F., Ineson, P. & Scott, A. (1998) Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global Change Biology* **4**, 43–54.
- Curtis, P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell Environment* **19**, 127–137.
- Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia* **113**, 299–313.
- Denne, M.P., Calahan, C.M. & Aebischer, D.P. (1999) Influence of growth rate and cambial age on density of Rauli (*Nothofagus nervosa*) in relation to vessel lumen areas and numbers. *Holzforchung* **53**, 199–203.
- Dickson, R.E. & Isebrands, J.G. (1991) Leaves as regulators of stress response. *Response of Plants to Multiple Stresses* (eds H.A. Mooney, W.E. Winner & E.J. Pell). Academic Press, London, pp. 3–34.
- Dickson, R.E., Coleman, M.D., Riemenschneider, D.E., Isebrands, J.G., Hogan, G.D. & Karnosky, D.F. (1998) Growth of five hybrid poplar genotypes exposed to interacting elevated CO₂ and O₃. *Canadian Journal of Forest Research* **28**, 1706–1716.
- Dickson, R.E., Lewin, K.F., Isebrands, J.G., Coleman, M.D., Heilman, W.E., Riemenschneider, D.E., Söber, J., Host, G.E., Zak, D.R., Hendrey, G.R., Pregitzer, K.S. & Karnosky, D.F. (2000) *Forest atmosphere carbon transfer storage-II (FACTS II) – the aspen free-air CO₂ and O₃ enrichment (FACE) project: an overview*. General Technical Report NC-214. USDA Forest Service, North Central Research Station, Rhinelander, WI.
- Donnelly, A., Craigon, J., Black, C.R., Colls, J.J. & Landon, G. (2001) Elevated CO₂ increases biomass and tuber yield in potato even at high ozone concentrations. *New Phytologist* **149**, 265–274.
- Finlayson-Pitts, B.J. & Pitts, Jr (1997) Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particulates. *Science* **276**, 1045–1051.
- Fowler, D., Flechard, C., Skiba, U., Coyle, M. & Cape, J.N. (1998) The atmospheric budget of oxidized nitrogen and its role in ozone formation and deposition. *New Phytologist* **139**, 11–23.
- Fowler, D., Cape, J.N., Coyle, M., Flechard, C., Kuylentstierna, J., Hicks, K., Derwent, D., Johnson, C. & Stevenson, D. (1999a) The global exposure of forests to air pollutants. *Water, Air and Soil Pollution* **116**, 5–32.
- Fowler, D., Cape, J.N., Coyle, M., Smith, R.I., Hjellbrekke, A.-G., Simpson, D., Derwent, R.G. & Johnson, C.E. (1999b) Modelling photochemical oxidant formation, transport, deposition and exposure of terrestrial ecosystem. *Environmental Pollution* **100**, 43–45.
- Gagnon, Z.E., Karnosky, D.F., Dickson, R.E. & Isebrands, J.G. (1992) Effect of ozone on chlorophyll content in *Populus tremuloides*. *American Journal of Botany* **79**, 107.
- Hao, X., Hale, B.A., Ormrod, D.P. & Papadopoulos, A.P. (2000) Effects of pre-exposure to ultraviolet-B radiation on responses of ambient and elevated carbon dioxide. *Environmental Pollution* **110**, 217–224.
- Hättenschwiler, S., Schweingruber, F.H. & Körner, C. (1996) Tree ring responses to elevated CO₂ and increased N deposition in *Picea abies*. *Plant Cell and Environment* **19**, 1369–1378.
- Hatton, J.V. & Hunt, K. (1992) Wood density and chemical properties of second-growth lodgepole pine. *Tappi 1992 Pulping Conference Proceedings*, pp. 873–882. Tappi Press.

- Heagle, A.S., Miller, J.E. & Pursley, W.A. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment. III. Yield and seed quality. *Crop Science* **38**, 128–134.
- Heck, W.W., Furiness, C.S., Cowling, E.B. & Sims, C.K. (1998) Effects of ozone on crop, forest, and natural ecosystems: assessments of research needs. *EM; Air and Waste Management Association*, Pittsburgh, PA, pp. 11–22.
- Hendrey, G.R., Long, S.P., McKee, I.F. & Baker, N.R. (1997) Can photosynthesis respond to short-term fluctuations in atmospheric carbon dioxide? *Photosynthesis Research* **5**, 179–184.
- Hendrey, G.R., Ellsworth, D.S., Lewin, K.F. & Nagy, J. (1999) A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO₂. *Global Change Biology* **5**, 293–309.
- Hogsett, W.E., Weber, J.E., Tingey, D., Herstrom, A., Lee, E.H. & Laurence, J.A. (1997) An approach for characterizing tropospheric ozone risk to forests. *Environmental Management* **21**, 105–120.
- Hudak, C., Bender, J., Weigel, H.-J. & Miller, J. (1999) Interactive effects of elevated CO₂, O₃, and soil water deficient on spring wheat (*Triticum aestivum* L. cv. Nandu). *Agronomie* **19**, 677–687.
- Huyen, G. (1999) Age trends in genetic parameters of wood density in young Norway spruce. *Canadian Journal of Forest Research* **29**, 135–143.
- IPCC (2001) *A report of working group I of the Intergovernmental Panel on Climate Change*. <http://www.ipcc.ch/>
- Isebrands, J.G., McDonald, E.P., Kruger, E., Hendrey, G., Pregitzer, K., Percy, K., Söber, J. & Karnosky, D.F. (2001) Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. *Environmental Pollution* **115**, 359–371.
- Karnosky, D.F. (1976) Threshold levels for foliar injury to *Populus tremuloides* Michx. by sulfur dioxide and ozone. *Canadian Journal of Forest Research* **6**, 166–169.
- Karnosky, D.F., Gagnon, Z.E., Reed, D.D. & Witter, J.A. (1992) Growth and biomass allocation of symptomatic and asymptomatic *Populus tremuloides* clones in response to seasonal ozone exposures. *Canadian Journal of Forest Research* **22**, 1785–1788.
- Karnosky, D.F., Gagnon, Z.E., Dickson, R.E., Coleman, M.D., Lee, E.H. & Isebrands, J.G. (1996) Changes in growth, leaf abscission, and biomass associated with seasonable tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Canadian Journal of Forest Research* **16**, 23–27.
- Karnosky, D.F., Podila, G.K., Gagnon, Z., Pechter, P., Akkapeddi, A., Coleman, M., Dickson, R.E. & Isebrands, J.G. (1998) Genetic control of responses to interacting tropospheric ozone and CO₂ in *Populus tremuloides*. *Chemosphere* **36**, 807–812.
- Karnosky, D.F., Mankovska, B., Percy, K., Dickson, R.E., Podila, G.K., Söber, J., Noormets, A., Hendrey, G., Coleman, M.D., Kubiske, M.E., Pregitzer, K.S. & Isebrands, J.G. (1999) Effects of tropospheric O₃ on trembling aspen and interaction with CO₂; results from an O₃-gradient and a FACE experiment. *Water, Air, and Soil Pollution* **116**, 311–322.
- Karnosky, D.F., Gielen, B., Ceulemans, R., Schlesinger, W.H., Norby, R.J., Oksanen, E., Matyssek, R. & Hendrey, G.R. (2001) FACE systems for studying the impacts of greenhouse gases on forest ecosystems. *The Impacts of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems* (eds D.F. Karnosky, G. Scarascia-Mugnozza, R. Ceulemans & J. Innes). CABI Publishing, Wallingford, UK, pp. 297–324.
- Karnosky, D.F., Percy, K.E., Xiang, B., Callan, B., Noormets, A., Mankovska, B., Hopkin, A., Söber, J., Jones, W., Dickson, R.E. & Isebrands, J.G. (2002a) Interacting CO₂-tropospheric O₃ and predisposition of aspen (*Populus tremuloides* Michx.) to infection by *Melampsora medusae* rust. *Global Change Biology* **8**, 1–10.
- Karnosky, D.F., Percy, K., Mankovska, B., Pritchard, T., Noormets, A., Dickson, R.E., Jepsen, E. & Isebrands, J.G. (2002b) Ozone affects the fitness of trembling aspen. *Air Pollution, Global Change and Forests in the New Millennium* (eds Karnosky, D.F., Percy, K.E., Chappelka, A.H., Simpson, C.J. & Pikkarainen, J.M.). Elsevier Science, Oxford, UK, in press.
- Keeling, C.M., Whort, T.P., Wahlen, M. & Vander Pliet, J. (1995) International extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* **375**, 666–670.
- King, J.N., Cartwright, C., Hatton, J. & Yanchuk, A.D. (1998) The potential of improving western hemlock pulp and paper quality. I. Genetic control and interrelationships of wood and fibre traits. *Canadian Journal of Forest Research* **28**, 863–870.
- King, J.S., Pregitzer, K.S., Zak, D.R., Söber, J., Isebrands, J.G., Dickson, R.E., Hendrey, G.R. & Karnosky, D.F. (2001) Fine root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* **128**, 237–250.
- Kopper, B.J. (2001) *Consequences of elevated carbon dioxide and ozone for interactions between deciduous trees and lepidopteran folivores*. PhD thesis, University of Wisconsin-Madison.
- Kopper, B.J. & Lindroth, R.L. (2003) Responses of trembling aspen (*Populus tremuloides*) phytochemistry and aspen blotch leafminer (*Phyllonorycter tremuloidiella*) performance to elevated levels of atmospheric CO₂ and O₃. *Agricultural and Forest Entomology*, **5**, 17–26.
- Körner, C. (2000) Biosphere responses to CO₂ enrichment. *Ecological Applications* **10**, 1590–1619.
- Kubiske, M.E. & Godbold, D.L. (2001) Influence of carbon dioxide on the growth and function of roots and root systems. *The Impacts of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems* (eds D.F. Karnosky, G. Scarascia-Mugnozza, R. Ceulemans & J. Innes). CABI Publishing, Wallingford, UK, pp. 297–324.
- Kull, O., Söber, A., Coleman, M.D., Dickson, R.E., Isebrands, J.G., Gagnon, Z. & Karnosky, D.F. (1996) Photosynthetic response of aspen clones to simultaneous exposures of ozone and CO₂. *Canadian Journal of Forest Research* **16**, 639–648.
- Kurz, W.A. & Apps, M.J. (1999) A 70-year retrospective analysis of carbon fluxes in the Canadian forest sector. *Ecology Applications* **9**, 526–547.
- Larson, J.L., Zak, D.R. & Sinsabaugh, R.L. (2002) Microbial activity beneath temperate trees growing under elevated CO₂ and O₃. *Soil Science Society of America Journal* **66**, 1848–1856.
- Lawson, T., Craigon, J., Black, C.R., Colls, J.J., Tulloch, A.-M. & Landon, G. (2001) Effects of elevated carbon dioxide and ozone on the growth and yield of potatoes (*Solanum tuberosum*) grown in open-top chambers. *Environmental Pollution* **111**, 479–491.
- Lindroth, R.L., Kinney, K.K. & Platz, C.L. (1993) Responses of deciduous trees to elevated atmospheric CO₂: productivity, phytochemistry and insect performance. *Ecology* **74**, 763–777.
- Lindroth, R.L., Roth, S., Kruger, E.L., Volin, J.C. & Koss, P.A. (1997) CO₂-mediated changes in aspen chemistry: effects on gypsy moth performance and susceptibility to virus. *Global Change Biology* **3**, 279–289.
- Lindroth, R.L., Kopper, B.J., Parsons, W.F.J., Bockheim, J.G., Söber, J., Hendrey, G.R., Pregitzer, K.S., Isebrands, J.G. & Karnosky, D.F. (2001) Effects of elevated carbon dioxide and ozone on foliar chemical composition and dynamics

- in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution* **115**, 394–404.
- Lindroth, R.L., Wood, S.A. & Kopper, B.J. (2002) Response of quaking aspen genotypes to enriched CO₂: foliar chemistry and tussock moth performance. *Agricultural and Forest Entomology* **4**, 315–323.
- Mankovska, B., Percy, K. & Karnosky, D.F. (1998) Impact of ambient tropospheric O₃, CO₂, and particulates on the epicuticular waxes of aspen clones differing in O₃ tolerance. *Ekológia* **18**, 200–210.
- Martin, M.J., Host, G.E., Lenz, K.E. & Isebrands, J.G. (2001) Simulating the growth responses of aspen to elevated ozone: a mechanistic approach to scaling a leaf-level model of ozone effects on photosynthesis to a complex canopy architecture. *Environmental Pollution* **115**, 425–436.
- Matyssek, R. & Innes, J.L. (1999) Ozone – a risk factor for trees and forests in Europe? *Journal of Water, Air, and Soil Pollution* **116**, 199–226.
- McDonald, E.P., Kruger, E.L., Riemenschneider, D.E. & Isebrands, J.G. (2000) Consequences of elevated levels of atmospheric CO₂ and O₃ for growth of *Populus tremuloides* clones: the role of competition. *Proceedings of Air Pollution, Global Change and Forests in the New Millennium: 19th International Meeting for Specialists in Air Pollution Effects on Forest Ecosystems, Houghton, Michigan*, p. 58, Michigan Technological University.
- McDonald, E.P., Kruger, E.L., Riemenschneider, D.E. & Isebrands, J.G. (2002) Competitive status influences tree-growth responses to elevated CO₂ and O₃ in aggrading aspen stands. *Functional Ecology* **16**, 792–801.
- McKee, I.F., Bullimore, J.F. & Long, S.P. (1997) Will elevated CO₂ concentrations protect the yield of wheat from O₃ damage? *Plant, Cell and Environment* **20**, 77–84.
- McLeod, A.R. & Long, S.P. (1999) Free-air carbon dioxide enrichment (FACE) in global change research: a review. *Advances in Ecological Research* **28**, 1–56.
- McLeod, A.R., Fackrell, J.E. & Alexander, K. (1985) Open-air fumigation of field crops: criteria and design for a new experimental system. *Atmospheric Environment* **19**, 1639–1649.
- Miller, J.E., Heagle, A.S. & Pursley, W.A. (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment. II. Biomass and development. *Crop Science* **38**, 122–128.
- Moore, B.D., Cheng, S.H., Sims, D. & Seemann, J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation of elevated atmospheric CO₂. *Plant Cell and Environment* **22**, 567–582.
- Noormets, A. (2001) The effects of interacting CO₂ and O₃ on the carbon balance of aspen (*Populus tremuloides* Michx.) grown in an open-air CO₂ and O₃ enrichment system. PhD thesis, Michigan Technological University.
- Noormets, A., Podila, G.K. & Karnosky, D.F. (2000) Rapid response of antioxidant enzymes to O₃-induced oxidative stress in *Populus tremuloides* clones varying in O₃ tolerance. *Forest Genetics* **7**, 339–342.
- Noormets, A., Sober, A., Pell, E.J., Dickson, R.E., Podila, G.K., Sober, J., Isebrands, J.G. & Karnosky, D.F. (2001a) Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO₂ and/or O₃. *Plant, Cell and Environment* **24**, 327–336.
- Noormets, A., McDonald, E.P., Kruger, E.L., Sober, A., Isebrands, J.G., Dickson, R.E. & Karnosky, D.F. (2001b) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain in aspen. *Trees* **15**, 262–270.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W. & Ceulemans, R. (1999) Tree responses to rising CO₂ in field experiments: implications for the future forest. *Plant Cell and Environment* **22**, 683–714.
- Norby, R.J., Long, T.M., Hartz-Rubin, J.S. & O'Neill, E.G. (2000) Nitrogen resorption in senescing tree leaves in a warmer, CO₂-enriched atmosphere. *Plant and Soil* **224**, 15–29.
- Norby, R.J., Cotrufo, M.F., Ineson, P., O'Neill, E.G. & Canadell, J.G. (2001a) Elevated CO₂, litter chemistry, and decomposition: a synthesis. *Oecologia* **127**, 153–165.
- Norby, R.J., Todd, D.E., Fufts, J. & Johnson, D.W. (2001b) Allometric determination of tree growth in a CO₂-enriched sweetgum stand. *New Phytologist* **150**, 477–487.
- Oksanen, E., Söber, J. & Karnosky, D.F. (2001) Interactions of elevated CO₂ and ozone in leaf morphology of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in aspen FACE experiment. *Environmental Pollution* **115**, 437–446.
- Olczyk, D.M., Tibbitts, T.W. & Hertzberg, W.M. (1980) Environment in open-top chambers utilized for air pollution studies. *Journal of Environmental Quality* **9**, 610–615.
- Olczyk, D.M. & Wise, C. (1997) Interactive effects of elevated CO₂ and O₃ on rice and flacca tomato. *Agriculture, Ecosystems and Environment* **66**, 1–10.
- Oren, R., Ellsworth, D.S., Johnson, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G. & Katul, G.G. (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature* **411**, 469–472.
- Parsons, W.F.J., Bockheim, J.G. & Lindroth, R.L. (2000) *Populus tremuloides* and *Betula papyrifera* decomposition at Aspen-FACE (FACTS II): leaf litter production and decay under individual and interactive effects of elevated CO₂ and O₃. *Proceedings of Air Pollution, Global Change and Forests in the New Millennium: 19th International Meeting for Specialists in Air Pollution Effects on Forest Ecosystems, Houghton, Michigan*, p. 64.
- Percy, K.E., Mankovska, B., Hopkin, A., Callan, B. & Karnosky, D.F. (2002a) Ozone affects leaf-surface – pest interaction. *Air Pollution, Global Change and Forests in the New Millennium* (eds Karnosky, D.F., Percy, K.E., Chappelka, A.H., Simpson, C.J. & Pikkarainen, J.M.). Elsevier Science, Oxford, UK, in press.
- Percy, K.E., Awmack, C.S., Lindroth, R.L., Kubiske, M.E., Kopper, B.J., Isebrands, J.G., Pregitzer, K.S., Hendrey, G.R., Dickson, R.E., Zak, D.R., Oksanen, E., Sober, J., Harrington, R. & Karnosky, D.F. (2002b) Altered performance of forest pests under CO₂- and O₃-enriched atmospheres. *Nature* **420**, 403–407.
- Phillips, R.L., Zak, D.R. & Holmes, W.E. (2002) Microbial community composition and function beneath temperate trees exposed to elevated atmospheric CO₂ and O₃. *Oecologia* **131**, 236–244.
- Piva, R.J. (1996) *Pulpwood Production in the Lake States, 1994*. Research Note NC-368. USDA Forest Service, North Central Research Station, Rhinelander, WI.
- Rudorff, B.F.T., Mulchi, C.L., Lee, E.H., Rowland, R. & Pausch, R. (1996) Effects of enhanced O₃ and CO₂ enrichment on plant characteristics in wheat and corn. *Environmental Pollution* **94**, 53–60.
- Ryerson, T.B., Frainer, M., Holloway, J.S., Parrish, D.D., Huey, L.G., Sueper, D.T., Frost, G.J., Donnelly, S.G., Schaffler, S., Atlas, E.L., Kuster, W.C., Goldan, P.D., Hubler, G., Meagher, J.F. & Fehsenfeld, F.C. (2001) Observations of ozone formation in power plant plumes and implications for ozone control strategies. *Science* **272**, 719–723.
- Saxe, H., Ellsworth, D.S. & Heath, J. (1998) Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytologist* **139**, 395–436.
- Schlesinger, W.H. & Lichter, J. (2001) Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature* **411**, 466–469.
- Söber, A., Noormets, A., Isebrands, J.G., Sober, J. & Karnosky, D.F. (2000) Hydraulic properties and stomatal

- conductance of aspen leaves as affected by leaf senescence and elevated concentrations of carbon dioxide and ozone. *Proceedings of the Annual Meeting of the American Society of Plant Physiologists, San Diego, CA: Plant Biology* **2000**, 97–98.
- Söber, A., Noormets, A., Kull, O., Isebrands, J.G., Dickson, R.E., Söber, J. & Karnosky, D.F. (2003) Photosynthetic parameters in aspen grown with interacting elevated CO₂ and tropospheric ozone concentrations as affected by leaf nitrogen. *Tree Physiology*, in press.
- Stark, R.W., Miller, P.R., Cobb, F.W. Jr, Wood, D.L. & Parmeter, J.R. Jr (1968) Photochemical oxidant injury and bark beetle (Coleoptera: Scolytidae) infestation of ponderosa pine. I. Incidence of bark beetle infestation in injured trees. *Hilgardia* **39**, 121–126.
- Stevenson, D.S., Johnson, C.E., Collins, W.J., Derwent, R.G., Shine, K.P. & Edwards, J.M. (1998) Evolution of tropospheric ozone radiative forcing. *Geophysical Research Letters* **25**, 3819–3822.
- Stott, P.A., Tett, S.F.B., Jones, G.S., Allen, M.R., Mitchell, J.F.B. & Jenkins, G.J. (2000) External control of 20th century temperature by natural and anthropogenic forcings. *Science* **290**, 2133–2137.
- Takeuchi, Y., Kubiske, M.E., Isebrands, J.G., Pregitzer, K.S., Hendrey, G. & Karnosky, D.F. (2001) Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO₂ enrichment. *Plant Cell Environment* **24**, 1257–1268.
- Tjoelker, M.G., Oleksyn, J. & Reich, P.B. (1998) Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO₂ and temperature. *Tree Physiology* **18**, 715–726.
- Utriainen, J., Janhunen, S., Helmisaari, H.-S. & Holopainen, T. (2000) Biomass allocation, needle structural characteristics and nutrient composition in Scots pine seedlings exposed to elevated CO₂ and O₃ concentrations. *Trees* **14**, 475–484.
- Vasseur, L. & Potvin, C. (1998) Natural pasture community response to enriched carbon dioxide atmosphere. *Plant Ecology* **135**, 31–41.
- Volin, J.C. & Reich, P.B. (1996) Interaction of elevated CO₂ and O₃ on growth, photosynthesis and respiration of three perennial species grown in low and high nitrogen. *Physiologia Plantarum* **97**, 674–684.
- Volin, J.C., Reich, P.B. & Givnish, T.J. (1998) Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. *New Phytologist* **138**, 315–325.
- Wang, D., Karnosky, D.F. & Bormann, F.H. (1986) Effects of ambient ozone on the productivity of *Populus tremuloides* Michx. grown under field conditions. *Canadian Journal of Forest Research* **16**, 47–55.
- Wustman, B.A., Oksanen, E., Karnosky, D.F., Söber, J., Isebrands, J.G., Hendrey, G.R., Pregitzer, K.S. & Podila, G.K. (2001) Effects of elevated CO₂ and O₃ on aspen clones varying in O₃ sensitivity: can CO₂ ameliorate the harmful effects of O₃? *Environmental Pollution* **115**, 473–481.
- Zobel, B.J. & van Buijtenen, J.P. (1989) *Wood Variation, its Causes and Control*. Springer-Verlag, Berlin.

Received 22 August 2002; revised 18 October 2002; accepted 27 October 2002