

Scaling ozone responses of forest trees to the ecosystem level in a changing climate

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ABSTRACT

Many uncertainties remain regarding how climate change will alter the structure and function of forest ecosystems. At the Aspen FACE experiment in northern Wisconsin, we are attempting to understand how an aspen/birch/maple forest ecosystem responds to long-term exposure to elevated carbon dioxide (CO₂) and ozone (O₃), alone and in combination, from establishment onward. We examine how O₃ affects the flow of carbon through the ecosystem from the leaf level through to the roots and into the soil micro-organisms in present and future atmospheric CO₂ conditions. We provide evidence of adverse effects of O₃, with or without co-occurring elevated CO₂, that cascade through the entire ecosystem impacting complex trophic interactions and food webs on all three species in the study: trembling aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh), and sugar maple (*Acer saccharum* Marsh). Interestingly, the negative effect of O₃ on the growth of sugar maple did not become evident until 3 years into the study. The negative effect of O₃ effect was most noticeable on paper birch trees growing under elevated CO₂. Our results demonstrate the importance of long-term studies to detect subtle effects of atmospheric change and of the need for studies of interacting stresses whose responses could not be predicted by studies of single factors. In biologically complex forest ecosystems, effects at one scale can be very different from those at another scale. For scaling purposes, then, linking process with canopy level models is essential if O₃ impacts are to be accurately predicted. Finally, we describe how outputs from our long-term multispecies Aspen FACE experiment are being used to develop simple, coupled models to estimate productivity gain/loss from changing O₃.

Key-words: *Populus tremuloides*; carbon accumulation and allocation; carbon dioxide; C and N cycling; climate change; ecosystem scaling; modelling; pest interactions; trembling aspen; tropospheric ozone.

Abbreviations: AAQS, American air quality standards; AOT40, accumulated hourly O₃ exposure over 40 ppb; C¹⁴, radioactive isotope of carbon; CDIAC, Carbon Dioxide Information Analysis Center; CL, critical level; CWS, Canadian Wide Standard; ECOPHYS, an ecophysiological model to grow individual trees; FACE, Free-Air CO₂ Exposure; LAI, leaf area index; N, nitrogen; PAR, photosynthetically active radiation; ppb, parts per billion; ppbh, parts per billion hours; ppm, parts per million; SOM, soil organic matter; SUM60, accumulated hourly exposure over 60 ppb.

INTRODUCTION

Tropospheric ozone (O₃), a secondary pollutant generated downwind of major metropolitan areas from nitrogen oxides and volatile organic compounds reacting in the presence of sunlight, was first identified as a problem in the 1950s (Dunn 1959) for plant communities downwind of the Los Angeles area. Among the first forest ecosystems found to be impacted by O₃ was the pine-fir forest in the San Bernardino Mountains (Miller *et al.* 1963; Miller & Millecan 1971). Subsequently, O₃ effects on forest trees also were identified in the Sierra Nevada Mountains of southern California (Miller, McCutcheon & Milligan 1972). About the same time, researchers in the eastern United States identified visible symptoms of O₃ damage on sensitive genotypes of eastern white pine (Dochinger *et al.* 1970; Costonis 1970).

Since these early days, thousands of papers have been published on the effects of tropospheric O₃ on the physiology, biochemistry, community structure, and population dynamics of forest trees (see reviews by Chappelka & Samuelson 1998; Skärby *et al.* 1998; Miller & McBride 1999;

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Samuelson & Kelly 2001; Percy, Legge & Krupa 2003a). However, despite numerous studies on individual plants, still very little is known about the impacts of O_3 on forest ecosystem structure and function (Karnosky *et al.* 2003a; Laurence & Andersen 2003). This is especially true in light of rapid increases in atmospheric carbon dioxide (CO_2) (Keeling *et al.* 1995). The interaction between increasing CO_2 and O_3 is especially important because of large areas of Earth are impacted by O_3 (Fowler *et al.* 1999; Fig. 1). Furthermore, it is important to understand how tropospheric O_3 may affect carbon sequestration of forest trees exposed to elevated CO_2 (Beedlow *et al.* 2004).

The Aspen FACE experiment was established in 1997 in northern Wisconsin to examine the impacts of tropospheric O_3 , alone and in combination with elevated atmospheric CO_2 , on the structure and function of a northern forest ecosystem dominated by the rapid-growing, pioneer species trembling aspen (*Populus tremuloides* Michx.) but including also another rapid-growing, pioneer species paper birch (*Betula papyrifera* Marsh) and the slower-growing, later-successional species sugar maple (*Acer saccharum* Marsh). Trembling aspen is the most widely distributed tree species in North America. Aspen forest types make up over 8.8 million ha in the USA and 17.8 million ha in Canada. In Wisconsin, alone, where this experiment is located, aspen, birch and maple stands comprise over 50% of the State's vast forest resource. Aspen and birch comprise some 70%

of the pulpwood harvested in the states bordering the Great Lakes (Piva 1996).

The Aspen FACE experiment is unique in that it was established to examine the long-term effects of these two greenhouse gases on development of the ecosystem from seedling establishment onward. The experiment utilizes a state-of-the-art open-air fumigation system (Dickson *et al.* 2000) that is devoid of artifacts that often occur in chamber systems such as low-winds, higher-than-ambient temperature, altered hydrology, higher humidity, and reduced light levels (Hendrey *et al.* 1999; McLeod & Long 1999; Karnosky *et al.* 2001). Furthermore, the plot sizes (30-m-diameter rings) are large enough to examine effects that are difficult to detect in small chambers such as intraspecific and interspecific competition, carbon fluxes to soil and pest epidemiology. Trophic interactions are facilitated by the unencumbered movement of insects into and out of the rings.

In this paper, we present a synthesis of results from the first 7 years of this unique experiment including studies of above-ground physiology and growth, canopy development, stem wood quality, community dynamics, below ground growth, carbon flux and community structure, and pest interactions. Finally, we discuss ways to scale up our experimental data to landscape or regional levels via various modelling strategies and development of appropriate O_3 dose–response functions to project impacts of O_3 . These

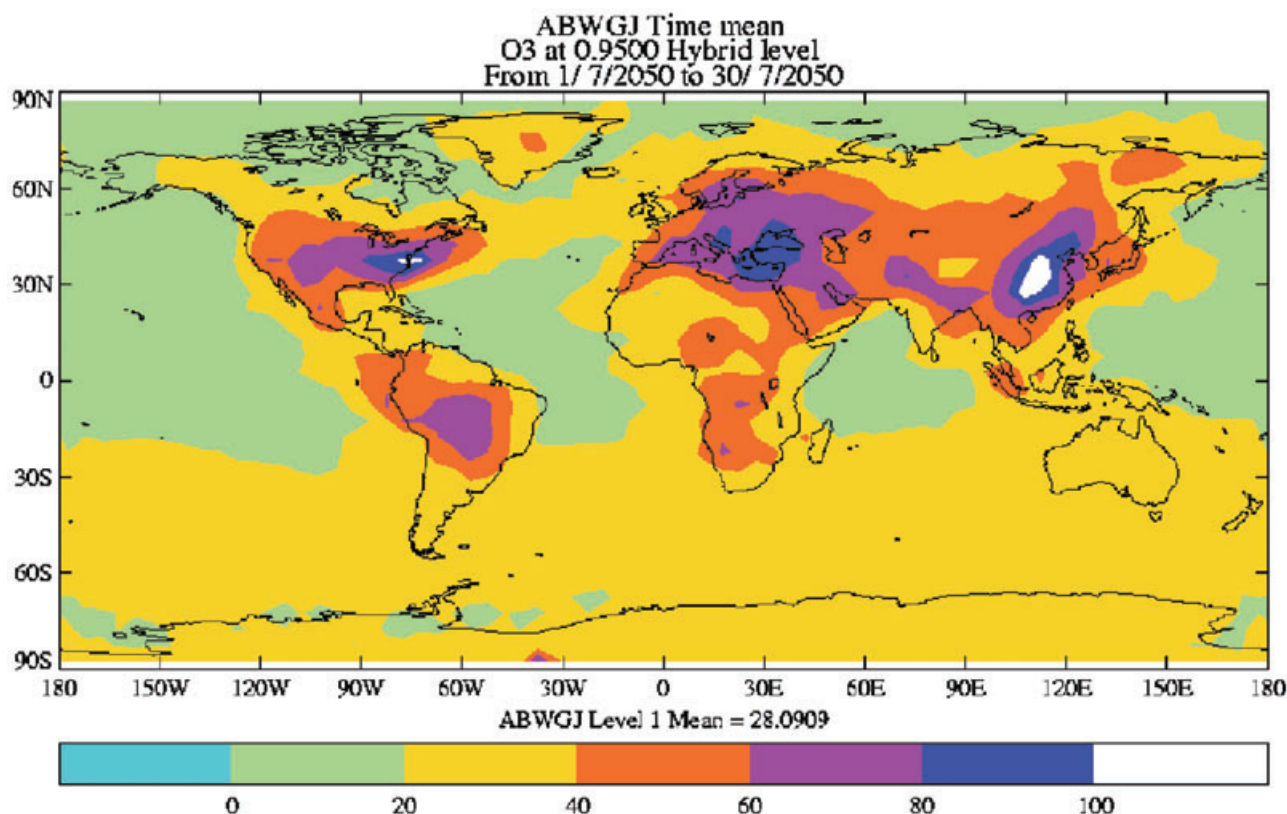


Figure 1. Projected maximum 1 h surface ozone values for July, 2050 from the model output of the STOCHEM model (C.E. Johnson and David Fowler, personal comm.).

could eventually be useful in determining O_3 standards to protect forest vegetation.

THE ASPEN FACE EXPERIMENT

The Aspen FACE experiment consists of a full factorial with 12 30-m-diameter treatment rings with three control rings, three rings with elevated O_3 , three rings with elevated CO_2 , and three rings with elevated O_3 + elevated CO_2 (Fig. 2); 100 m is the minimum distance between any two FACE rings. The rings were planted in late 1997 and treatments ran from budbreak to the end of each growing season from 1998 to 2004. The eastern one-half of each ring was randomly planted in two-tree plots at $1\text{ m} \times 1\text{ m}$ spacing with five aspen clones differing in O_3 tolerance (8L, 216 and 271 = relatively tolerant; 42E and 259 = relatively sensitive). The north-western quadrant of each ring was planted at the

same spacing with alternating aspen clone 216 and sugar maple seedlings, and the south-western quadrant of each ring was planted as above with aspen clone 216 and paper birch seedlings.

Carbon dioxide and O_3 are delivered via a computer-controlled system modified from Hendrey *et al.* (1999) during the daylight hours with our target CO_2 being 560 ppm, which is about 200 ppm above the daylight ambient CO_2 concentration. Ozone was applied at a target of $1.5 \times$ ambient and was not delivered during days when the maximum temperatures were projected to be less than 15°C or when plants were wet from fog, dew, or rain events. Additional details of the experimental design and pollutant generation and monitoring can be found in Karnosky *et al.* (2003b). Actual treatment summaries for CO_2 and O_3 were published in Karnosky *et al.* (2003b). Hourly O_3 values for one control and one O_3 ring (Fig. 3) show that our O_3 expo-

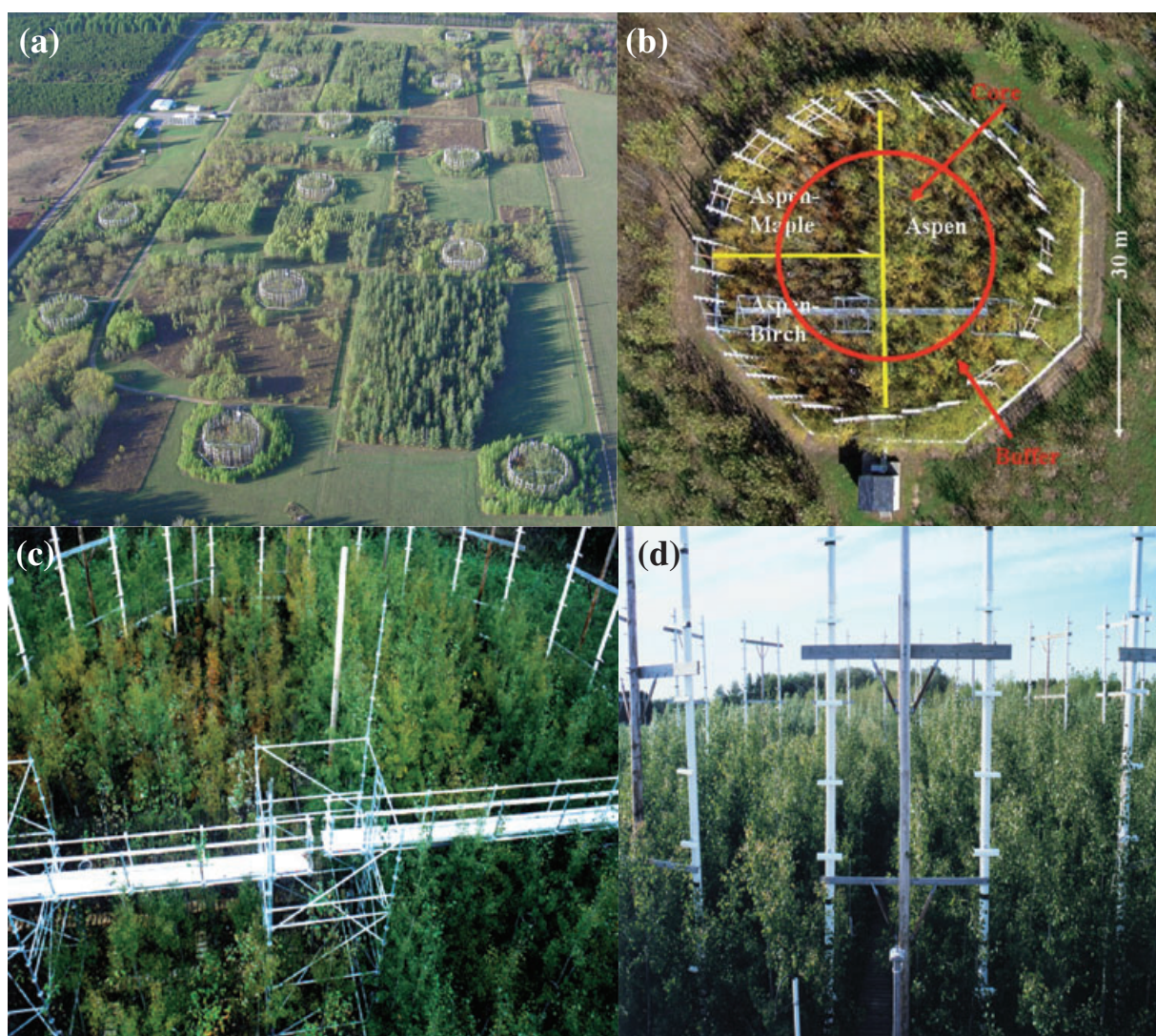


Figure 2. (a) Overview of the 12 rings of the Aspen FACE project. (b) Distribution of the aspen, aspen-birch and aspen-maple communities in each Aspen FACE ring. Each ring is surrounded by a buffer of hybrid poplar trees. (c) Canopy access walkways in each ring allow for canopy sampling in the aspen and aspen-birch communities. (d) Slots and baffles over them along the vertical vent pipes allow for gas dispensing to the outside of each ring. Wind then blows the gases into the rings.

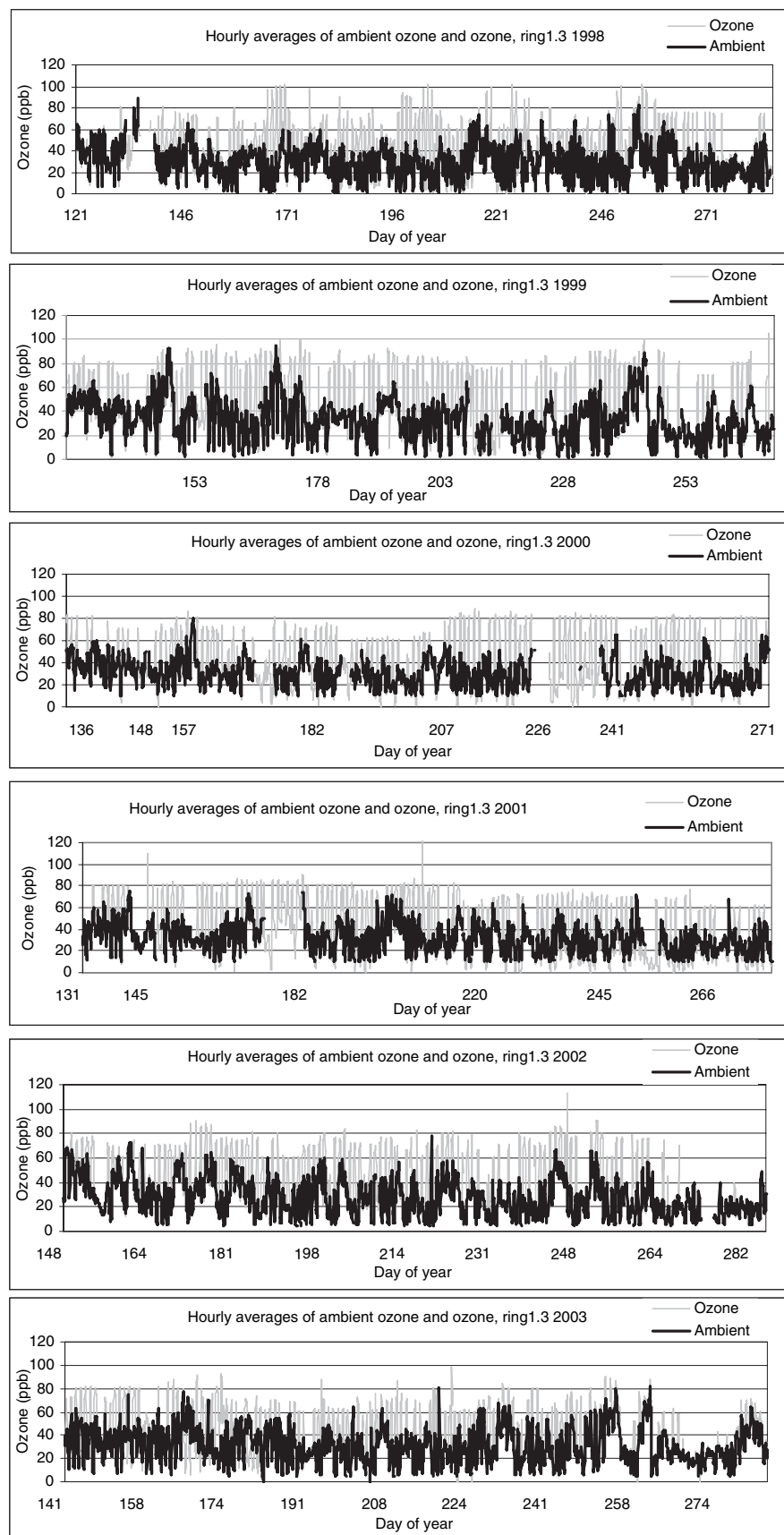


Figure 3. Hourly O_3 concentrations from ambient air (black) and one elevated O_3 ring (grey) in the Aspen FACE project during 1998–2003.

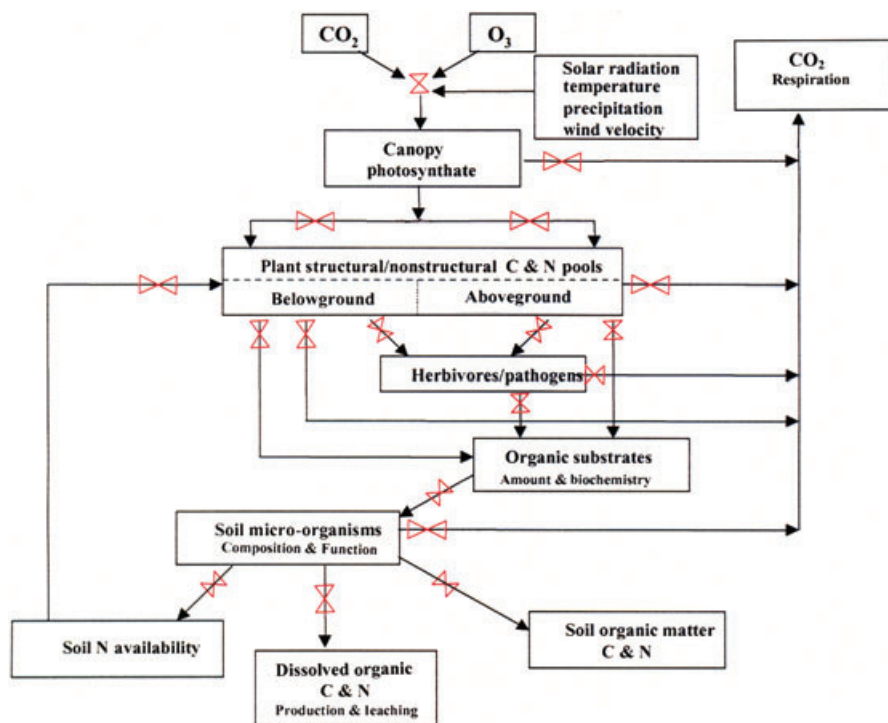


Figure 4. A conceptual model developed for the Aspen FACE project describing the flow of carbon from photosynthesis through forest ecosystems. The red switches indicate processes that serve as control points where we hypothesize that genotypic and species-specific responses to atmospheric CO_2 and O_3 will alter the flow of carbon to and through above-ground and below-ground food webs.

sure levels matched our $1.5 \times$ ambient target quite well. In addition, we monitored a number of micrometeorological parameters at our site including wind speed, wind direction, PAR, net radiation, relative humidity, rainfall, air temperature at five heights to 20.0 m and soil temperature at the soil surface and at five depths to 2.0 m and soil moisture. Relevant web sites for the Aspen FACE project include: (1) the general site for the Aspen FACE (<http://aspen-face.mtu.edu>); (2) the micrometeorology data collected at the Aspen FACE site (<http://climate.usfs.msu.edu/FACE/meteorology/>); (3) the treatment gas concentrations are shown at the BNL web site (http://www.face.bnl.gov/FACE_Site_Data_Archive/FACESites/FACTSII.htm) and the CDIAC data depository web site (<http://cdiac.ornl.gov/programs/FACE/facts-IIdata/factsIIdata.html>).

EXAMINATION OF CARBON FLOWS

In order to prioritize research at the Aspen FACE project, our site's investigator team spent a considerable amount of time developing a conceptual model of carbon cycling in our ecosystem (Fig. 4). Our research team has focused attention on control points (red triangles) in an attempt to develop a comprehensive understanding of ecosystem carbon flow under elevated CO_2 and O_3 . We hypothesized that ecosystem level responses to these two greenhouse gases would be driven by the responsiveness of the three key-stone tree species (Karnosky 2005). In this paper, we will summarize the effects of O_3 on carbon uptake, allocation, distribution, and release at background and elevated levels of CO_2 . Finally, we discuss our progress scaling our results to regional levels and we present our results in terms of O_3 metrics as best predictors of O_3 effects.

ABOVE-GROUND RESPONSES

Gas exchange

All carbon flows into trees through leaves via the process of photosynthesis. Thus, the impacts of greenhouse gases on leaf morphology, chlorophyll content, Rubisco content, stomatal density, stomatal conductance, leaf area in the canopy, and phenology of leaf display play critical roles in carbon budgets of forest trees. In Fig. 5, it is shown that there is interspecific variability in the effects of elevated O_3 and/or CO_2 on maximal photosynthesis as the rapid-growing pioneer species, trembling aspen and paper birch, are both quite responsive to these two greenhouse gases but the slower-growing, later-successional species sugar

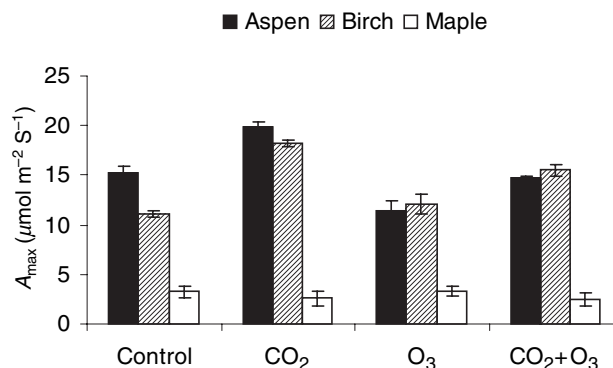


Figure 5. Light-saturated CO_2 assimilation rates of aspen, birch and maple growing under experimental atmospheric CO_2 and O_3 treatments. Data represent the mean and SE of three trees from each of three replicates for three to five measurement times over the 1999 and 2000 growing seasons (From: Karnosky *et al.* 2003b).

maple is not very responsive to either gas (Noormets *et al.* 2001a,b; Karnosky *et al.* 2003b; Sharma *et al.* 2003).

The effects of O₃ on photosynthesis have generally been negative for aspen at either CO₂ concentration (Karnosky *et al.* 2003b), whereas birch photosynthesis was most affected at the elevated CO₂. The depression of photosynthesis under elevated CO₂ + O₃ suggests co-occurring elevation of O₃ could decrease the projected stimulation of photosynthesis under future CO₂ concentrations (Kull *et al.* 1996).

Ozone effects on photosynthesis vary by aspen genotype, leaf age (Noormets *et al.* 2001a,b) and crown position (Sharma *et al.* 2003). Average maximal photosynthesis was depressed by 29–40%, depending on the aspen clone at ambient CO₂ and by 19–31% under elevated CO₂, compared with photosynthesis at elevated CO₂ alone. Older leaves tended to be more severely affected by O₃ at either CO₂ concentration (Noormets *et al.* 2001a,b). The limitations to maximal photosynthesis were primarily driven by altered mesophyll processes, primarily by impacts on Rubisco (Noormets *et al.* 2001b). In addition, O₃ decreased

chloroplast size, starch content, and altered foliar ultrastructure (Oksanen, Sober & Karnosky 2001) in aspen but not in birch leaves. More recently, O₃-induced decreases in mesophyll cell wall thickness and starch grain size were detected for both aspen and birch trees (Oksanen *et al.* 2003).

While no effects of elevated CO₂ have been found in foliar respiration at our site (Davey *et al.* 2004), increased levels of foliar respiration have been detected in O₃-damaged aspen leaves, particularly later in the growing season (Noormets *et al.* 2001a,b).

Growth

Since we have shown decreased maximal photosynthesis for aspen (see above), it is not surprising that we have documented a relatively consistent and statistically significant, O₃-induced 10–15% decrease in both height and diameter growth in aspen from O₃ at both ambient and elevated CO₂ (Isebrands *et al.* 2001; Percy *et al.* 2002; Fig. 6). In contrast, birch has not shown adverse effects of

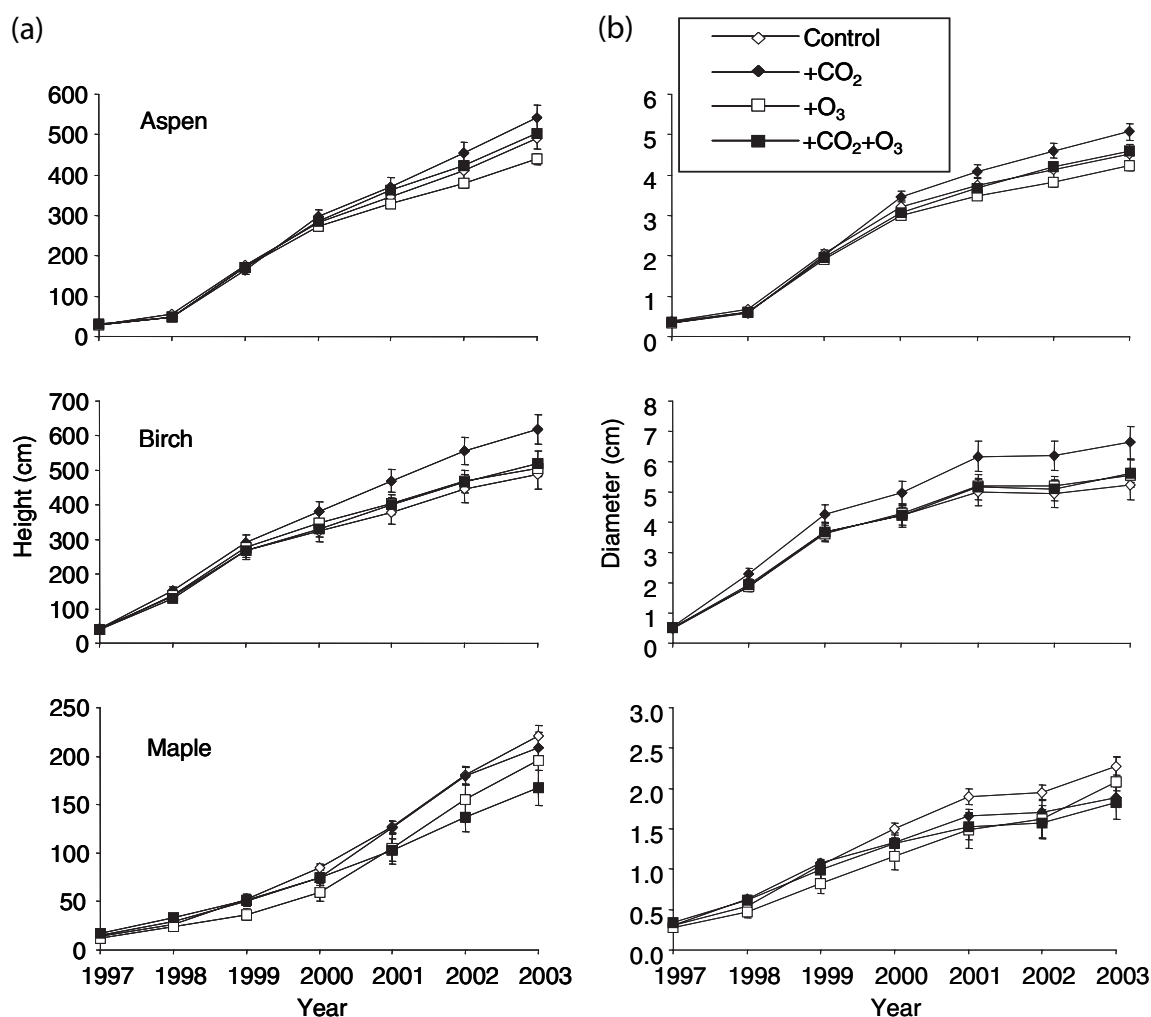


Figure 6. Height (a) and diameter (b) growth for trembling aspen, paper birch and sugar maple seedlings exposed to elevated CO₂ and/or O₃ at the Aspen FACE project. Means ± SE.

O_3 in ambient CO_2 . However, consistent and significant 14–18% decreases in growth of birch have been seen for elevated O_3 in the elevated CO_2 environment compared to birch in elevated CO_2 alone.

The most interesting growth response we have found was that of sugar maple in that no significant O_3 effects were detected for the first few years but growth has been significantly decreased since year four. After age 5, O_3 had significantly decreased both height and diameter growth of sugar maple, regardless of the CO_2 treatment. While we have not yet determined the cause for either the stronger O_3 response in birch in the $CO_2 + O_3$ combination treatment or the significant long-term decrease in sugar maple growth under elevated O_3 . These two findings show the importance of long-term studies of interacting stresses in decreasing uncertainties about the impacts of O_3 on forest ecosystems and the need to examine interacting stresses as the responses found were not predicted from the single-factor treatments.

Carbon allocation

Shifts in carbon allocation under elevated O_3 are expected in our experiment because O_3 often decreases carbon allocation to root systems (Andersen 2003). Our previous open-top chamber research with aspen suggested that O_3 may alter C^{14} distribution to roots (Coleman *et al.* 1995) and that O_3 differentially impacts root growth (Karnosky *et al.* 1996; Coleman *et al.* 1996). While a trend was seen in decreased soil respiration under elevated O_3 suggesting possible adverse effects on root growth for our site (King *et al.* 2001), we did not detect any shifts in carbon allocation in sample whole-tree biomass harvests in 2000 (Pregitzer, unpublished) and 2002.

Canopy dynamics and phenology

In addition to photosynthetic rates, leaf area and leaf display duration contribute to carbon gain potential in forest trees. In our study, we have shown that O_3 can have major negative impacts on both LAI (Fig. 7) and leaf display duration. LAI was significantly decreased under elevated O_3 throughout the growing season and this is due to several factors. First, bud break is delayed so that leaf out is later in the spring, as has been previously reported by Oksanen (2003). Second, leaf senescence and leaf abscission are accelerated under elevated O_3 , regardless of CO_2 concentration, as can be seen in late-season aerial photos (Fig. 8). Accelerated leaf aging is a well-documented phenomenon with O_3 (Pell *et al.* 1990; Bielenberg, Lynch & Pell 2002) and we have previously reported on it in aspen (Karnosky *et al.* 1996). Third, the reduced LAI may have been partially due to decreased tree sizes (Isebrands *et al.* 2001; Percy *et al.* 2002), reduced branch and leaf sizes (Oksanen *et al.* 2001), or changes in crown architecture (Dickson *et al.* 2001).

Competitive interactions

Competition for light, nutrients, and water in a forest environment can have major impacts on the responses of trees to elevated levels of O_3 and CO_2 (Matyssek & Sandermann 2003). At the Aspen FACE experiment, we are testing both the effects of interspecific as well as intraspecific competition. Clear evidence has been presented from our project that the magnitude of the O_3 effects on growth depended on the particular aspen genotype and its competitive status (McDonald *et al.* 2002). After 6 years of treatments, we can readily see the effects of CO_2 and O_3

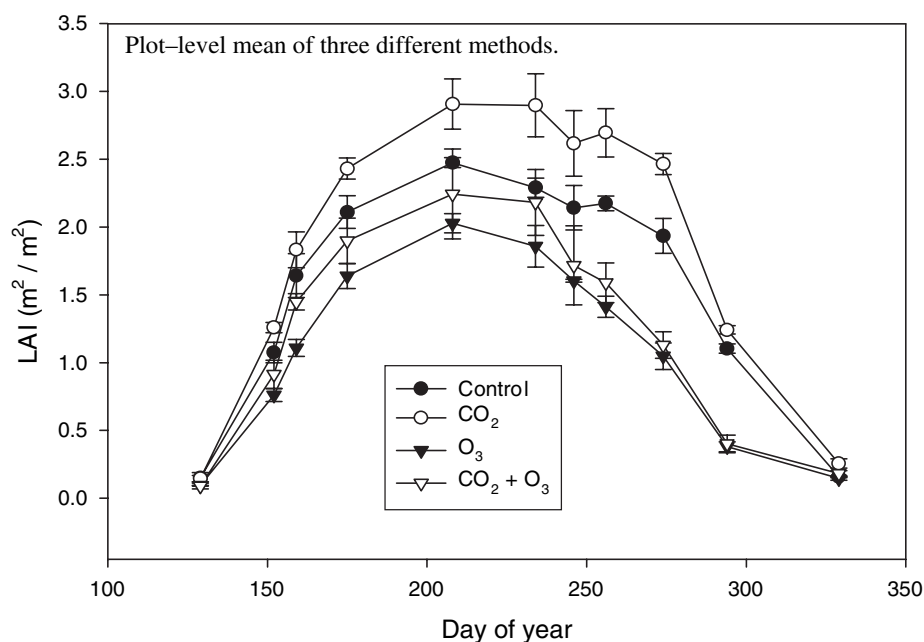


Figure 7. Estimated seasonal trends for LAI for aspen stands in the Aspen FACE experiment in northern Wisconsin during the 2002 growing season based on plot-level means in the aspen one-half of each ring for litter-fall, optical (hemispherical fisheye approach), and ceptometer data. Values are means $\pm$ SE. (From Giardina *et al.* unpublished)

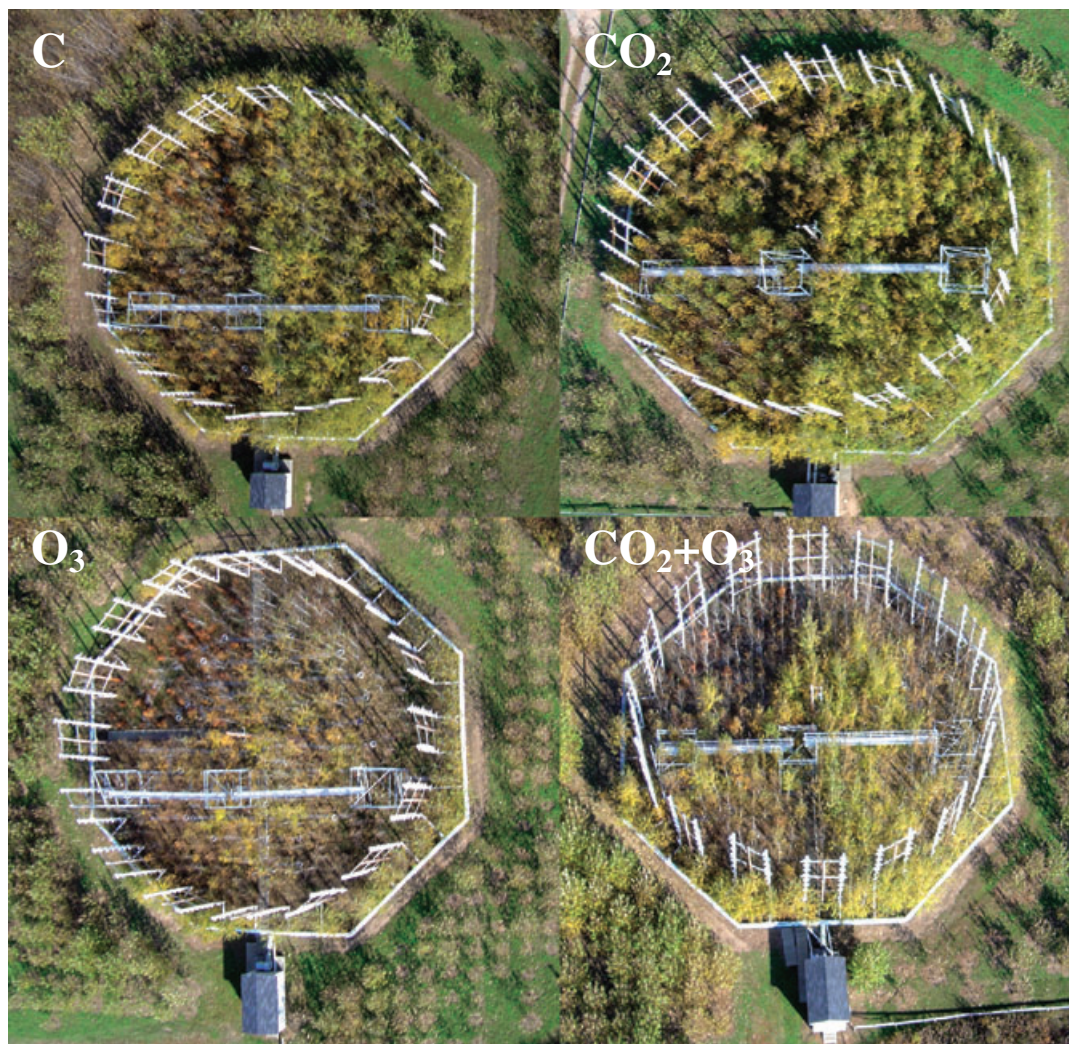


Figure 8. Ozone-induced accelerated leaf abscission at the canopy level is seen in these aerial photos of replicate three of the Aspen FACE experiment taken on 10 October 2003. The right one-half of each ring is aspen. The top left one-quarter of each ring is a mix of aspen and maple and the bottom left one-quarter of each ring is a mix of aspen and birch.

on the competitive ability of aspen clones (Fig. 9). Clone 259 is not a good competitor in our experiment as seen by its poor growth in all treatments, but we have evidence that its fitness is particularly decreased under elevated O_3 as evidenced by its decreased survival and inability to produce seed (Karnosky *et al.* 2003c). Interestingly, clone 8L appears to thrive in elevated O_3 as it is growing at faster rates under high O_3 , regardless of CO_2 treatment. We have previously seen enhanced growth in low levels of O_3 (i.e. in $0.5 \times$ ambient versus ' O_3 -free air' produced by charcoal filtering) (Karnosky *et al.* 1996) that was attributed to adaptation to elevated background O_3 exposures at the location from which the clone originated. In general, however, the lowest growth rate of aspen is in the elevated O_3 treatment and the $CO_2 + O_3$ treatment is nearly identical to growth under control conditions (Isebrands *et al.* 2001; Fig. 9).

Stem wood properties

Ozone significantly decreased volume of xylem as shown by decreased distance from the pith to the bark in our juvenile aspen stems but did not affect birch or maple stems (Kaakinen *et al.* 2004). Gravimetric lignin concentration increased under elevated O_3 in four of five aspen clones and in birch (Kaakinen *et al.* 2004). This suggests a possible change in carbon allocation leading to enhanced activity of the phenylpropanoid biosynthetic pathway as we previously documented in our Aspen FACE project with increased PAL transcripts under elevated O_3 (Wustman *et al.* 2001).

Another wood structure change illicit by O_3 was that vessel lumen diameter decreased, which could decrease water transport efficiency in the xylem (Kaakinen *et al.* 2004). In birch, hemicellulose increased under elevated O_3 .

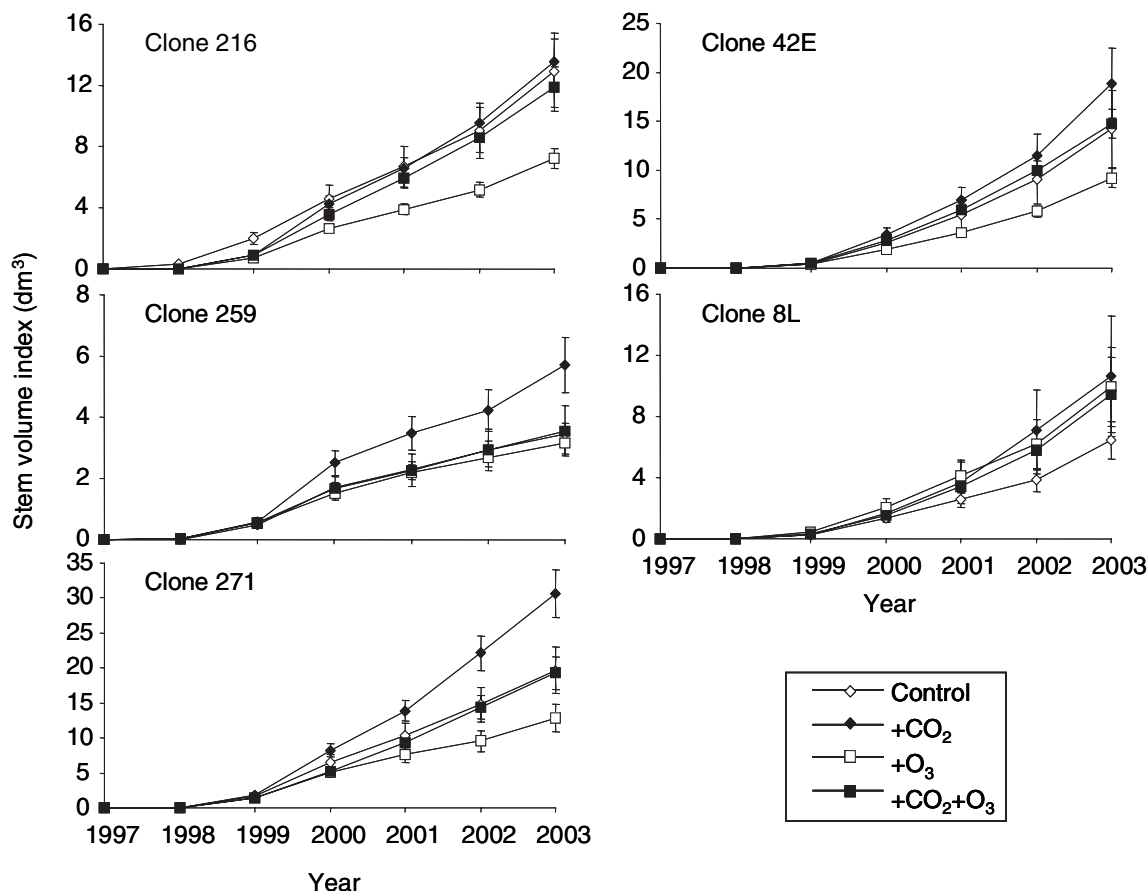


Figure 9. Impacts of elevated CO_2 and O_3 , alone and in combination, on volume growth, estimated by d^2h , from five aspen clones in the Aspen FACE experiment. Means $\pm$ SE.

Elevated CO_2 largely offset adverse effects of O_3 on both aspen and birch wood structure and chemistry (Kaakinen *et al.* 2004).

Pest interactions

A large uncertainty for future forests growing under elevated levels of O_3 and CO_2 is how forest pests will be affected (Harrington, Woiwood & Sparks 1999; Chakraborty, Tiedemann & Teng 2000). Our Aspen FACE results suggest that elevated O_3 can impact forest pests in several ways that can result in increased or decreased pest occurrence, depending on the pest. For example, O_3 altered the nutrient and defense compound composition of aspen and birch foliage in our study (Lindroth *et al.* 2001) and O_3 decreased the tremulacin concentration of aspen by about 20%, adversely affecting aspen blotch minor development (Kopper & Lindroth 2003a). However, the performance (as measured by pupal mass and fecundity) of the forest tent caterpillar, a major pest of aspen-birch forests in the Lake States region, was enhanced under elevated O_3 (Kopper & Lindroth 2003b; Holton, Lindroth & Nordheim 2003). Similarly, aphid populations were significantly higher on aspen trees under elevated O_3 (Percy *et al.* 2002; Awmack, Har-

ington & Lindroth 2004; Mondor *et al.* 2004). Interestingly, the impact of O_3 on aphids appeared to be an indirect one on their natural predators (Percy *et al.* 2002; Awmack *et al.* 2004) or due to exaggerated escape behaviour due to increased production of alarm pheromones under elevated O_3 (Mondor *et al.* 2004).

For diseases, Karnosky *et al.* (2002) have reported a three- to five-fold increase in aspen leaf rust, caused by *Melampsora medusae*, under elevated O_3 at the Aspen FACE over several growing seasons. This disease was particularly enhanced under O_3 for O_3 -sensitive aspen clones. We believe that the mechanisms for this rust infection increase is that the surface of aspen leaves is altered structurally by elevated O_3 (Mankovska, Percy & Karnosky 1998, 2003; Karnosky *et al.* 1999) and chemically (Percy *et al.* 2002) making the leaves more wettable (Percy *et al.* 2002) and more conducive to leaf infection (Percy *et al.* 2003b). These effects do not appear to be alleviated under elevated CO_2 (Mankovska *et al.* 1998, 2003).

BELOW-GROUND RESPONSES

While the role of tropospheric O_3 in altering plant growth and development has been thoroughly studied over the

recent decades, there is still limited understanding regarding the effects of O_3 on tree roots and soil processes (Andersen 2003). From our open-top chamber research with aspen, we knew that carbon allocation and partitioning to roots could be dramatically altered by O_3 (Coleman *et al.* 1995). This, in turn, could lead to decreased root growth of aspen (Coleman *et al.* 1996) and altered root/shoot ratios (Karnosky *et al.* 1996). Thus, we expected going into the Aspen FACE experiment that we would likely be able to detect effects of O_3 on root growth in aspen but did not know if birch or maple would be adversely affected. Furthermore, we were uncertain as to how interacting elevated CO_2 and O_3 would affect root growth and soil processes of any of the three species.

Root development

Early in our Aspen FACE experiment, we found that live fine-root biomass (<1.0 mm) averaged over community types was 263 g^{-2} , of which 81% was composed of roots <0.5 mm in diameter (King *et al.* 2001). Elevated O_3 resulted in a decrease in fine root biomass, which was marginally significant after three growing seasons (King *et al.* 2001). Elevated O_3 has increased fine root turnover (Pregitzer, unpublished), as was previously found from our open-top chamber studies of O_3 effects on aspen fine root dynamics (Coleman *et al.* 1996). As with our above-ground studies, elevated CO_2 alleviated the negative effects of O_3 on aspen roots. Conversely, elevated O_3 offset increases in soil respiration caused by elevated CO_2 when the two gases were applied in combination (King *et al.* 2001). Taken together, root studies at Aspen FACE suggest that elevated CO_2 increases the flux of carbon from root systems to the soil, while elevated O_3 alters whole plant source-sink relationships, resulting in more rapid root turnover and a smaller crop of standing fine root biomass (Karnosky & Pregitzer 2005).

Soil carbon

Carbon inputs to the Aspen FACE soils were increased under elevated CO_2 as seen by the increase in soil respiration (King *et al.* 2001, 2004). However, under the combination of elevated CO_2 + O_3 , stable soil carbon formation was decreased by 50% compared to that under elevated CO_2 alone (Loya *et al.* 2003; Fig. 10). This suggests that NPP-induced changes in the atmosphere may have a significant impact on the formation of stable soil carbon. These findings have begun to influence air pollution control strategies in Europe (see Grennfelt 2004).

Decomposition of leaf litter is another important part of carbon input into the forest ecosystem. Elevated CO_2 altered birch litterfall chemistry at Aspen FACE, decreasing nitrogen content, and increasing condensed tannins, regardless of O_3 concentration (Parsons, Lindroth & Bockheim 2004). Nitrogen content in decomposing litter from elevated CO_2 treatments remained consistently lower up to

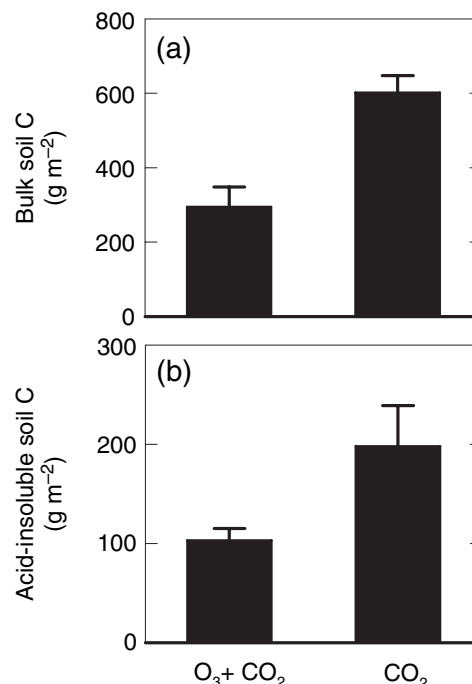


Figure 10. Total carbon incorporated into soils during 5 years of exposure to elevated O_3 + CO_2 and elevated CO_2 (a). Carbon incorporated into the stable acid-insoluble fraction of soils during 5 years of exposure to elevated O_3 + CO_2 and elevated CO_2 (b). Values are means with 1 SE bars; $P < 0.01$. Data from Loya *et al.* 2003.

350 d. Ozone alone did not affect birch leaf litter quality or litter decomposition (Parsons *et al.* 2004).

Soil fauna and micro-organisms

Since O_3 adversely affected aspen growth at Aspen FACE and since O_3 impacts litter quality in aspen (Lindroth *et al.* 2001), Loranger, Pregitzer & King (2004) hypothesized that O_3 could impact the abundance of soil fauna. They found significant decreases in Acari (−47%) under elevated O_3 , but these responses were alleviated by elevated CO_2 (Loranger *et al.* 2004).

Phillips, Zak & Holmes (2002) presented evidence from Aspen FACE that O_3 may be impacting microbial community composition at Aspen FACE. While microbial respiration was increased under elevated CO_2 , this increase was negated by the presence of co-occurring O_3 (Phillips *et al.* 2002; Fig. 11). Fungal abundance declined under elevated O_3 in both the aspen and aspen-birch communities but not in the aspen-maple community (Phillips *et al.* 2002).

Holmes *et al.* (2003) found that soil nitrogen transformations were impacted by O_3 (Fig. 12). Elevated O_3 significantly decreased gross N mineralization and microbial biomass N. Effects were consistent across all three communities (Holmes *et al.* 2003). The authors suggested that by decreasing plant litter inputs, O_3 appeared to be decreasing microbial biomass and activity.

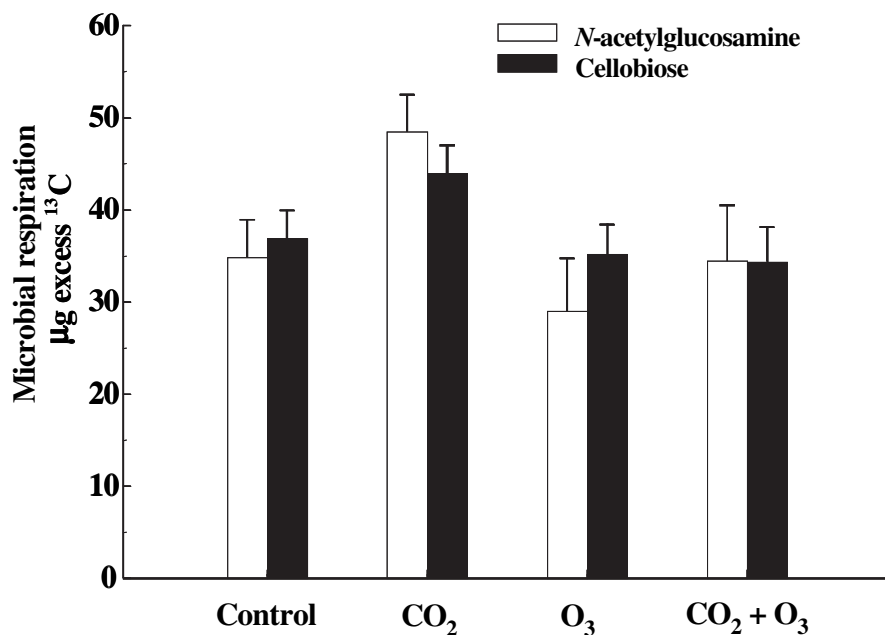


Figure 11. Microbial respiration is determined by metabolism of ^{13}C -labelled cellobiose and *N*-acetylglucosamine beneath plants exposed to elevated CO_2 and O_3 . Data from Phillips *et al.* (2002).

SCALING ASPEN FACE RESPONSES WITH MODELS

Scaling responses from 30 m plots studied over 7 years at Aspen FACE to the landscape or regional levels over decades or centuries requires making a series of assumptions to bridge these gaps in scale. Through simulation modelling we can extrapolate the consequences of these assumptions over space and time (Laurence & Andersen 2003). Since the direct effects of O_3 and CO_2 occur as physiological effects on photosynthesis in individual leaves (Chappelka & Samuelson 1998), using a leaf-level model is a logical starting point for scaling. The ways that changes in leaf physiology can in turn affect growth of trees and their ability to compete within forest canopies can then be evaluated by using forest community or forest productivity models.

Two alternative models have been used at Aspen FACE to take leaf-level responses and predict their significance for whole-tree health. First, Martin *et al.* (2001) have developed a process-based model that predicts the relative effects of O_3 on the photosynthetic rate and growth of an O_3 -sensitive aspen clone. Modifying the model ECOPHYS, developed by Rausher *et al.* (1990), Host *et al.* (1996), and Isebrands *et al.* 2000, Martin *et al.* (2001) estimate seasonal growth, biomass accumulation, and leaf drop under various O_3 profiles. Further adaptations enable the simulation of root growth and below-ground water redistribution (Theiseira *et al.* 2003).

A second model is TREGRO (Weinstein, Beloin & Yanai 1991), which simulates carbon, water, and nutrient flows of an individual plant in response to changes in temperature, drought, nutrient deficiency, and exposure to pollutants and CO_2 levels. The TREGRO model evaluates whether a reduction in the rate of photosynthesis in direct proportion to the

cumulative O_3 uptake would prevent an individual tree from meeting its carbon demands for growth. TREGRO then calculates how the plant is likely to shift its carbon allocation as a result of the lowered supply of carbon and mobilized stored carbon reserves to continue tissue growth.

Forest community or production models are then used to extrapolate the predictions of these physiological models to tree performance in the presence of competing species. One model that has been used for this purpose is ZELIG (Urban 1990; Urban *et al.* 1991), a gap-succession of forest growth simulation that predicts whole canopy and landscape processes. The link of TREGRO with ZELIG has been used to evaluate the regional impact of O_3 on several tree species (Laurence *et al.* 2001; Weinstein *et al.* 2001a; Weinstein, Gollands & Retzlaff 2001b; Weinstein *et al.* 2005).

The ZELIG model was applied to extrapolate the results from the Aspen FACE studies to regional forests of Wisconsin over 100 years of simulated stand development. Ozone was predicted to cause *P. tremuloides* basal area in the approximately 1.4 million hectares of aspen-birch stands throughout Wisconsin to decrease by over 1 million m^2 cross-sectional area or 12% of the abundance expected in the absence of O_3 (Fig. 13). This level of reduction was expected because of the physiological sensitivity of *P. tremuloides* to O_3 . However, predictions for *B. papyrifera* demonstrate that scaling is not a simple matter of extrapolating directly from experimental results. *Betula papyrifera* was predicted to increase in abundance despite the absence of sensitivity to O_3 . The scaling methodology clearly must be capable of considering changes in the competitive opportunities among species, since in this case *B. papyrifera* thrived (11% increase) despite O_3 because *P. tremuloides* was injured more severely. *Betula papyrifera* was then able to compete for resources in situations where previously it had not been able.

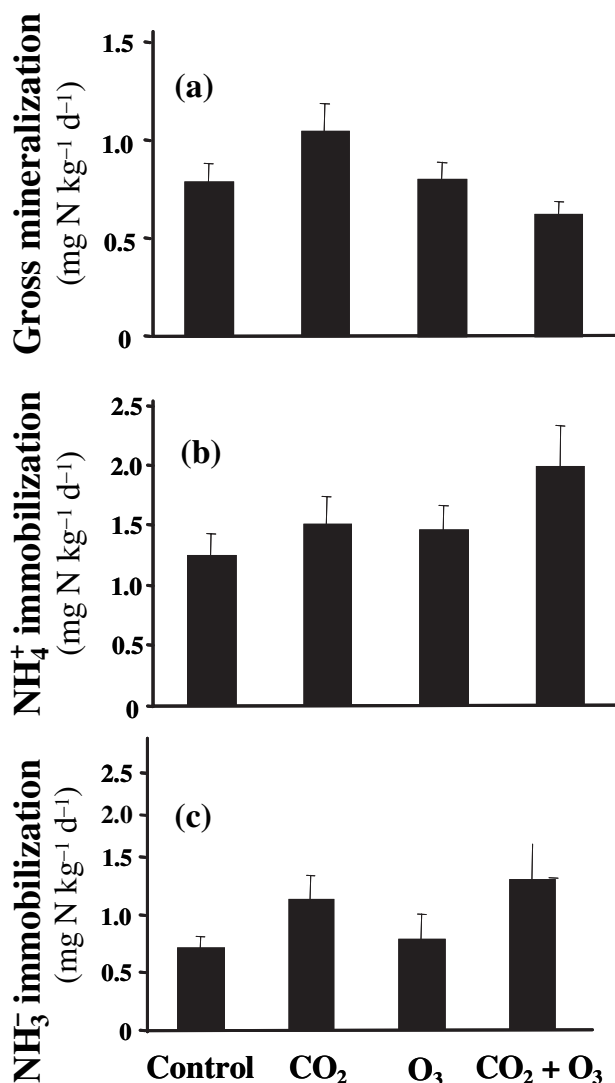


Figure 12. Gross N mineralization (a), NH₄⁺ immobilization (b), and NO₃⁻ immobilization (c) in the Aspen FACE Experiment (Holmes *et al.* 2003). Data have been averaged across aspen, aspen-birch, and aspen-maple ring sections.

Elevated levels of CO₂ caused 20% growth increases in *B. papyrifera* and 30% increases in *P. tremuloides* (Fig. 13). For *P. tremuloides*, this amounted to an increase of over 1 million m², similar in size but opposite in direction to the effect of O₃. Simultaneous exposure to O₃ and CO₂ had offsetting effects in *B. papyrifera* and *P. tremuloides*. Unpredictably, the competitive advantages *B. papyrifera* was simulated to have under O₃ exposure disappeared when CO₂ was also present. In another result from the landscape level that would not have been predictable from the physiological effects, the presence of CO₂ exacerbated the negative effect of O₃ on *Acer saccharum* abundance, causing it to decrease by over 30%.

How predictable were these landscape level effects from the individual plot responses? While it is somewhat difficult to compare 5 years of tree plot results to 100 years of landscape predictions, the changes in the landscape abundance of species were generally a reflection of responses that had been noted in average growth in the experimental plots (Table 1). For example, *P. tremuloides* decreased by 14% in the experimental plots but only by 12% in abundance by year 2100 in the simulation. In no case did the competitive dynamics among species on the landscape predicted in the simulation result in the opposite response to that observed in the plots. However, *P. tremuloides* was predicted to have twice the response to CO₂ on the landscape as the plots suggested, and *B. papyrifera* had twice the response to O₃.

SCALING FOR RISK ANALYSIS

North America and Europe have adopted different approaches to ambient O₃ standard setting, with the latter opting for an approach to specifically protect vegetation. In North America, ambient air quality standards (AAQS) are used for compliance purposes and are balanced against social, economic and political considerations (see Ashmore 2005). AAQS do not implicitly assume the existence of a concentration threshold for receptor (i.e. tree) response (Percy *et al.* 2003a), and therefore, target values are often substituted for regulatory purposes. In North America, the

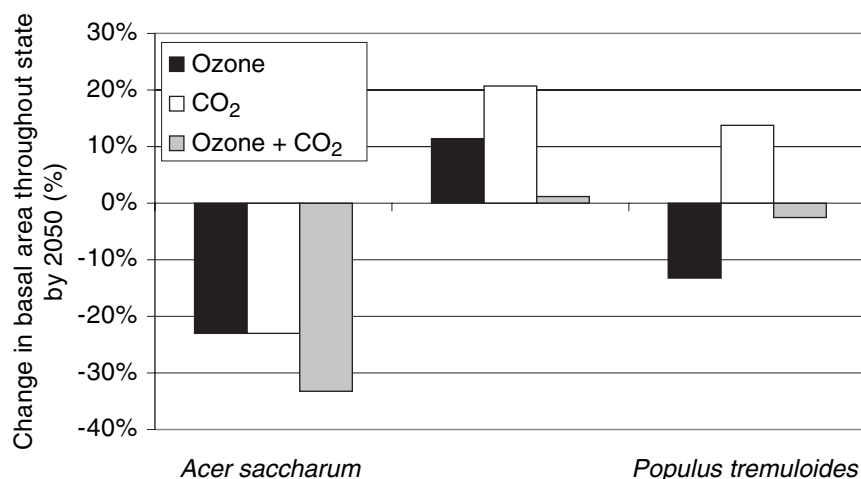


Figure 13. Simulated effects of Aspen FACE treatments on major species in aspen forests of Wisconsin, 2050, expressed as a percent change. The simulations were based on TREGRO/ZELIG model runs parameterized with responses from the Aspen FACE experiment.

Table 1. Comparison of responses at the scale of physiology, plot, and predicted landscape

Treatments	<i>Acer saccharum</i>	<i>Betula papyrifera</i>	<i>Populus tremuloides</i>
FACE Amax reductions			
O ₃	0%	0%	-20%
CO ₂	0%	64%	27%
O ₃ + CO ₂	0%	36%	-7%
Face plot effects measured			
O ₃	-18%	5%	-14%
CO ₂	-15%	26%	15%
O ₃ + CO ₂	-25%	3%	-3%
ZELIG landscape effects predicted in 2100			
O ₃	-28%	11%	-12%
CO ₂	-23%	18%	31%
O ₃ + CO ₂	-35%	4%	-1%

current primary O₃ National AAQS is set at 0.08 ppm (80 ppb) and 0.065 (65 ppb) calculated as the 3-year average of the annual fourth highest daily maximum 8-h O₃ concentrations for the US (Federal Register 1997) and Canada (CCME 2000), respectively.

In contrast, Europe has adopted critical levels (CLs) that assume a threshold concentration for receptor (tree) response exists (UN-ECE 1988; Tema Nord 1994). The CL concept implicitly requires that all adverse effects should be prevented regardless of the economic costs of reducing primary pollutant emissions. The current European CL, based upon the accumulated mean hourly exposure over time above an O₃ concentration threshold of 40 ppb (AOT40), for forests is 10 000 ppbh (10 ppmh) and is calculated for daylight hours with global clear-sky radiation during a 6-month (April–September) period (Kärenlampi & Skärby 1996). The continuously evolving UN-ECE process recently concluded that the CL for forest trees should be based upon stomatal uptake (Karlsson, Sellden & Pleijel 2003; see Ashmore 2005).

Independent of the various experimental methods used, however, there remain a number of uncertainties. First of all, hourly ambient O₃ concentrations follow a rather complex, three-parameter Weibull distribution (Nosal, Legge & Krupa 2000). Secondly, weekly or bi-weekly O₃ concentration means or seasonal concentration summation methods cannot capture the dynamic changes of the atmosphere and plant biology (Krupa & Kickert 1997). Thirdly, cause–effect relationships that have been established have used empirical/statistical, and mechanistic/process models, the former relying heavily on correlations and multivariate linear regression models. However, even a statistically significant correlation does not necessarily mean causality. The effect of O₃ on tree growth is a complex phenomenon and its quantitative characterization will require more complex, non-linear regression models. Fourthly, in most investigations saplings rather than mature tree responses have been examined (Samuelson & Kelly 2001; Kolb & Matyssek 2001). Finally, even though O₃ may have a statistically significant effect on tree growth, it is usually not the ecologically dominant factor. There are meteorological (e.g.

precipitation, soil moisture, solar radiation, temperature, etc.) factors, co-occurring air pollutants, and many other variables that exert large effects on tree growth.

In order to meaningfully assess the risk of increasing O₃ concentrations, it is vital to build a predictive model comprising all important predictors. Krupa *et al.* (2003) have recently developed a multivariate statistical model including meteorological variables (global radiation, air temperature, relative humidity and wind speed, variables that influence plant O₃ uptake through stomata). With the addition of soil moisture data to the main meteorological variables listed, an approximation of first-order atmospheric O₃ flux can be achieved. After verification of validity and significance of such a model, it is then necessary to factor out the effect of O₃ itself, while controlling all other predictors. This is a complicated procedure requiring integrated experiments such as Aspen FACE, which provide systematic and reliable monitoring of pertinent predictors.

Coincidentally, evidence from our Aspen FACE experiment is pointing to the multitropic nature of forest ecosystem responses to long-term, low-level O₃ exposures. Feedbacks to growth have included a large reduction in both aspen height (-12%) and diameter (-13%) growth at the stand level (Percy *et al.* 2002). We have now taken the initial steps towards linking a multivariate statistical model (Krupa *et al.* 2003) with multipoint plant response data. After computing O₃ exposure within each of the three replicate FACE rings using established AAQS, CL, and related descriptors, O₃ dose–response functions relevant to regulatory processes have been calculated.

Initial analysis using The Best Subsets Regression Algorithm (Percy *et al.* unpublished) suggests the best predictor of aspen growth was the 4th highest daily maximum 8 h O₃ concentration (Table 2) followed by tree age. As a second step in the process, meteorological data (T, RH, PAR, precipitation) are being combined with soil moisture data at the stand level to develop an approximation of first-order atmospheric O₃ flux and aspen stomatal uptake. If such efforts are coupled to multipoint plant response measurements, meaningful cause–effect relationships can be derived regarding the nature of the so-called background

Table 2. Best subsets regression analysis of dependence of aspen clone growth on a variety of ozone exposure indexes and tree age for trees growing in the Aspen FACE project^a

Growth variable	Best single predictor	Adj. R-Sq %	Second best	Adj. R-Sq % single predictor
HT	4th ^b	76.5	age	68.6
DIA	age ^c	78.7	4th	70.3
HT	age	48.1	4th	41.1
DIA	4th	72.1	age	72.1
HT	4th	80.1	max 8 h ^d	61.9
DIA	age	80.1	4th	78.3
HT	4th	71.1	age	61.9
DIA	age	83.5	4th	77.9
HT	age	91.6	4th	64.8
DIA	age	88.4	4th	71.5

^aMaximum 1 h O₃ concentration; maximum 8 h O₃ concentration; SUM60; 4th highest daily maximum 8 h O₃ concentration; AOT40 (various 3-month sums); AOT40 (6 month); and tree age (1–5 years). ^b4th, 3-year average of the annual fourth highest daily maximum 8-h O₃ concentrations; from used to calculate US EPA and CWS AAQS. ^cage, aspen tree age (1–5 years). ^dmax 8 h: maximum 8 h O₃ concentration.

O₃ concentrations and their significance in more remote forested areas (Krupa *et al.* 2003). The future development of new flux-based critical levels in Europe and biologically based dose–response functions in North America will allow policy makers and regulators for the first time to more accurately predict O₃ risk to the world's forests in the future.

SUMMARY AND CONCLUSIONS

We have examined over 7 years the effects of elevated levels of O₃ at two atmospheric CO₂ levels, current ambient (360 ppm) and 560 ppm (projected for about year 2050), on northern Wisconsin aspen, aspen-birch, and aspen-maple forest communities. In this paper, we elucidate how O₃ affects the flow of C from the leaf and canopy level through tree roots to soil and soil microorganisms, under ambient and elevated CO₂. Our long-term, multidisciplinary research project has consistently shown adverse effects of O₃ on the above-ground growth and physiology of all three species. These impacts on above-ground biochemistry, physiology and morphology with feedbacks to growth and pest occurrence have cascaded through the ecosystem via multiple food webs and trophic levels ultimately affecting ecosystem C cycling. While CO₂ generally moderated the detrimental responses of O₃, there were some noticeable exceptions, including the long-term growth suppression of sugar maple and paper birch, which could not have been predicted by studies of these two important greenhouse gases applied singly or for a short-term.

Our results suggest that fine roots are key mediators of ecosystem response to these greenhouse gases, regardless of forest community type. Secondly, they highlight the importance of bottom-up changes caused by the combined effects of CO₂ and O₃ on food quality and the long-term population dynamics of forest pests. Furthermore, they suggest establishing links between net primary productivity,

the biochemical constituents of plant litter, and the metabolic responses of microbial communities which are crucial to a mechanistic understanding of how these greenhouse gases will alter soil C and N cycling, as well as the long-term forest ecosystem productivity.

We are attempting to scale up our results to project O₃ responses of forest regions using various process-based models linked to canopy gap models. In addition, we are using a regression approach to find the best policy-relevant predictors of our observed O₃ effects on growth.

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REFERENCES

- Andersen C.P. (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* **157**, 213–228.
- Ashmore M.R. (2005) Assessing the future global impacts of ozone on vegetation. *Plant, Cell and Environment* **28** doi: 10.1111/j.1365-3040.2005.01342.x
- Awmack C.S., Harrington R. & Lindroth R.L. (2004) Individual

- performance does not predict aphid population responses to elevated atmospheric CO₂ or O₃. *Global Change Biology* **10**, 1414–1423.
- Beedlow P.A., Tingey D.T., Phillips D.L., Hogsett W.E. & Olszyk D.M. (2004) Rising atmospheric CO₂ and carbon sequestration in forests. *Frontiers in Ecology and the Environment* **2**, 315–322.
- Bielenberg D.G., Lynch J.P. & Pell E.J. (2002) Nitrogen dynamics during O₃-induced accelerated senescence in hybrid poplar. *Plant, Cell and Environment* **25**, 501–512.
- CCME (Canadian Council of Ministers of the Environment) (2000) *Canada-Wide Standards for Particulate Matter (PM) and Ozone*. Available at http://www.ccme.ca/assets/pdf/pmozone_standard_e.pdf.
- Chakraborty S., Tiedemann A.V. & Teng P.S. (2000) Climate change: Potential impact on plant diseases. *Environmental Pollution* **108**, 317–326.
- 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. (1995) Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* **15**, 593–604.
- Coleman M.D., Dickson R.E., Isebrands J.G. & Karnosky D.F. (1996) Root growth and physiology of potted and field-grown trembling aspen exposed to tropospheric ozone. *Tree Physiology* **16**, 145–152.
- Costonis A.C. (1970) Acute foliar injury of eastern white pine induced by sulfur dioxide and ozone. *Phytopathology* **60**, 994–999.
- Davey P.A., Hunt S., Graham J.H., DeLucia E.H., Drake B.G., Karnosky D.F. & Long S.P. (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO₂], but is increased with long-term growth in the field at elevated [CO₂]. *Plant Physiology* **134**, 520–527.
- Dickson R.E., Coleman M.D., Pechter P. & Karnosky D.F. (2001) Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. *Environmental Pollution* **115**, 319–334.
- Dickson R.E., Lewin K.F., Isebrands J.G., *et al.* (2000) *Forest Atmosphere Carbon Transfer Storage-II (FACTS II) – The Aspen Free-air CO₂ and O₃ Enrichment (FACE) Project in an Overview*. General Technical Report NC-214. USDA Forest Service North Central Research Station, Rhinelander, WI, USA.
- Dochinger L.S., Bender F.W., Fox F.L. & Heck W.W. (1970) Chlorotic dwarf of eastern white pine caused by an ozone and sulphur dioxide interaction. *Nature* **225**, 476.
- Dunn D.B. (1959) Some effects of air pollution on *Lupinus* in the Los Angeles area. *Ecology* **40**, 621.
- Federal Register (1997) *National Ambient Air Quality Standards for Ozone, Rules and Regulations*, 40 CFR Part 50, Final Rule. 62 (138), July 18 1997, p. 38856. Environmental Protection Agency, Washington, DC, USA.
- Fowler D., Cape J.N., Coyle M., Flechard C., Kuylensstierna J., Hicks K., Derwent D., Johnson C. & Stevenson D. (1999) The global exposure of forests to air pollutants. *Water, Air, and Soil Pollution* **116**, 5–32.
- Grennfelt P. (2004) New directions: Recent research findings may change control policies. *Atmospheric Environment* **38**, 2215–2216.
- Harrington R., Woiwod I. & Sparks T. (1999) Climate change and trophic interactions. *Trees* **14**, 146–150.
- 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.
- Holmes W.E., Zak D.R., Pregitzer K.S. & King J.S. (2003) Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO₂ and O₃. *Global Change Biology* **9**, 1743–1750.
- Holton M.K., Lindroth R.L. & Nordheim E.V. (2003) Foliar quality influences tree–herbivore–parasitoid interactions: effects of elevated CO₂, O₃, and genotype. *Oecologia* **137**, 233–244.
- Host G.E., Isebrands J.G., Theseira G.W., Kiniry J.R. & Graham R.L. (1996) Temporal and spatial scaling from individual trees to plantations: a modelling strategy. *Biomass and Bioenergy* **11**, 233–243.
- Isebrands J.G., Host G.E., Lenz K.E., Wu G. & Stech H.W. (2000) Hierarchical, parallel computing strategies using component object model for process modelling responses to forest plantations to interacting multiple stresses. In *Forest Ecosystem Modelling, Upscaling, and Remote Sensing* (eds R.J.M. Ceulemans, F. Veroustraete, V. Gond & J.B.H.F. VanRensbergen), pp. 123–135. SPB Academic Publishing, The Hague, The Netherlands.
- Isebrands J.G., McDonald E.P., Kruger E., Hendrey G., Pregitzer K., Percy K., Sober J. & Karnosky D.F. (2001) Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. *Environmental Pollution* **115**, 359–371.
- Kaakinen S., Kostianinen K., Ek F., Saranpää P., Kubiske M.E., Sober J., Karnosky D.F. & Vapaavuori E. (2004) Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after three years of treatments to elevated carbon dioxide and ozone. *Global Change Biology* **10**, 1513–1525.
- Kärenlampi L. & Skärby L. (1996) *Critical Levels for Ozone in Europe: Testing and Finalizing the Concepts*, UN-ECE Workshop Report. UN-ECE Convention on Long-Range Transboundary Air Pollution. Kuopio, Finland, April 15–17, 1996. Department of Ecology and Environmental Science. University of Kuopio, Kuopio, Finland.
- Karlsson P.E., Selldén G. & Pleijel H. (eds) (2003) *Establishing Ozone Critical Levels II UNECE Workshop Report*. IVL report B 1523. IVL Swedish Environmental Research Institute, Gothenburg, Sweden.
- Karnosky D.F. (2005) Ozone effects on forest ecosystems under a changing global environment. *Journal of Agricultural Meteorology* **60**, 353–358.
- 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 seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Canadian Journal of Forest Research* **26**, 23–37.
- Karnosky D.F., Gielen G., 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. In *The Impact of Carbon Dioxide and Other Greenhouse Gases on Forest Ecosystems* (eds D.F. Karnosky, G. Scarascia-Mugnozza, R. Ceulemans & J. Innes), pp. 297–324. CABI Publishing, New York, USA.
- Karnosky D.F., Mankovska B., Percy K., *et al.* (1999) Effects of tropospheric O₃ on trembling aspen and interaction with CO₂: Results from an O₃-gradient and a FACE experiment. *Journal of Water, Air and Soil Pollution* **116**, 311–322.
- Karnosky D.F., Percy K.E., Chappelka A.H. & Krupa S.V. (2003a) Air pollution and global change impacts on forest ecosystems: Monitoring and research needs. In *Air Pollution, Global Change and Forests in the New Millennium* (eds D.F. Karnosky, K.E. Percy, A.H. Chappelka, J. Pikkariainen & C.J. Simpson), pp. 447–459. Elsevier, Oxford, UK.
- Karnosky D.F., Pregitzer K.S., Hendrey G.R., *et al.* (2003b) Impacts of interacting CO₂ and O₃ on trembling aspen: results from the aspen FACE experiment. *Functional Ecology* **17**, 289–304.

- Karnosky D.F., Percy K.E., Mankovska B., Prichard T., Noormets A., Dickson R.E., Jepsen E. & Isebrands J.G. (2003c) Ozone affects the fitness of trembling aspen. In *Air Pollution, Global Change and Forests in the New Millennium* (eds D.F. Karnosky, K.E. Percy, A.H. Chappelka, J. Pikkarainen & C.J. Simpson), pp. 199–209. Elsevier, Oxford, UK.
- Karnosky D.F., Percy K.E., Xiang B., *et al.* (2002) Interacting elevated CO₂ and tropospheric O₃ predisposes aspen (*Populus tremuloides* Michx.) to infection by rust (*Melampsora medusae* f.sp. *tremuloidae*). *Global Change Biology* **8**, 329–338.
- Karnosky D.F. & Pregitzer K.S. (2005) Impacts of elevated CO₂ and O₃ on northern temperate forest ecosystems: Results from the Aspen FACE experiment. In *Managed Ecosystems and CO₂: Case Studies, Processes and Perspectives. Ecological Studies*. (eds J. Nösberger, S.P. Long, G.R. Hendry, M. Stitt, R.J. Norby & H. Bum). Springer-Verlag, Berlin, Germany, 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.S., Hanson P.J., Bernhardt E., DeAngelis P., Norby R.J. & Pregitzer K.S. (2004) A multi-year synthesis of soil respiration responses to elevated atmospheric CO₂ from four forest FACE experiments. *Global Change Biology* **10**, 1027–1042.
- King J.S., Pregitzer K.S., Zak D.R., Karnosky D.F., Isebrands J.G., Dickson R.E., Hendrey G.R. & Sober J. (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.
- Kolb T.E. & Matyssek R. (2001) Limitations and perspectives about scaling ozone impacts in trees. *Environmental Pollution* **115**, 373–393.
- Kopper B.J. & Lindroth R.L. (2003a) 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.
- Kopper B.J. & Lindroth R.L. (2003b) Effects of elevated carbon dioxide and ozone on the genotypic response of aspen phytochemistry and the performance of an herbivore. *Oecologia* **134**, 95–103.
- Krupa S.V. & Kickert R.N. (1997) Ambient ozone (O₃) and adverse crop response. *Environmental Review* **5**, 55–77.
- Krupa S., Nosal M., Ferdinand J.A., Stevenson R.E. & Skelly J.M. (2003) A multi-variate statistical model integrating passive sampler and meteorology data to predict the frequency distributions of hourly ambient ozone (O₃) concentrations. *Environmental Pollution* **124**, 173–178.
- Kull O., Sober 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.
- Laurence J.A. & Andersen C.P. (2003) Ozone and natural systems: understanding exposure, response and risk. *Environment International* **29**, 155–160.
- Laurence J.A., Retzlaff W.A., Kern J.S., Lee E.H., Hogsett W.E. & Weinstein D.A. (2001) Predicting the regional impact of ozone and precipitation on the growth of loblolly pine and yellow-poplar using linked TREGRO and ZELIG models. *Forest Ecology and Management* **146**, 251–267.
- Lindroth R.L., Kopper B.J., Parsons W.F.J., Bockheim J.G., Sober 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**, 395–404.
- Loranger G.I., Pregitzer K.S. & King J.S. (2004) Elevated CO₂ and O₃ concentrations differentially affect selected groups of the fauna in temperate forest soils. *Soil Biology and Biochemistry* **36**, 1521–1524.
- Loya W.M., Pregitzer K.S., Karberg N.J., King J.S. & Giardina C.P. (2003) Reduction of soil carbon formation by tropospheric ozone under elevated carbon dioxide. *Nature* **425**, 705–707.
- 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 (Bratislava)* **18**, 200–210.
- Mankovska B., Percy K. & Karnosky D.F. (2003) Impact of greenhouse gases on epicuticular waxes of *Populus tremuloides* Michx. Results from an open-air exposure and a natural O₃ gradient. *Ekológia (Bratislava)* **22** (Suppl. 1), 235–243.
- Martin M.J., Host G.E., Lenz K.E. & Isebrands J.G. (2001) Simulating the growth response 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. & Sandermann H. (2003) Impact of ozone on trees: an ecophysiological perspective. In *Progress in Botany* **64** (eds K. Esser, U. Lüttge, W. Beyschlag & F. Hellwig), pp. 349–404. Springer-Verlag, Heidelberg, Germany.
- 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.
- 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.
- Miller P.R. & McBride J.R. (eds) (1999) *Oxidant Air Pollution Impacts in the Montane Forests of Southern California*, pp. 397–416. Springer-Verlag, New York, USA.
- Miller P.R., McCutchan M.H. & Milligan H.P. (1972) Oxidant air pollution in the Central Valley, Sierra Nevada foothills, and Mineral King Valley of California. *Atmospheric Environment* **6**, 623–633.
- Miller P.R. & Millecan A.A. (1971) Extent of oxidant air pollution damage to some pine and other conifers in California. *Plant Disease Reporter* **55**, 555–559.
- Miller P.R., Parmeter J.R. Jr, Taylor O.C. & Cardiff E.A. (1963) Ozone injury to foliage of *Pinus ponderosa*. *Phytopathology* **53**, 1072–1076.
- Mondor E.B., Tremblay M.N., Awmack C.S. & Lindroth R.L. (2004) Divergent pheromone-mediated insect behaviour under global atmospheric change. *Global Change Biology* **10**, 1820–1824.
- Noormets A., McDonald E.P., Dickson R.E., Kruger E.L., Sober A., Isebrands J.G. & Karnosky D.F. (2001a) 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.
- Noormets A., Sober A., Pell E.J., Dickson R.E., Podila G.K., Sober J., Isebrands J.G. & Karnosky D.F. (2001b) 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.
- Nosal M., Legge A.H. & Krupa S.V. (2000) Application of a stochastic, Weibull probability generator for replacing missing data on ambient concentrations of gaseous pollutants. *Environmental Pollution* **108**, 439–446.
- Oksanen E. (2003) Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. *Tree Physiology* **23**, 603–614.
- Oksanen E., Häikiö E., Sober J. & Karnosky D.F. (2003) Ozone-

- induced H₂O₂ accumulation in field-grown aspen and birch is linked to foliar ultrastructure and peroxisomal activity. *New Phytologist* **161**, 791–799.
- Oksanen E., Sober 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.
- Parsons W.F.J., Lindroth R.L. & Bockheim J.G. (2004) Decomposition of *Betula papyrifera* leaf litter under the independent and interactive effects of elevated CO₂ and O₃. *Global Change Biology* **10**, 1666–1677.
- Pell E.J., Enyedi A., Eckardt N. & Landry L. (1990) Ozone-induced alterations in quantity and activity of rubisco: implications for foliar senescence. In *Biology Oxidation Systems* (eds B.G. Fox & J.D. Lipscomb), pp. 389–403. Academic Press, New York, USA.
- Percy K.E., Awmack C.S., Lindroth R.L., *et al.* (2002) Altered performance of forest pests under CO₂- and O₃-enriched atmospheres. *Nature* **420**, 403–407.
- Percy K.E., Legge A.H. & Krupa S.V. (2003a) Tropospheric ozone: A continuing threat to global forests? In *Air Pollution, Global Change and Forests in the New Millennium* (eds D.F. Karnosky, K.E. Percy, A.H. Chappelka, J. Pikkariainen & C.J. Simpson), pp. 85–118. Elsevier, Oxford, UK.
- Percy K.E., Mankovska B., Hopkin A., Callan B. & Karnosky D.F. (2003b) Ozone affects leaf surface pest interactions. In *Air Pollution, Global Change and Forests in the New Millennium* (eds D.F. Karnosky, K.E. Percy, A.H. Chappelka, J. Pikkariainen & C.J. Simpson), pp. 247–257. Elsevier, Oxford, UK.
- 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, USA.
- Rausher H.M., Isebrands J.G., Host G.E., Dickson R.E., Dickmann D.I., Crow T.R. & Michael D.A. (1990) ECOPHYS: an ecophysiological growth process model for juvenile poplar. *Tree Physiology* **7**, 255–281.
- Samuelson L. & Kelly J.M. (2001) Scaling ozone effects from seedlings to forest trees. *New Phytologist* **149**, 21–41.
- Sharma P., Sober A., Sober J., Podila G.K., Kubiske M.E., Mattson W.J., Isebrands J.G. & Karnosky D.F. (2003) Moderation of [CO₂]-induced gas exchange responses by elevated tropospheric O₃ in trembling aspen and sugar maple. *Ekologia (Bratislava)* **22** (Suppl. 1), 318–331.
- Skärby L., Ro-Poulsen H., Wellburn F.A.M. & Sheppard L.J. (1998) Impacts of ozone on forests: a European perspective. *New Phytologist* **139**, 109–122.
- Tema Nord (1994) *Critical Levels for Tropospheric Ozone – Concepts and Criteria Tested for Nordic Conditions*. L. Skärby (Coordinator). Nordic Council of Ministers, Copenhagen, Denmark.
- Theseira G.W., Host G.E., Isebrands J.G. & Whisler F.D. (2003) SOILPSI: a potential-driven three-dimensional soil water redistribution model – description and comparative evaluation. *Environmental Software and Modeling* **18**, 13–23.
- UN-ECE (1988) *ECE Critical Levels Workshop*, Final Draft Report. March 14–18, 1988. UN-ECE, Bad Harzburg, Federal Republic of Germany.
- Urban D.L. (1990) *A versatile model to simulate forest pattern: A user's guide to Zelig, Version 1.0*. Environmental Sciences Department. University of Virginia, Charlottesville, VA., USA.
- Urban D.L., Bonan G.B., Smith T.M. & Shugart H.H. (1991) Spatial applications of gap models. *Forest Ecology and Management* **42**, 95–110.
- Weinstein D.A., Beloin R.M. & Yanai R.D. (1991) Modeling changes in red spruce carbon balance and allocation in response to interacting ozone and nutrient stress. *Tree Physiology* **9**, 127–146.
- Weinstein D.A., Gollands B. & Retzlaff W.A. (2001b) The effects of ozone on a lower slope forest of the Great Smoky Mountain National Park: Simulations Linking an Individual Tree Model to a Stand Model. *Forest Science* **47**, 29–42.
- Weinstein D.A., Laurence J.A., Retzlaff W.A., Kern J.S., Lee E.H., Hogsett W.E. & Weber J. (2005) Predicting the effects of tropospheric ozone on regional productivity of ponderosa pine and white fir. *Forest Ecology and Management* **205**, 73–89.
- Weinstein D.A., Woodbury P.B., Gollands B., King P., Lepak L. & Pendleton D. (2001a) *Assessment of Effects of Ozone on Forest Resources in the Southern Appalachian Mountains*, Final Report. Southern Appalachian Mountain Initiative, Asheville, NC, USA.
- Wustman B.A., Oksanen E., Karnosky D.F., Sober 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.

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