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

D. F. KARNOSKY[1], K. S. PREGITZER[1], D. R. ZAK[2], M. E. KUBISKE[3], G. R. HENDREY[4], D. WEINSTEIN[5], M. NOSAL[6] & K. E. PERCY[7]

[1]*School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, Michigan 49931, USA,* [2]*School of Natural Resources & Environment, The University of Michigan, 430 E. University, Ann Arbor, Michigan 48109–1115, USA,* [3]*USDA Forest Service, North Central Research Station, Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, Wisconsin 54501, USA,* [4]*Brookhaven National Laboratory and Queens College CUNY, 65–30 Kissena Blvd., Flushing, NY 11367, USA,* [5]*Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, New York 14853, USA,* [6]*Department of Mathematics and Statistics, University of Calgary, Calgary, Alberta, Canada T2N 1 N4 and* [7]*Natural Resources Canada, Canadian Forest Service-Atlantic Forestry Centre, PO Box 4000, Fredericton, New Brunswick, Canada E3B 5P7*

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 (CO_2) and ozone (O_3), alone and in combination, from establishment onward. We examine how O_3 affects the flow of carbon through the ecosystem from the leaf level through to the roots and into the soil microorganisms in present and future atmospheric CO_2 conditions. We provide evidence of adverse effects of O_3, with or without co-occurring elevated CO_2, 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 O_3 on the growth of sugar maple did not become evident until 3 years into the study. The negative effect of O_3 effect was most noticeable on paper birch trees growing under elevated CO_2. 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 O_3 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 O_3.

Correspondence: David F. Karnosky. Fax: +1 906-487-2897; e-mail: karnosky@mtu.edu

INTRODUCTION

Tropospheric ozone (O_3), 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 O_3 was the pine-fir forest in the San Bernardino Mountains (Miller *et al*. 1963; Miller & Millecan 1971). Subsequently, O_3 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 O_3 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 O_3 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;

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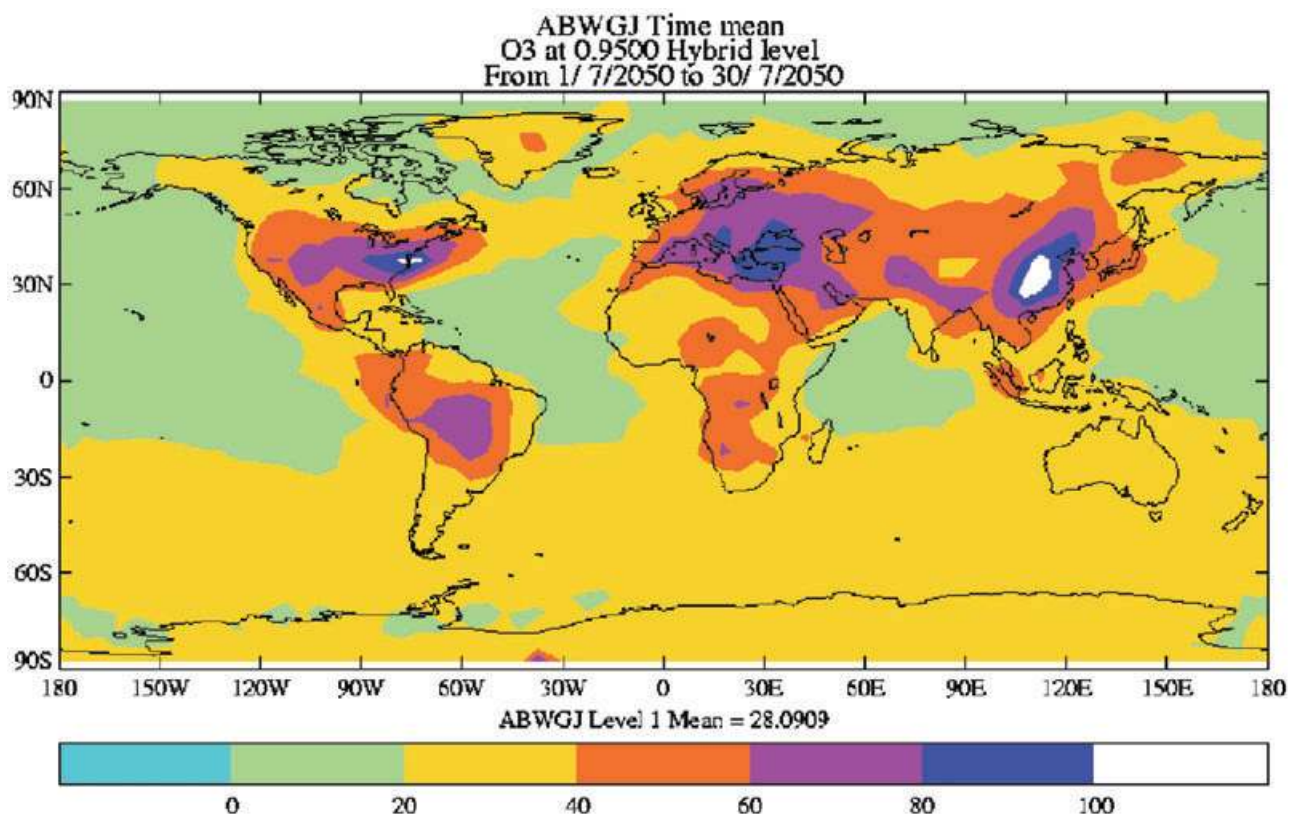
Figure 1. Projected maximum 1 h surface ozone values for July, 2050 from the model output of the STOCHEM model (C.E. Johnson and David Fowler, personal comm.).

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 m × 1 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 × 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-
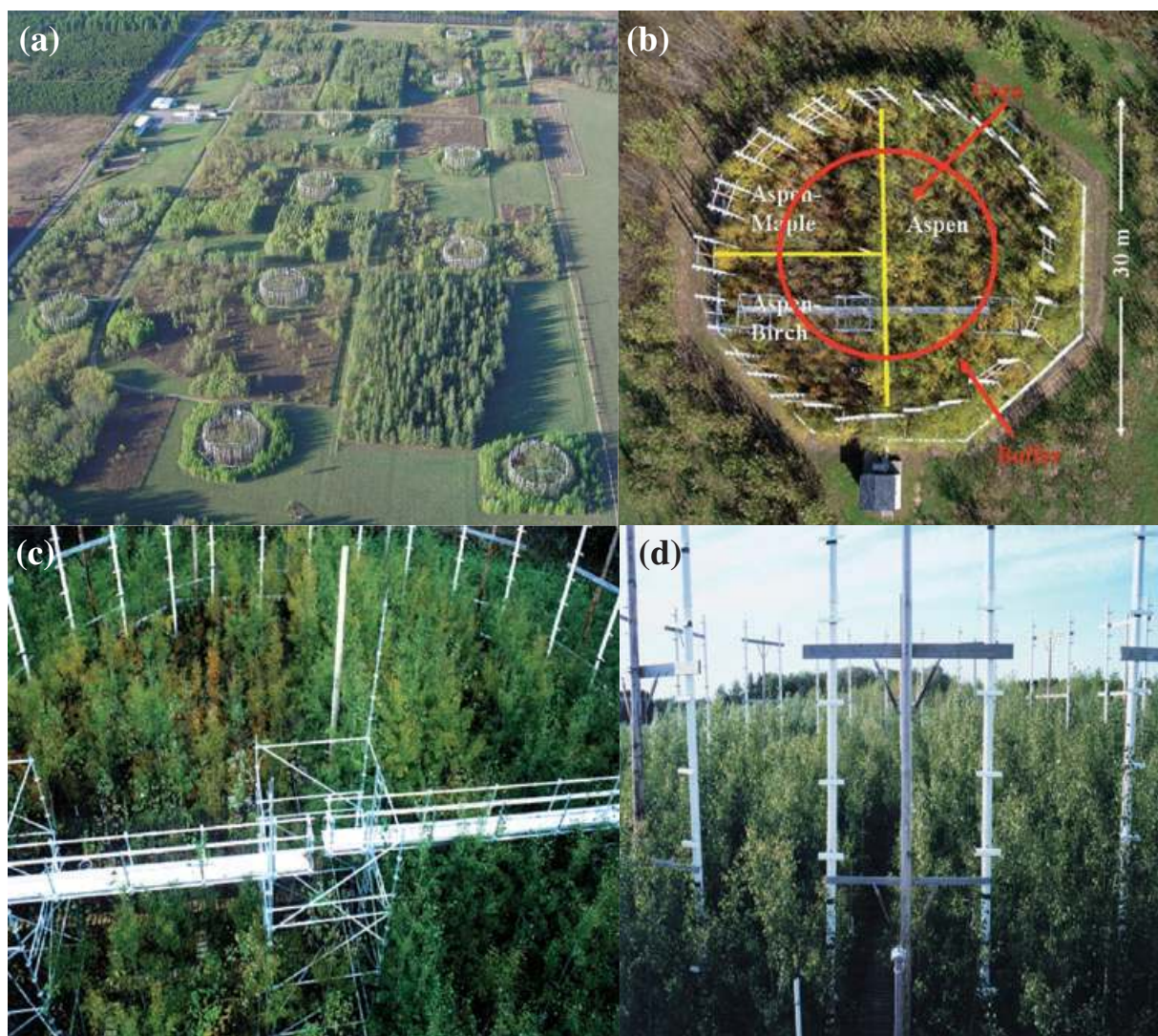
Figure 2. (a) Overview of the 12 rings of the Aspen FACE project. (b) Distribution of the aspen, aspen-birch and aspen-maple communities in each Aspen FACE ring. Each ring is surrounded by a buffer of hybrid poplar trees. (c) Canopy access walkways in each ring allow for canopy sampling in the aspen 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.
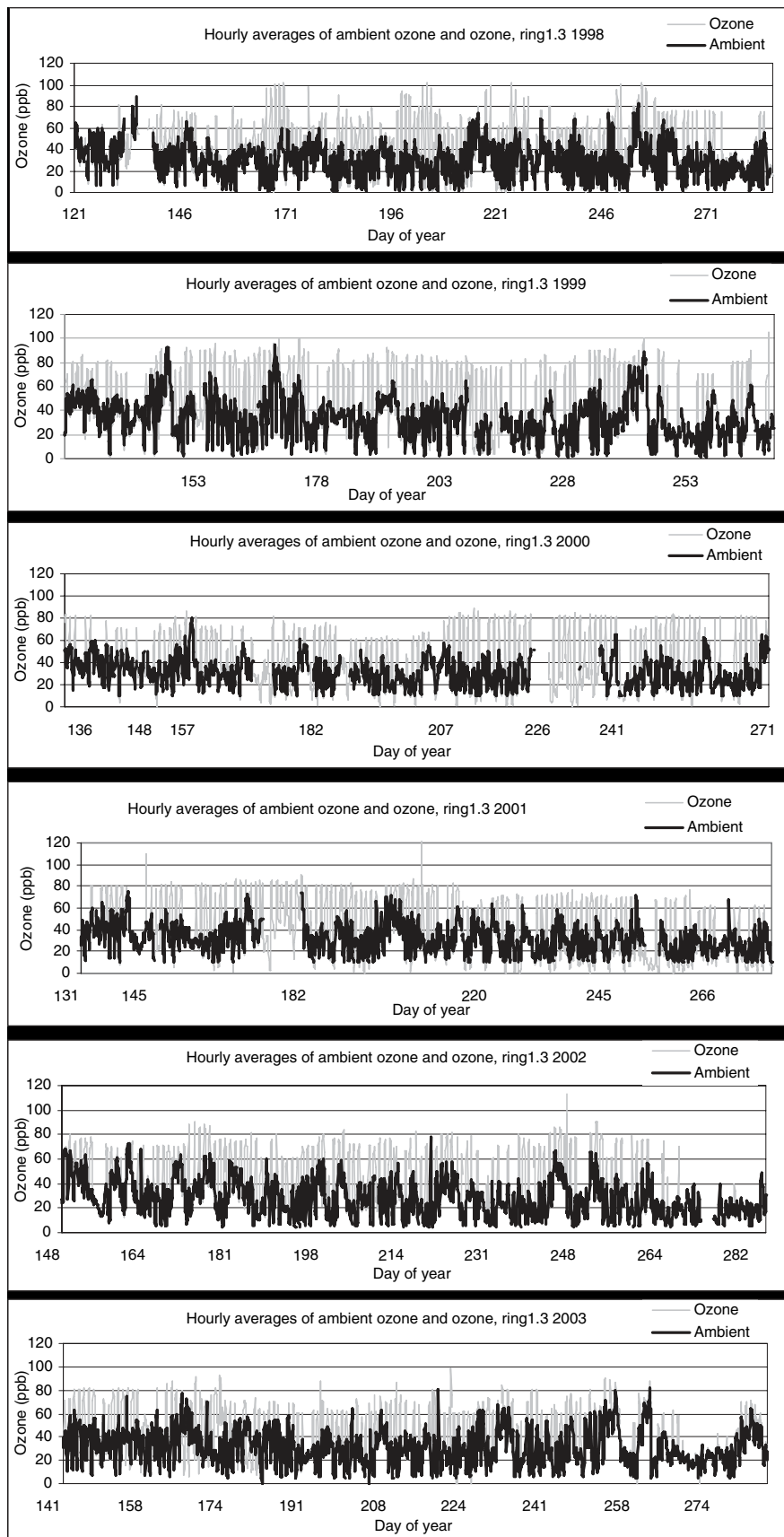
Figure 3. Hourly O_3 concentrations from ambient air (black) and one elevated O_3 ring (grey) in the Aspen FACE project during 1998–2003.
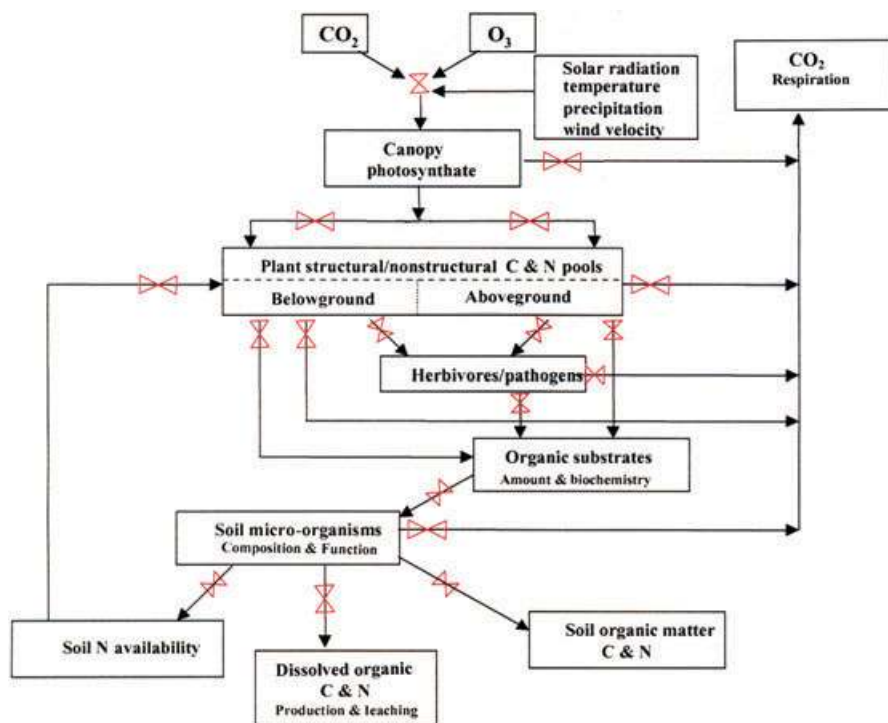
Figure 4. A conceptual model developed for the Aspen FACE project describing the flow of carbon from photosynthesis through forest ecosystems. The red switches indicate processes that serve as control points where we hypothesize that genotypic and species-specific responses to atmospheric CO_2 and O_3 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 keystone 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
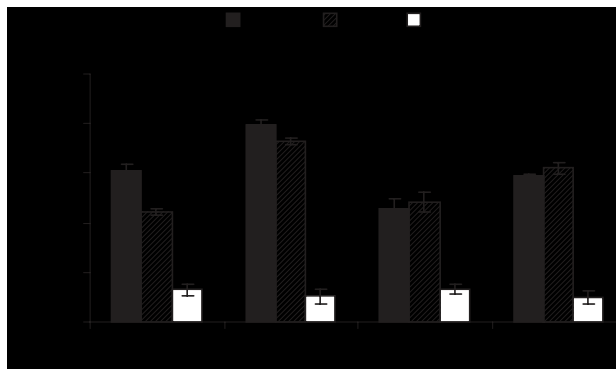
Figure 5. Light-saturated CO_2 assimilation rates of aspen, birch and maple growing under experimental atmospheric CO_2 and O_3 treatments. Data represent the mean and SE of three trees from each of three replicates for three to five measurement times over the 1999 and 2000 growing seasons (From: Karnosky *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 O_3 on photosynthesis have generally been negative for aspen at either CO_2 concentration (Karnosky *et al.* 2003b), whereas birch photosynthesis was most affected at the elevated CO_2. The depression of photosynthesis under elevated $CO_2 + O_3$ suggests co-occurring elevation of O_3 could decrease the projected stimulation of photosynthesis under future CO_2 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 CO_2 and by 19–31% under elevated CO_2, compared with photosynthesis at elevated CO_2 alone. Older leaves tended to be more severely affected by O_3 at either CO_2 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, O_3 decreased chloroplast size, starch content, and altered foliar ultrastructure (Oksanen, Sober & Karnosky 2001) in aspen but not in birch leaves. More recently, O_3-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 CO_2 have been found in foliar respiration at our site (Davey *et al.* 2004), increased levels of foliar respiration have been detected in O_3-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, O_3-induced 10–15% decrease in both height and diameter growth in aspen from O_3 at both ambient and elevated CO_2 (Isebrands *et al.* 2001; Percy *et al.* 2002; Fig. 6). In contrast, birch has not shown adverse effects of
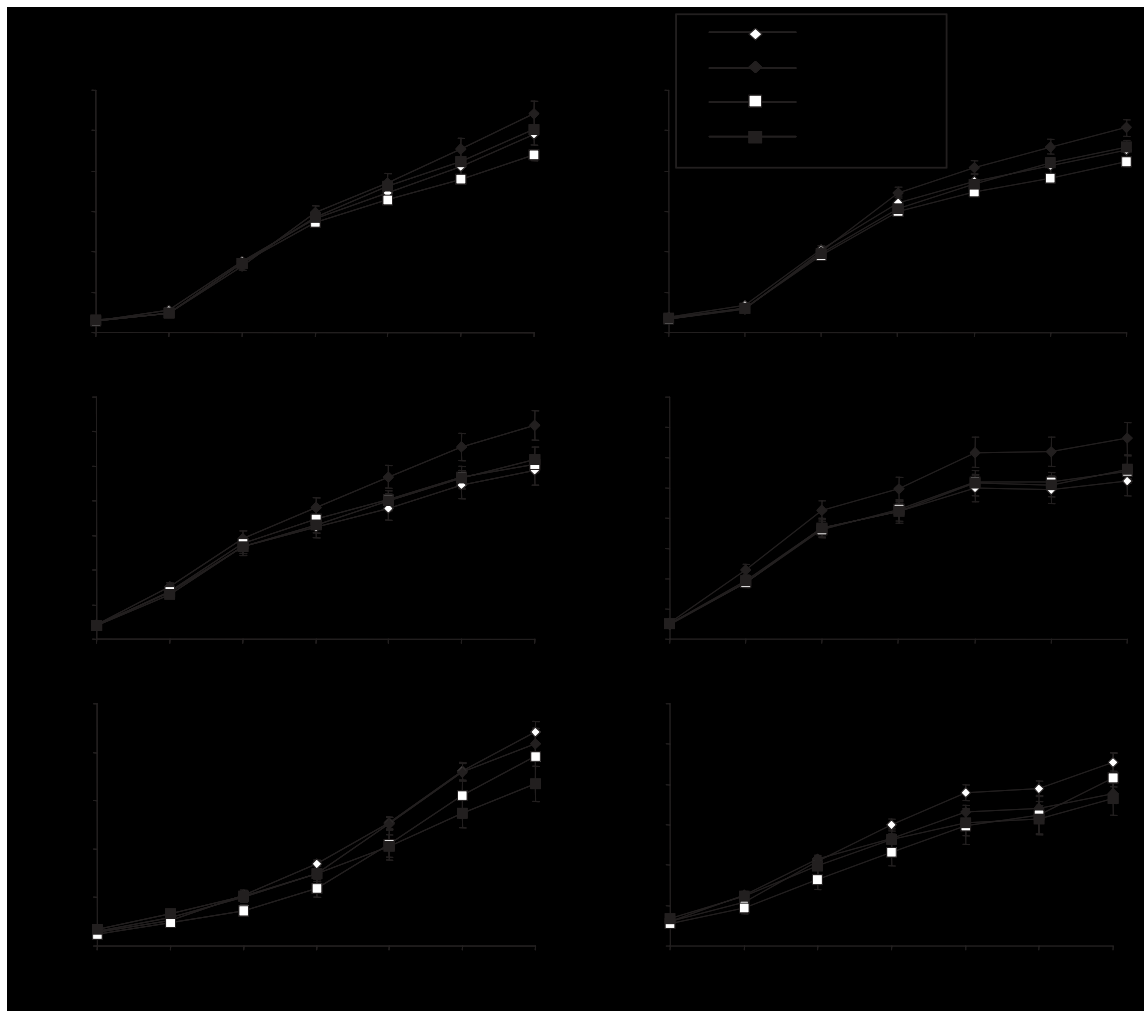
Figure 6. Height (a) and diameter (b) growth for trembling aspen, paper birch and sugar maple seedlings exposed to elevated CO_2 and/ or O_3 at the Aspen FACE project. Means ± SE.

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
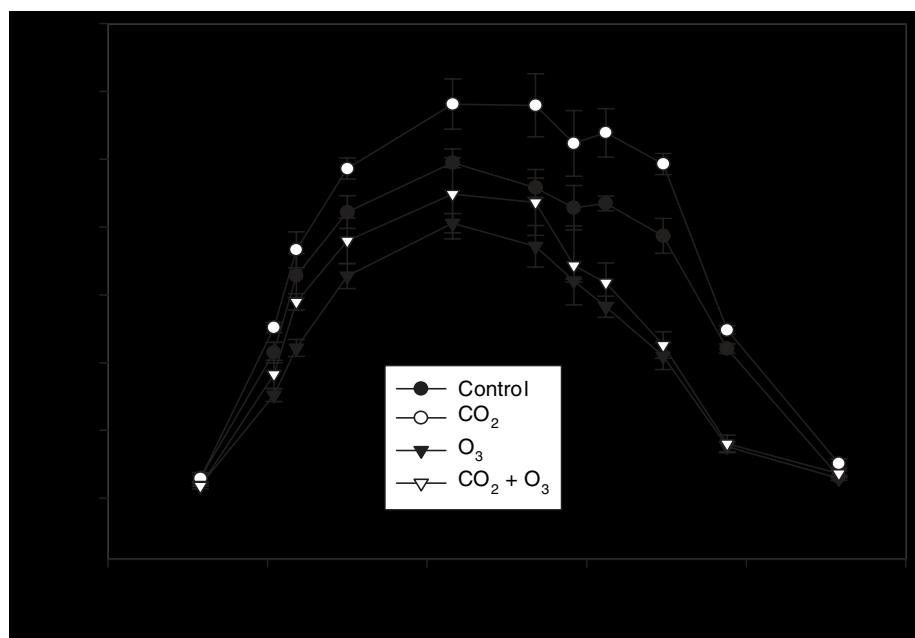
Figure 7. Estimated seasonal trends for LAI for aggrading aspen stands in the Aspen FACE experiment in northern Wisconsin during the 2002 growing season based on plot-level means in the aspen one-half of each ring for litterfall, optical (hemispherical fisheye approach), and ceptometer data. Values are means ± 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 illicited 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.
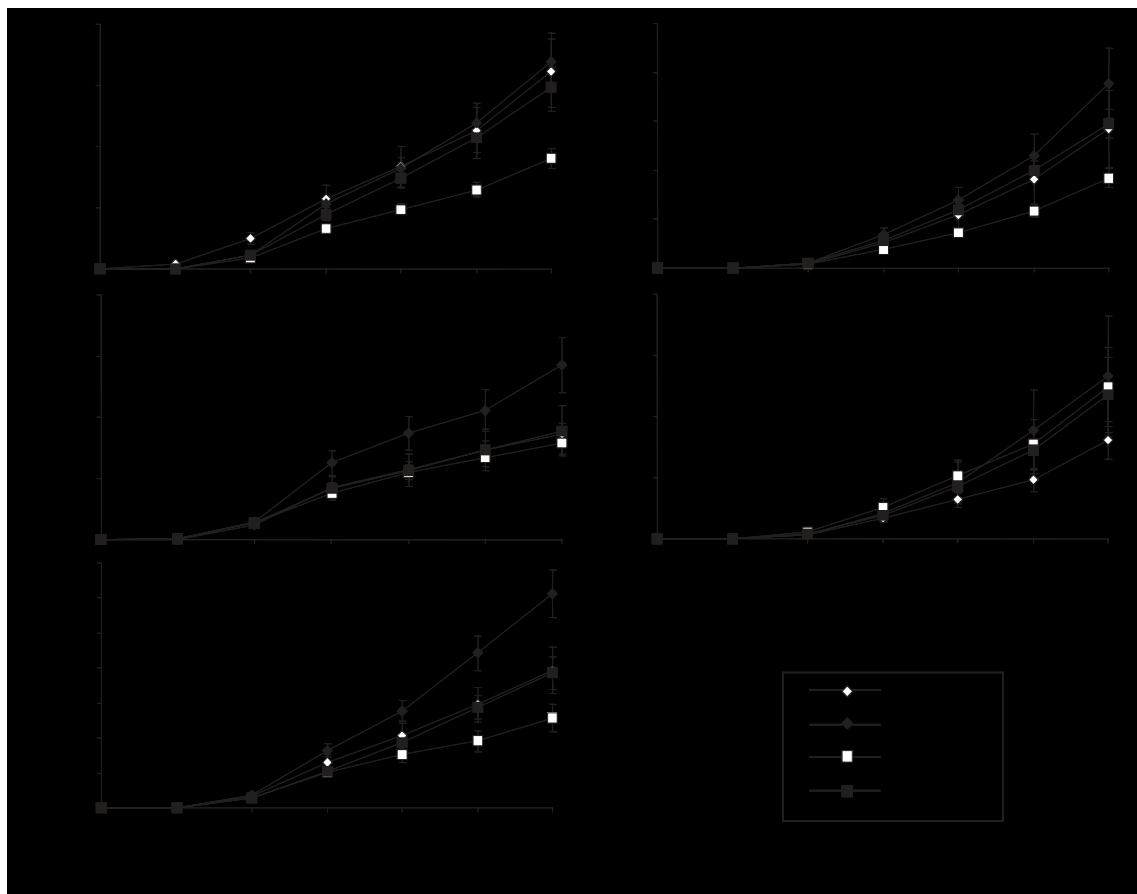
Figure 9. Impacts of elevated CO$_2$ and O$_3$, alone and in combination, on volume growth, estimated by d^{2}h, from five aspen clones in the Aspen FACE experiment. Means ± SE.

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-

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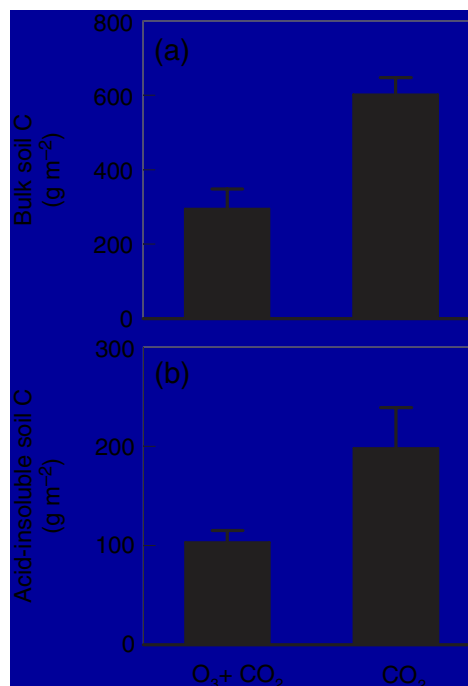
Figure 10. Total carbon incorporated into soils during 5 years of exposure to elevated $O_3 + CO_2$ and elevated CO_2 (a). Carbon incorporated into the stable acid-insoluble fraction of soils during 5 years of exposure to elevated $O_3 + CO_2$ and elevated CO_2 (b). Values are means with 1 SE bars; $P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

350 d. Ozone alone did not affect birch leaf litter quality or litter decompositon (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.
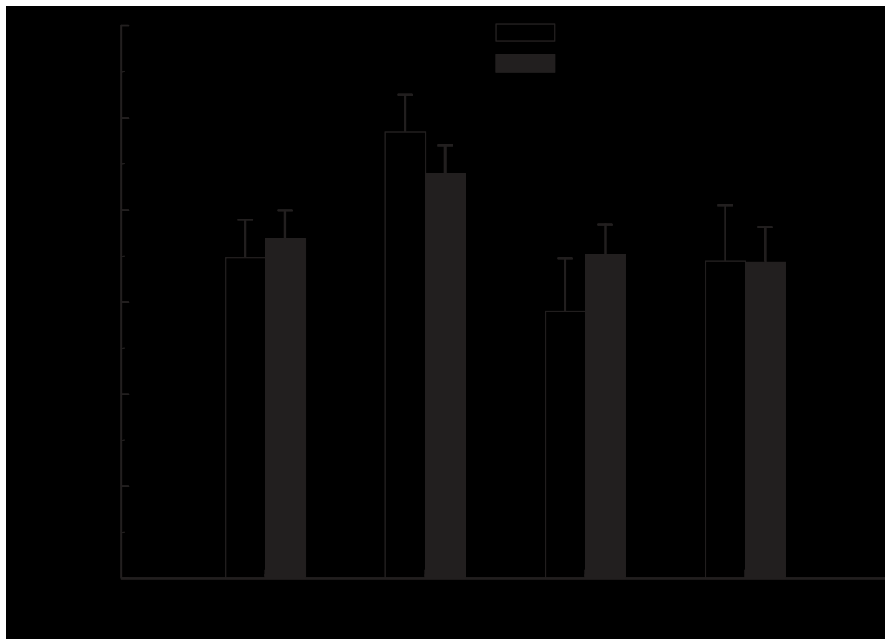
Figure 11. Microbial respiration is determined by metabolism of ^{13}C-labelled cellobiose and *N*-acetylglucosamine beneath plants exposed to elevated CO_2 and O_3. Data from Phillips *et al*. (2002).

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 (Theseira *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 mobilize 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.
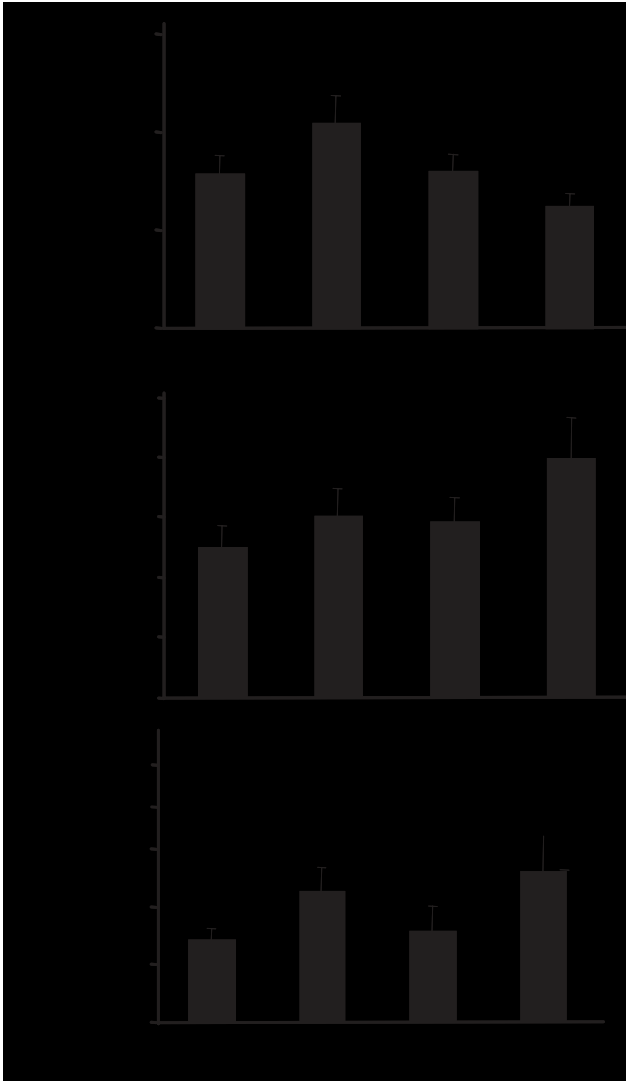
Figure 12. Gross N mineralization (a), NH_4^+ immobilization (b), and NO_3^- immobilization (c) in the Aspen FACE Experiment (Holmes *et al*. 2003). Data have been averaged across aspen, aspen-birch, and aspen-maple ring sections.

Elevated levels of CO_2 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 m^2, similar in size but opposite in direction to the effect of O_3. Simultaneous exposure to O_3 and CO_2 had offsetting effects in *B. papyrifera* and *P. tremuloides*. Unpredictably, the competitive advantages *B. papyrifera* was simulated to have under O_3 exposure disappeared when CO_2 was also present. In another result from the landscape level that would not have been predictable from the physiological effects, the presence of CO_2 exacerbated the negative effect of O_3 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 CO_2 on the landscape as the plots suggested, and *B. papyrifera* had twice the response to O_3.

SCALING FOR RISK ANALYSIS

North America and Europe have adopted different approaches to ambient O_3 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
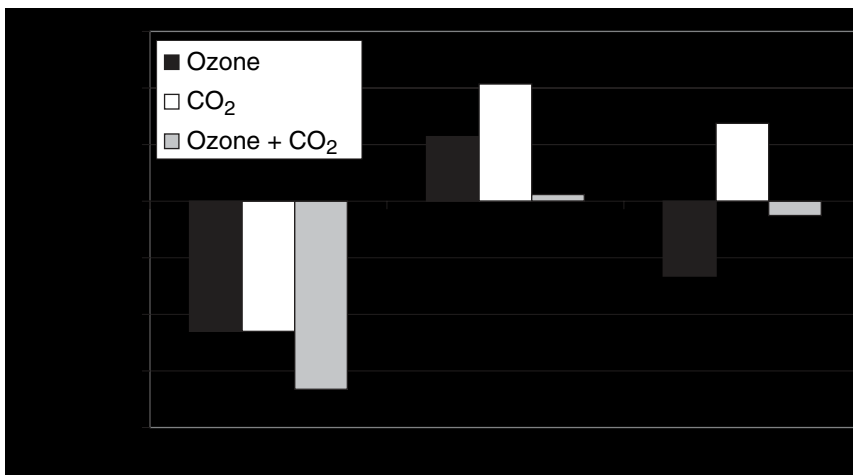
Figure 13. Simulated effects of Aspen FACE treatments on major species in aspen forests of Wisconsin, 2050, expressed as a percent change. The simulations were based on TREGRO/ZELIG model runs parameterized with responses from the Aspen FACE experiment.

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

Treatments	*Acer saccharum*	*Betula papyrifera*	*Populus tremuloides*
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 O_3 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 O_3 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 O_3 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, Selldén & Pleijel 2003; see Ashmore 2005).

Independent of the various experimental methods used, however, there remain a number of uncertainties. First of all, hourly ambient O_3 concentrations follow a rather complex, three-parameter Weibull distribution (Nosal, Legge & Krupa 2000). Secondly, weekly or bi-weekly O_3 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 O_3 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 O_3 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 O_3 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 O_3 uptake through stomata). With the addition of soil moisture data to the main meteorological variables listed, an approximation of first-order atmospheric O_3 flux can be achieved. After verification of validity and significance of such a model, it is then necessary to factor out the effect of O_3 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 O_3 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 O_3 exposure within each of the three replicate FACE rings using established AAQS, CL, and related descriptors, O_3 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 O_3 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 O_3 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

[a]Maximum 1 h O_3 concentration; maximum 8 h O_3 concentration; SUM60; 4th highest daily maximum 8 h O_3 concentration; AOT40 (various 3-month sums); AOT40 (6 month); and tree age (1–5 years). [b]4th, 3-year average of the annual fourth highest daily maximum 8-h O_3 concentrations; from used to calculate US EPA and CWS AAQS. [c]age, aspen tree age (1–5 years). [d]max 8 h: maximum 8 h O_3 concentration.

O_3 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 O_3 risk to the world's forests in the future.

SUMMARY AND CONCLUSIONS

We have examined over 7 years the effects of elevated levels of O_3 at two atmospheric CO_2 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 O_3 affects the flow of C from the leaf and canopy level through tree roots to soil and soil microorganisms, under ambient and elevated CO_2. Our long-term, multidisciplinary research project has consistently shown adverse effects of O_3 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 CO_2 generally moderated the detrimental responses of O_3, 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 CO_2 and O_3 on food quality and the long-term population dynamics of forest pests. Futhermore, 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 O_3 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 O_3 effects on growth.

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