Free-Air Exposure Systems to Scale up Ozone Research to Mature Trees

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Abstract: **Because seedlings and mature trees do not necessarily respond similarly to O_3 stress, it is critically important that exposure systems be developed that allow exposure of seedlings through to mature trees. Here we describe three different O_3 Free-Air Exposure Systems that have been used successfully for exposure at all growth stages. These systems of spatially uniform O_3 release have been shown to provide reliable O_3 exposure with minimal, if any, impact on the microclimate. This methodology offers a welcome alternative to chamber studies which had severe space constraints precluding stand or community-level studies and substantial chamber effects on the microclimate and, hence physiological tree performance.**

Key words: **Free-air, greenhouse gases, ozone, mature trees, sugar maple *(Acer saccharum),* paper birch *(Betula papyrifera),* trembling aspen *(Populus tremuloides),* European beech *(Fagus sylvatica),* Norway spruce *(Picea abies),* European white birch *(Betula pendula).***

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, is an important stressor of over 30% of the world's forests (Fowler et al., 1999; IPCC, 2001). Given the world's continued dependence on fossil fuels and the increasing worldwide distribution of O_3 precursors, it is likely that the toxic impacts of O_3 will continue to threaten the world's forests well into this current century (Percy et al., 2003; Ashmore, 2005; Felzer et al., 2005; Karnosky et al., 2006).

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Our understanding of the influences of O_3 on forest tree growth and metabolism is based largely on seedling research (Hanson et al., 1994; Samuelson and Kelly, 1996; Matyssek and Innes, 1999; Kolb and Matyssek, 2001). Furthermore, most data on tree response to O_3 are from controlled exposures of young trees, often constrained in pots in either indoor or outdoor chambers that do not experience the actual rigors of natural site conditions, including competition (Kolb and Matyssek, 2001). Thus, it is difficult to extrapolate the results of such previous growth studies to the growth trends for trees over their lifetimes or for forest stands over their rotation, given the short time frames of observations and the limited representativeness of studied ontogenetic stages and growth conditions. Conclusions from such experimental setups about the performance of adult, large trees under forest conditions or changing environmental conditions are uncertain at best (Norby et al., 1999; Hendrey et al., 1999; Karnosky et al., 2001; Schaub et al., 2005). In addition, the appropriateness of using seedlings as surrogates for mature trees is questionable, as these ontogenetic stages substantially differ in morphological and physiological characteristics, which, in turn, strongly influences plant response to O_3 (Samuelson and Edwards, 1993; Hanson et al., 1994; Grulke and Miller, 1994; Samuelson and Kelly, 1996; Wieser et al., 2002; Oksanen, 2003 a, b).

The differences between O_3 sensitivity of seedlings and mature trees are not consistent across all species. In giant sequoia, O_3 sensitivity decreases with tree age as stomatal conductance decreases in the older trees (Grulke and Miller, 1994). Greater sensitivity of younger rather than older black cherry trees has also been reported (Fredericksen et al., 1995, 1996). Similarily, Wieser et al. (2002) reported greater O_3 sensitivity in young Norway spruce trees and they associated this with changes in needle morphology, lowered cumulative O_3 uptake, and increased detoxification capacity of older spruce trees. However, in another northern conifer species, Scots pine, the O_3 responses increased with increasing exposure time and tree age (Utriainen and Holopainen, 2001 b). In contrast, red oak seedlings have lower stomatal conductance than mature trees and older trees have greater O_3 uptake and more injury to the pho-

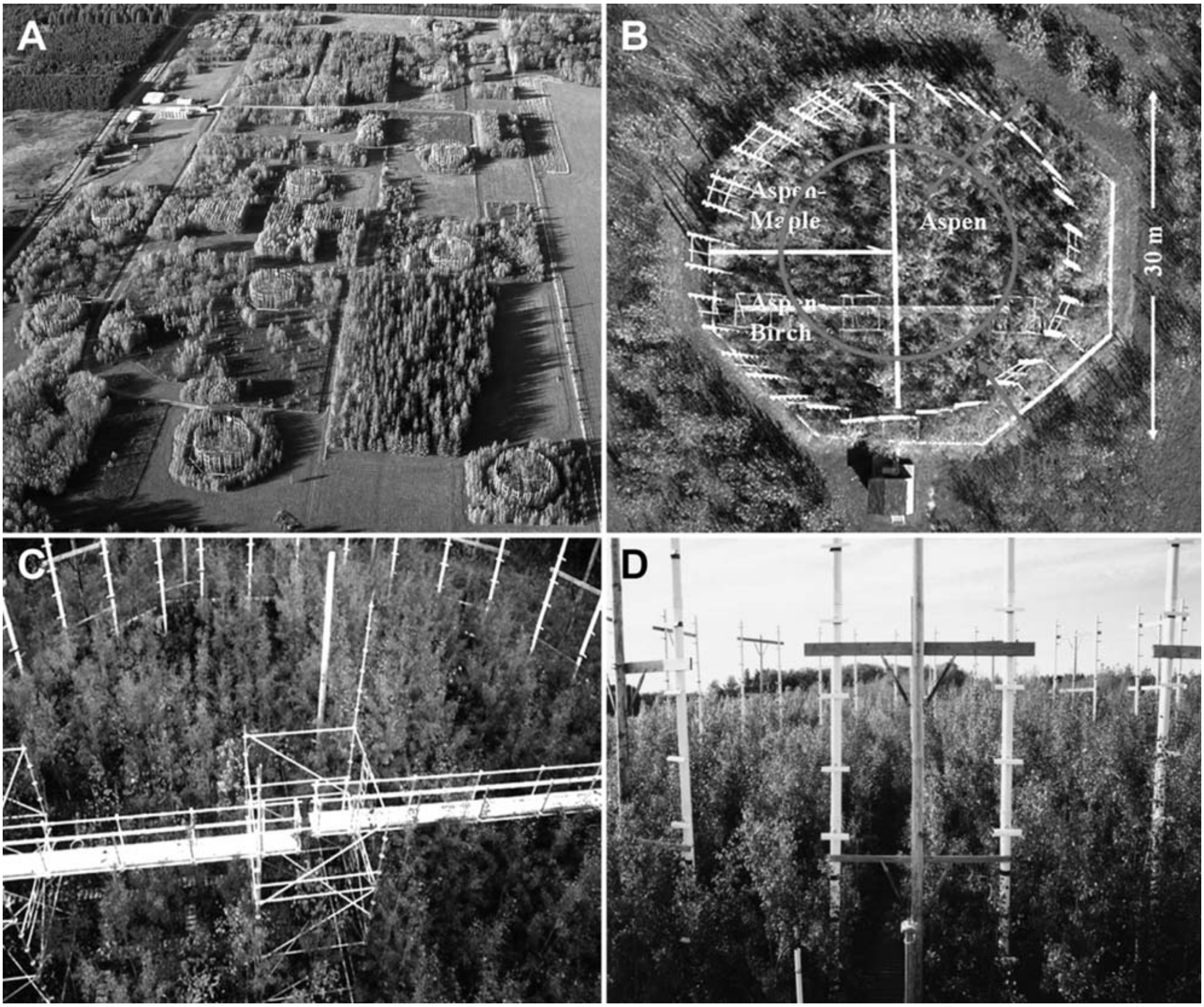

Fig. 1 (**A**) Overview of the 12 rings of the Aspen FACE project in northern Wisconsin. (**B**) Distribution of the aspen, aspen-birch, and aspen-maple communities in each Aspen FACE ring. Each ring is surrounded by a buffer of hybrid poplar trees. (**C**) Canopy access walkways in each ring allow for canopy sampling in the aspen and aspen-birch communities. (**D**) Slots and baffles along the vertical vent pipes allow for gas dispensing to the outside of each ring. Wind then blows the gases into the rings. (Modified from Karnosky et al., 2005.)

tosynthetic system than seedlings (Samuelson and Edwards, 1993; Hanson et al., 1994; Samuelson and Kelly, 1996). Similarly, Oksanen (2003 a, b) found increasing O_3 sensitivity of selected birch clones over her six-year study, suggesting that O_3 sensitivity in European white birch increases with increasing exposure time, tree size, and tree age. Identical O_3 sensitivities were seen for young and old European beech trees, once cumulative O_3 flux was determined (Baumgarten et al., 2000).

Thus, literature on the influence of age on the response of trees to O_3 is quite variable. To be able to predict long-term forest tree growth under rising O_3 concentrations worldwide, a greater understanding of O_3 sensitivity versus tree age is needed (Wieser et al., 2002). The main reason that age effects on tree responses to O_3 remains poorly understood is the difficulty of experimentally exposing large trees to O_3 (Samuelson and Edwards, 1993; Grulke and Miller, 1994; Samuelson and Kelly, 2001; Kolb and Matyssek, 2001; Wieser et al., 2002). In this paper, we describe three free-air O_3 fumigation systems that offer opportunities to examine the impacts of O_3 on trees of differing ages, from young seedlings through to mature trees, while reliably delivering target O_3 concentrations throughout the entire canopy in the absence of major disturbance to the ambient environmental conditions.

Aspen FACE

The Aspen FACE experiment consists of a full factorial design with twelve 30-m diameter treatment rings: 3 control rings, 3 rings with elevated O_3, 3 rings with elevated CO_2, and 3 rings with elevated O_3 + elevated CO_2 (Fig. **1**); where 100 m is the minimum distance between any two FACE rings (Dickson et al., 2000). The rings were planted in late 1997 and treatments ran from budbreak to the end of each growing season from

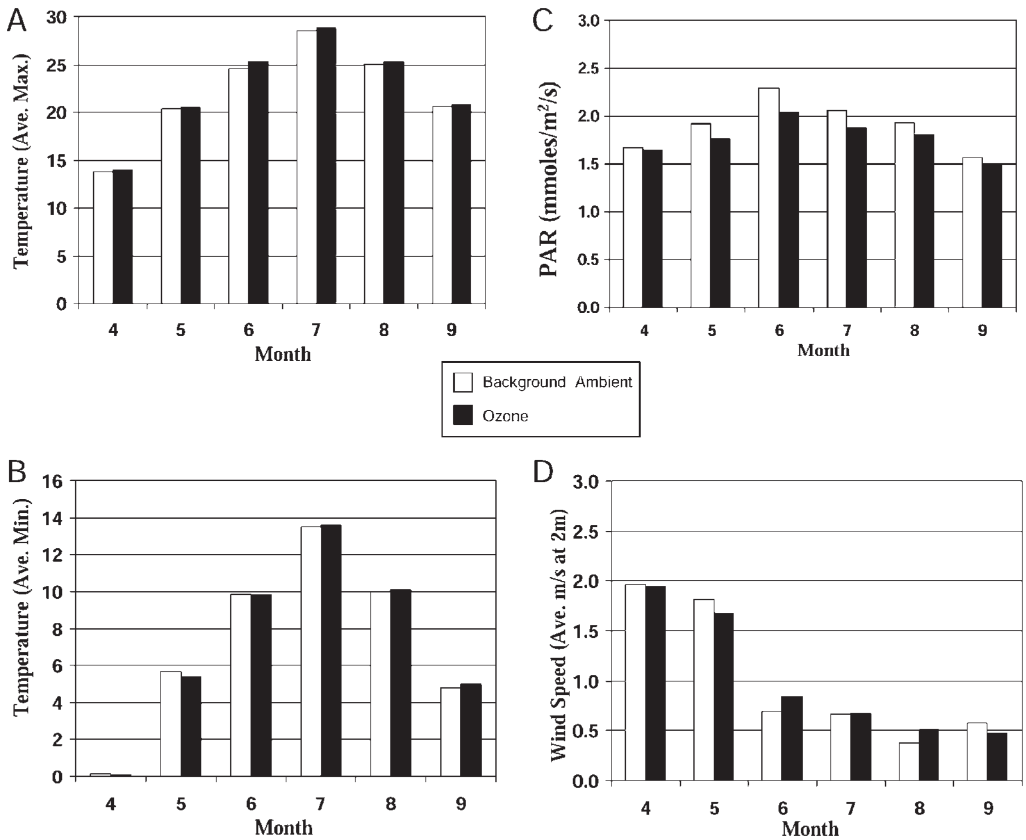

Fig. 2 The Aspen FACE experimental rings have had minimal impact on micrometeorology, as shown in these comparisons for background ambient conditions and an O_3 ring for maximum (**A**) and minimum air temperature (**B**), PAR (**C**), and wind speed (**D**). All values were taken in the mid-canopy height of the aspen section in 1999 and are monthly averages.

1998 – 2006. The eastern half of each ring was randomly planted in two-tree plots at 1 m × 1 m spacing with 5 trembling aspen (*Populus tremuloides* Michx.) clones differing in O_3 tolerance (8L, 216, and 271 = relatively tolerant; 42E and 259 = relatively sensitive). The northwestern quadrant of each ring was planted at the same spacing with alternating aspen clone 216 and sugar maple (*Acer saccharum* Marsh.) seedlings, and the southwestern quadrant of each ring was planted as above with aspen clone 216 and paper birch (*Betula papyrifera* Michx.) seedlings.

Carbon dioxide and O_3 were delivered during the daylight hours using a computer-controlled system modified from Hendrey et al. (1999), with the target CO_2 being 560 µl CO_2 l^{-1}, about 200 µl CO_2 l^{-1} above the daylight ambient CO_2 concentration. Ozone was applied at a target of 1.5 × ambient. Ozone was not delivered during days when the maximum temperatures were projected to be less than 15 °C or when leaf surfaces were wet from fog, dew, or rain events, or when wind speeds were < 0.4 m/s or > 4.0 m/s. Thus, O_3 was fumigated on only 48.7 – 51.6% of the potential growing season days of the study (Percy et al., 2006). Additional details of the experimental design and pollutant generation and monitoring can be found in Karnosky et al. (2003). Treatment summaries for CO_2 and O_3 were published in Karnosky et al. (2003, 2005). In addition, investigators monitored a number of micrometeorological parameters at the site, including wind speed, wind direction, photosynthetically active radiation (PAR), net radiation, relative humidity, rainfall, air temperature at five heights to 20.0 m, soil temperature at the soil surface and at five depths to 2.0 m and soil moisture. Relevant websites for the Aspen FACE project include (1) the general site for the Aspen FACE project (http://aspenface.mtu.edu), (2) the micrometeorology data collected at the Aspen FACE site (http://www.ncrs.fs.fed.us/4401/focus/face/meteorology/), (3) the treatment gas concentrations are shown on the BNL web site (http://www.face.bnl.gov/FACE_Site_Data_Archive/FACESites/FACTSII.htm) and the CDIAC data depository website (http://cdiac.ornl.gov/programs/FACE/facts-IIdata/factsIIdata.html).

Micrometeorology and ozone characterization

The Aspen FACE experiment was originally established to examine the interacting effects of elevated CO_2 and O_3 on the structure and function of northern temperate forest ecosystems. In 2001, the experiment was designated part of a distributed user facility by the U.S. Department of Energy. Key research results have been summarized in Karnosky et al. (2003, 2005).

Aspen FACE ambient air micrometeorological conditions and conditions within the treatment rings were not significantly different from one another (Fig. **2**), suggesting minimal or no effect of the FACE rings on the microclimate at the experiment's start. Treatment effects on tree growth, leaf production and duration, and other physiological parameters have since altered micrometeorological conditions but this has been due to the atmospheric gases and not due to the apparatus.

A representative seasonal cumulative O_3 exposure for Aspen FACE is shown in Fig. **3**. Typical accumulated O_3 exposure over a threshold of 40 nl O_3 l^{-1} (AOT40s) for ambient conditions at

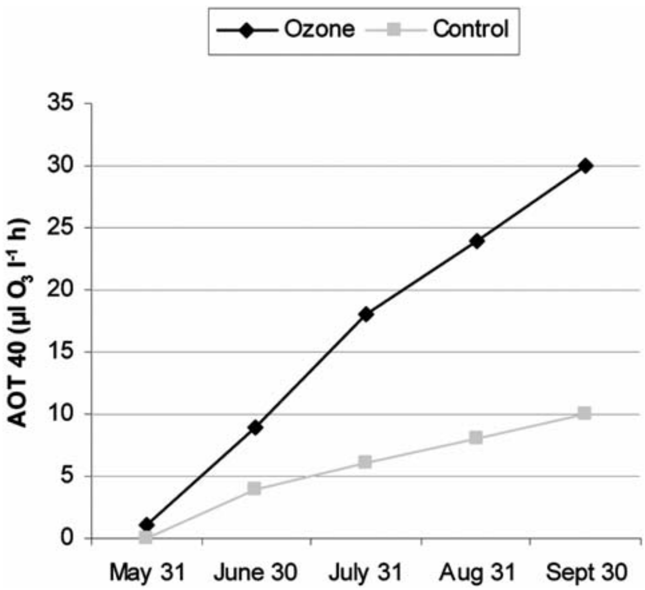

Fig. 3 The ability to deliver a reliable O_3 treatment at Aspen FACE is shown by the 2005 growing season O_3 dose accumulation as depicted by AOT. Here, data from Ring 1.3 is compared to our control monitor.

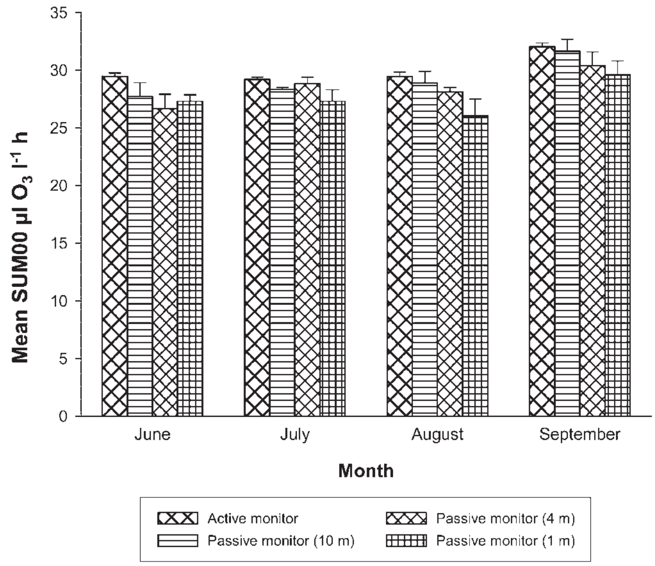

Fig. 4 2005 0zone exposure at ring centre averaged across three FACE rings. Data are shown as mean ± 1 SE. SUM00 cumulative values were measured: 1) using an active ozone monitor with inlet tube co-located above (10 m) the tree canopy with a Can Oxy Plate™ passive monitor; and 2) within (at 4 m) the canopy and below (at 1 m) the canopy using Can Oxy Plate™ passive (24 h) ozone monitors. There was no significant difference (paired *t*-test; $p = 0.127$) in SUM00 between the co-located active and passive monitors. There was ($p = 0.001$) a significant difference in SUM00 due to month. Ozone SUM00 was higher in September due to ozone-induced accelerated senescence of aspen leaves.

Rhinelander, WI have been in the range of 8 to 12 μl O_3 l^{-1} h. Treatment AOT40s have ranged between 25 and 30 μl O_3 l^{-1} h. Mean growing season daylight hour concentrations for ambient and $+O_3$ rings have averaged between 34.6 to 36.9 nl O_3 l^{-1} and 48.9 to 54.5 nl O_3 l^{-1}, respectively, over 1998 – 2005.

Passive sampling using Can Oxy Plate™ passive monitors (Cox and Malcolm, 1999) were used to characterize vertical and horizontal O_3 distribution in O_3 treatment rings (Fig. **4**). Passive samplers co-located with the active O_3 monitor inlet at the top of the canopy in the centre of each ring were always in close agreement ($p = 0.127$). There were no significant ($p < 0.05$) differences between passive samplers situated 1 m and 4 m above ground on individual trees (n = 9) growing within the core area where all biological data are collected by Aspen FACE investigators. In July 2005 (maxium LAI), O_3 exposure ranged from 23.7 to 29.7 μl O_3 l^{-1} h (4 m) and from 20.4 μl O_3 l^{-1} h to 26.3 μl O_3 l^{-1} h (1 m) in ring 2.3. Individual Can Oxy Plate™ passive monitor July 2005 monthly accumulated hourly exposures over 0 nl l^{-1} (SUM00) O_3 values collected at 1 m and 4 m heights have been mapped (Fig. **5**) using ESRI's Arc Map version 9.1. Data were interpolated using a tension spline with the weight of the tension being 0.1.

The Kranzberg Ozone Fumigation Experiment (KROFEX)

KROFEX was installed during 1999/2000 at the Kranzberger Forst research site, about 35 km northeast of Munich (48°25′N, 11°39′E, 490 m a.s.l.) (Werner and Fabian, 2002; Nunn et al., 2002). Scaffolding placed into the mixed stand allowed access to the shade and sun crowns of about 30 trees of European beech (*Fagus sylvatica* Ehrh.) and Norway spruce (*Picea abies* [L.] Karst.). In 1994, beech trees were 51 years old and 24 m high, and spruce trees 44 years old and up to 27 m high (detailed site and stand descriptions in Pretzsch et al., 1998). The scaffolding consisted of three 30-m towers connected through platforms, at 17, 19, 21, and 23 m above ground and a separate 32 m high tower which altogether were positioned approximately as a trapezoid. Since 2001, a canopy research crane with 50-m jib permitted access to the tree canopies of the whole monitoring area of 50 m × 100 m (Matyssek and Häberle, 2002; Häberle et al., 2003). KROFEX was initiated to serve three major interdisciplinary research projects, namely CASIROZ (see www.casiroz.de), the outcome of which is being summarized in this special issue of "Plant Biology" (Matyssek et al., 2007); a preceding study on "Risk assessment of chronically enhanced O_3 exposure by means of free-air fumigation in a mixed spruce/beech stand" (Nunn et al., 2005 a – c, 2006); and the integrated research centre of "SFB 607: Growth and parasite defence – competition for resources in economic plants from agronomy and forestry" (Matyssek et al., 2002, 2005; www.sfb607.de). The latter project aimed at unravelling competitive mechanisms of resource allocation within and between plants, including the adult forest trees of Kranzberger Forst, by making use of O_3 as well as naturally occurring drought or light gradients as stressors or disturbants of the regulatory control of resource allocation (e.g., Reiter et al., 2005; Löw et al., 2006).

The "Free-Air Canopy O_3 Exposure System" (FACOS) of KROFEX enabled a group of 10 neighbouring trees, five beech and five spruce individuals, to be experimentally treated with an enhanced, chronic O_3 regime which was superimposed on the ambient O_3 levels ($1 \times O_3$ regime) of the forest site. The enhancement factor was 2 (2 × ambient O_3 regime = $2 \times O_3$), as based on the online levels of the $1 \times O_3$ regime which were tracked during the diurnal courses throughout the growing season (preventing levels > 150 nl O_3 l^{-1} from $2 \times O_3$ to exclude

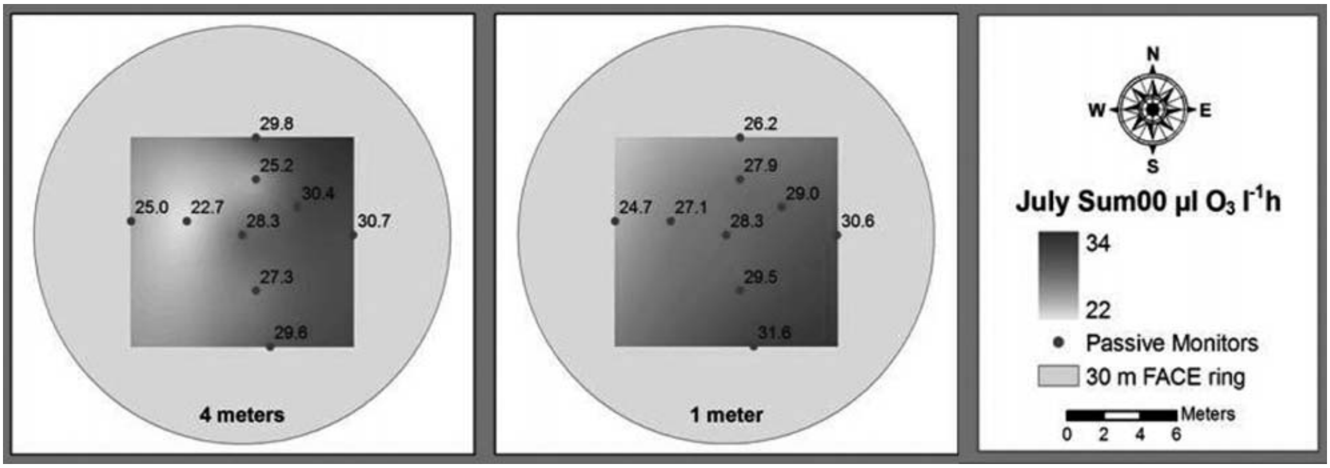

Fig. 5 Horizontal and vertical O_3 exposure profile for the core study area of Aspen FACE ring 2,3 in July 2005. Data are splined as SUM00 µl O_3 l^{-1} h as measured using the Can Oxy Plate™ passive monitors. Measurements at 4 m, 1 m above ground at the same locations are shown separately and in 3-D together. The grey area outside the O_3 profile is the outline of the 30-m diameter FACE rings.

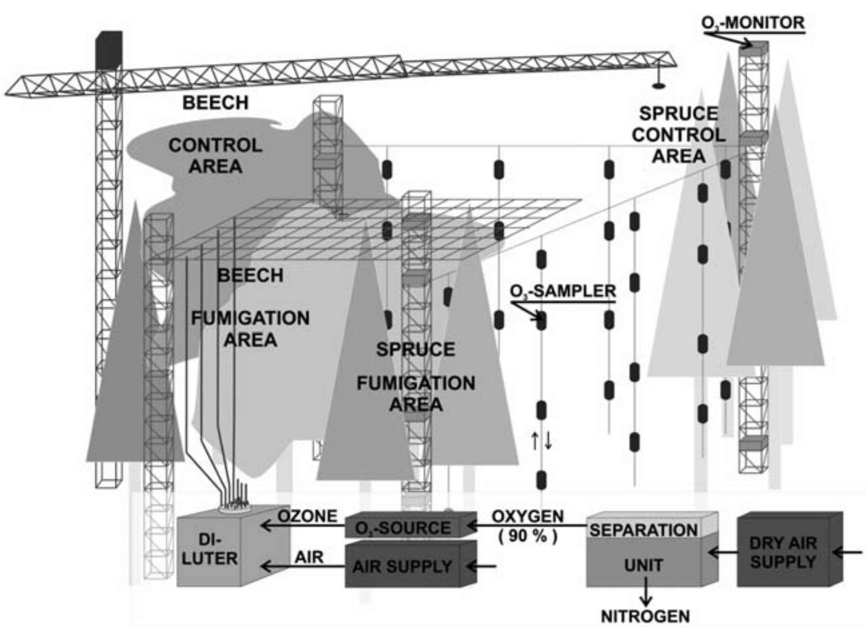

Fig. 6 The experimental setup of the KROFEX experiment, located near Munich, Germany. Ozone is produced by a commercial ozone generator fed with 90% oxygen, which is separated on site from dry purified air. Diluted ozone is pumped to the fumigated canopy volume (approx. 2000 m^3) by means of 117 PTFE tubes and distributed via approx. 6000 calibrated outlets. Ozone monitoring is done continuously by 8 online monitors and 100 passive samplers replaced at weekly intervals.

acute O_3 effects; cf. Reich, 1987). Another group of five beech and spruce trees each under 1 × O_3, adjacent to the O_3-fumigated canopy zone, served as "control". Comparison between the two O_3 regimes allowed a quantitative examination of a broad spectrum of molecular, biochemical, and ecophysiological tree responses under the given site conditions for incipient O_3 injury (see Nunn et al., 2002 and Matyssek et al., 2007 for further details on the rationale of the CASIROZ project).

The KROFEX delivery system is depicted in Fig. **6**. Ozone was produced by means of a commercial O_3 generator, the output of which was computer-controlled between 0 and 70 g/h. In order to prevent oxides of nitrogen from being formed, the O_3 generator was supplied with 90% oxygen rather than air. Based on the pressure swing adsorption (PSA) technique, as mediated through a molecular sieve, oxygen was purified and enriched to 90% from ambient air, after having been passed over a dryer and subsequent VOC filter. The O_3 generator output was fed into a 1000-l mixing tank, with a constant flow of 1500 l/min of ambient air which was added by means of a blower maintaining a tank pressure of 1.2 bar.

In May 2000, fumigation was started with an O_3 distribution system of 100 PTFE tubes (enlarged to 117 tubes in 2002) fitted to the mixing tank with a manifold to conduct the O_3/air mixture directly into the canopy of the study trees. The tubes were fixed in a horizontally mounted grid above the canopy, hanging downwards vertically, at distances of about 100 cm relative to each other. Every tube was equipped with 45 outlets which were each calibrated to the same rate of air release (being corrected for the pressure gradient within the tubes) at distances of 33 cm relative to each other. Outlets were PTFE capillaries of

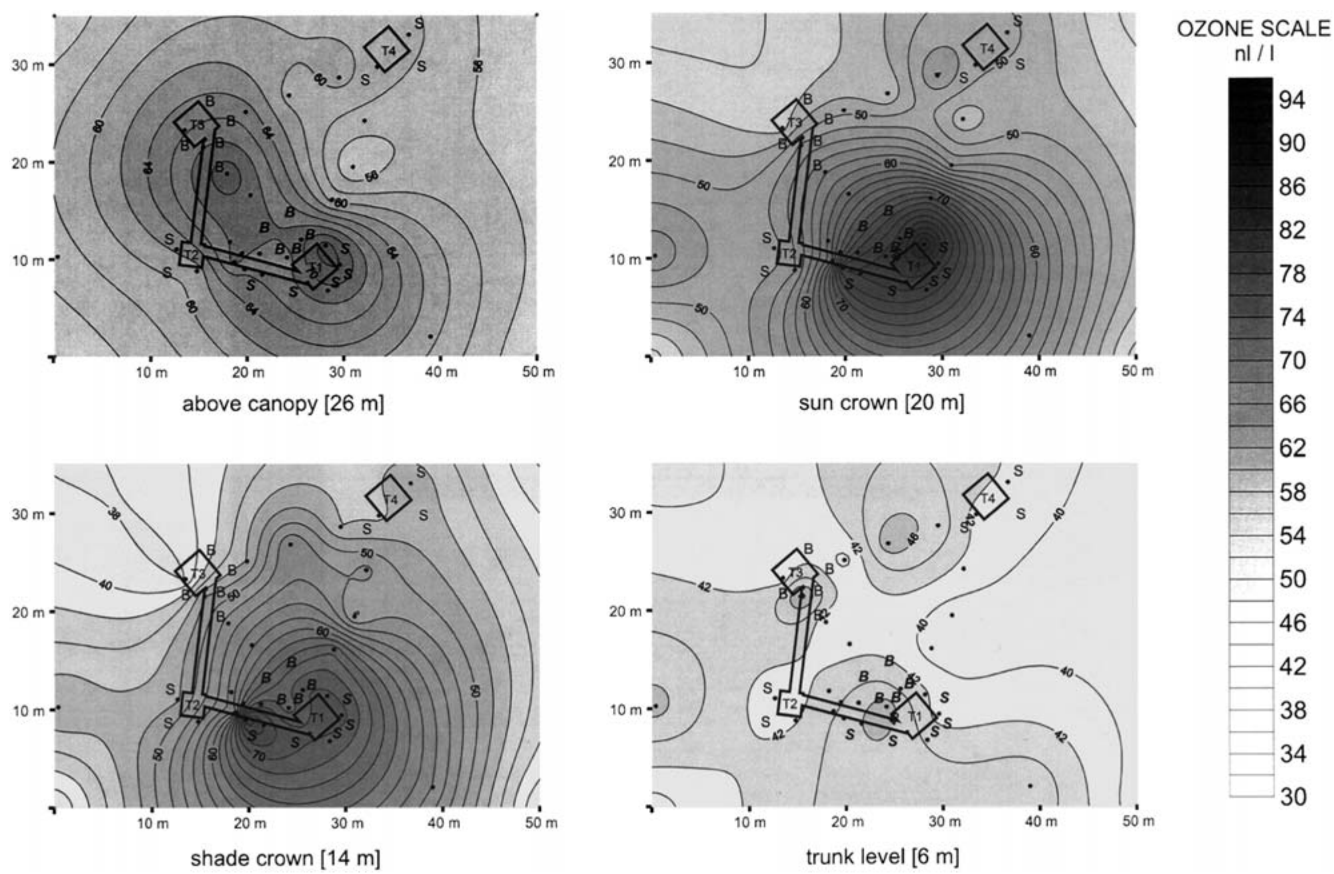

Fig. 7 Contour plots computed by a linear kriging algorithm from passive sampler results in four ozone monitoring levels (6, 14, 20, and 26 m above ground). Sample time was the first week in August 2004. Scaffolding and towers are shown in top view. Ozone is applied around tower T1 and half way between T1 and T2. Passive sampler sites are symbolized by filled circles. Stem positions of fumigated beech (B) and spruce (S) are given in bold italic letters, positions of control trees in regular style. Please note: Due to ozone transport, mixing, and decay the ozone plume is fading out within a few meters, as shown in the contour plots "trunk level" and "above canopy". Much better than a 2-D linear kriging algorithm would be a 3-D non-linear approach including canopy structure and ozone reduction effects. This is the subject of future developments.

0.5 mm inner diameter fitted to the PTFE tubes such that the outflow was oriented downwards. The length of the capillaries decreased along the tubes from bottom to top to compensate for the decline in pressure.

Given this setup, a fumigated canopy volume of about 10 × 13 × 15 m^3 was accomplished. KROFEX was designed to maintain about 2 × ambient O_3 levels across the crowns of the 10 neighbouring trees in this treatment (however, preventing increase above 150 nl O_3 l^{-1}; see above). The system has operated day and night from late April/early May through the first week of November in each year since 2000.

Four continuously-measuring online O_3 monitors were installed at four levels within the fumigated volume, at 6 m (below the fumigated canopies), 14 m (shade crown), 20 m (sun crown), and 26 m (above the canopy), while an additional four monitors were operated within the control plot of the site with the other 10 study trees under unchanged ambient air. For recording the spatial O_3 distribution of the experimentally enhanced O_3 regime and of the control, in addition, an array of 100 (in campaigns up to 200) passive samplers (Werner, 1992; Heerdt, 2006) were deployed at four levels above ground, providing cumulative O_3 exposure as integrated over weekly sampling intervals. Fig. **7** exemplifies passive sampler results within cross-sections of the four O_3 monitoring levels. Data were standardized per unit of time and given as "nl O_3 l^{-1}" rather than "nl O_3 l^{-1} h", as the calibration of as much as 15 000 passive samplers (exposure time of 168 h ± 1 h each) against eight online O_3 monitors (being deployed in the restricted volume of the 2 × O_3 regime and in the surrounding 1 × O_3 regime) yielded a coefficient of determination, $R^2 > 0.9$.

An important issue related to any fumigation system was the formation of "hot spots" due to the fact that O_3 concentrations at the gas outlets needed to be higher than the O_3 target levels to be achieved. Measurements carried out near or between the outlets of adjacent PTFE tubes showed horizontal O_3 gradients to stay confined within a maximum distance of about 5 cm around the outlets. Thus, it can be concluded that KROFEX achieved a fairly homogeneous O_3 distribution, with hot spots, if occurring at all, being restricted to rather short radii around the outlets.

In parallel to the online monitoring of O_3 data at 10-s intervals, a number of micrometeorological parameters were recorded at the site, namely two vertical profiles (five heights) of temperature and air humidity, including wind velocity and direction above the canopy. Particular attention was directed to radiation measurement. In addition to the assessment of global radiation above the canopy, a novel multi-channel system for spectral analysis of solar radiation gave highly resolved spectral information within and above the stand at up to 264 recording points (Reitmayer et al., 2002; Leuchner et al., 2005). Cooperating groups collected further data on rainfall as well as soil and vegetation parameters. Findings on air temperature, precipitation, and continuous O_3 measurements were reported elsewhere (Bahnweg et al., 2005), and cumulative O_3 data are given in (Matyssek et al., 2007).

Open Field O_3 Exposure System in Kuopio, Finland

The Kuopio open field exposure system consisted of eight circular 10-m diameter plots with four replicates of both ambient air and elevated O_3 (Fig. **8**). The free-air system was established in 1989 and has been operating in its current form with eight plots since 2002. The plots were established on a former field and the minimum distance between any two plots is 18 m (mean distance 28 m). The O_3 dispensing system consisted of both horizontal and vertical pipe systems, with total height of 2.2 m. Ozone was generated from pure oxygen with a generator and delivered via a computer-controlled system during daylight hours (between 8 am to 10 pm). Wind direction and wind speed were measured in each O_3 plot, and the O_3 delivery was focused in the upwind direction of each pipe system. The target concentration of the elevated O_3 was 2 × ambient and the target concentration of each exposure plot was individually measured and controlled. Ozone delivery was stopped during calm weather (wind speed < 0.1 m/s), during heavy rain, and when the ambient O_3 concentration was less than 10 nl O_3 l^{-1}. The total O_3 exposure has been 1.3 – 1.7 × ambient due to these cut-off periods. The O_3 concentrations, wind speed, and wind direction were measured from each plot, and PAR, relative humidity, precipitation, and air temperature were measured at the centre of the experimental field. Typical accumulation of AOT40 exposure in control and elevated O_3 plots of the Kuopio exposure field is presented in Fig. **9A**. The hourly mean O_3 concentration of the ambient air rarely exceeding 40 nl O_3 l^{-1} resulting in very low AOT40 exposures (ranging from 0.3 to 1.0 µl O_3 l^{-1} h per growing season) of the control plants. AOT40 doses in the elevated O_3 plots ranged from 7.0 to 22.0 µl O_3 l^{-1} h per growing season (Oksanen, 2003 b). Measurements on leaf-level PAR values, air temperature within the canopy, and volumetric soil moisture content indicated there were no significant differences between the ambient air and the elevated O_3 plots within the experimental field (Fig. **9**; Oksanen, 2003 b).

Potted seedlings were used in most experiments as experimental material. Soil-planted young birch (Oksanen, 2003 a, b) and aspen saplings up to several years old were exposed. The experimental plants were placed in the central parts of the plots, with the diameter of the effective area in each plot being 7 – 8 m, to avoid the higher O_3 concentrations occurring near the pipe system (i.e., hot spots). The potted plants were rotated at each plot during the growth periods. The O_3 exposure operated from the beginning of the growth period (mid-late May) to the end of the growth period (from the end of September to early October). In the longest experiment at the Kuopio site, two European white birch (***Betula pendula*** Roth.) genotypes showing different O_3 sensitivities were exposed to 1.4 – 1.7 × ambient O_3 over seven growing seasons (Oksanen et al., 2007). Data from this long-term birch experiment were used in comparing the efficiency of flux-based $AF_{st}Y$ (Accumulated Flux through stomata above a threshold of X) and AOTX-(Accumulated Over a Threshold of X nl O_3 l^{-1}) based indices at several experimental sites across Europe (Karlsson et al., 2004; Uddling et al., 2004), aiming to improve O_3 risk assessment. Furthermore, birch data from Kuopio were involved in comparison of different stomatal conductance algorithms for O_3 flux modelling (Büker et al., 2006). In the late 1990s, several experiments with conifer seedlings, concentrating on the interaction of nutrient availability and O_3 exposure, were performed on the Kuopio exposure site (Kainulainen et al., 2000; Utriainen and Holopainen, 2001 a, b). Recently, several interaction studies were performed within the Kuopio exposure site, including O_3 × frost experiments with birch and O_3 × soil nitrogen studies with aspen.

Fig. 8 An ozone fumigation plot within the Kuopio experimental site in May (**A**) and July (**B**) 2002.

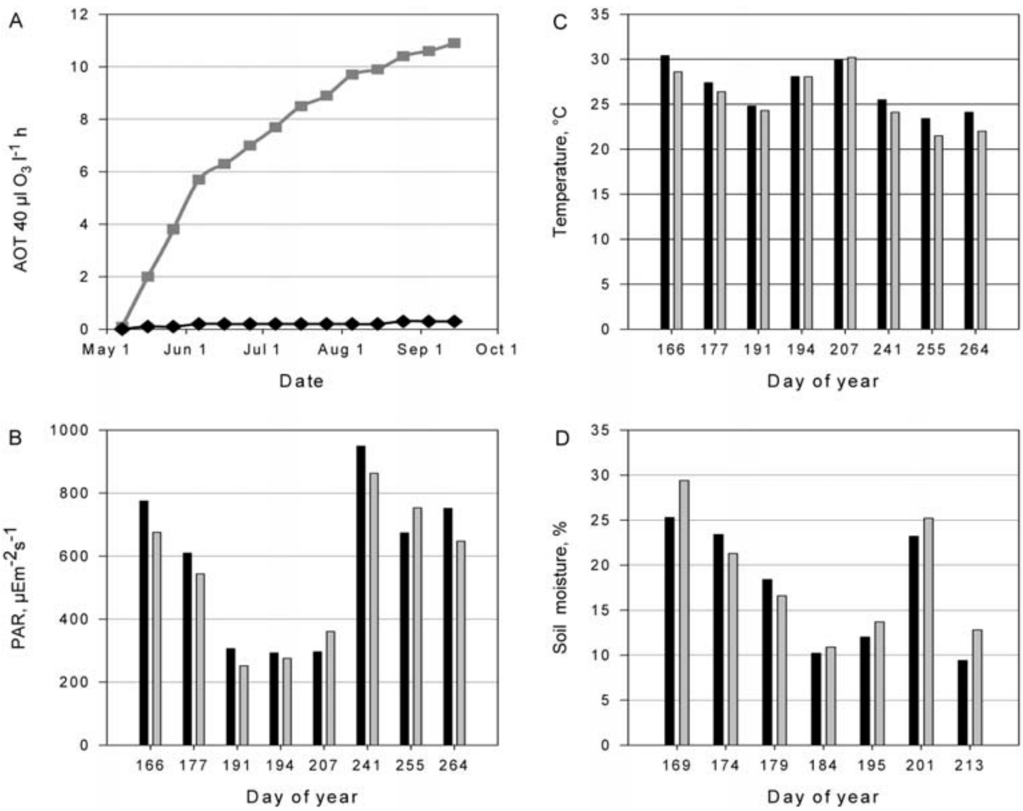

Fig. 9 Typical O_3 dose accumulation pattern given as AOT40 value for elevated ozone fields (■) and ambient ozone fields (■) (**A**), mean daylight leaf-level PAR values (**B**), mean daylight air temperature at canopy level (**C**), and mean volumetric soil moisture content (% of dry weight) (**D**) measurements in Kuopio exposure site in the growing season of 2001.

Conclusions

Here, we have presented three free-air systems differing in design but each with a capability of efficiently delivering desired O_3 concentrations to trees ranging from small stature to mature trees, while having minimal impact on the micrometeorological conditions of the plots. These systems have already been used extensively for studies on forest productivity and ecosystem structure and function. They provide the scientific community with a viable option to study O_3 impacts on older trees, which, until now, have proven difficult to do. Together, the papers from these three free-air systems are helping to develop a clearer picture for scaling up O_3 effects to older trees.

Acknowledgements

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